Planta (1985) 163:527–543

Planta

Control of the rate of cell enlargement: Excision, wall relaxation, and growth-induced water potentials

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Abstract. A new guillotine thermocouple psychrometer was used to make continuous measurements of water potential before and after the excision of elongating and mature regions of dark-grown soybean (*Glycine max* L. Merr.) stems. Transpiration could not occur, but growth took place during the measurement if the tissue was intact. Tests showed that the instrument measured the average water potential of the sampled tissue and responded rapidly to changes in water potential. By measuring tissue osmotic potential (Ψ_s), turgor pressure (Ψ_p) could be calculated. In the intact plant, Ψ_s and Ψ_p were essentially constant for the entire 22 h measurement, but Ψ_s was lower and Ψ_p higher in the elongating region than in the mature region. This caused the water potential in the elongating region to be lower than in the mature region. The mature tissue equilibrated with the water potential of the xylem. Therefore, the difference in water potential between mature and elongating tissue represented a difference between the xylem and the elongating region, reflecting a water potential gradient from the xylem to the epidermis that was involved in supplying water for elongation. When mature tissue was excised with the guillotine, Ψ_s and Ψ_p did not change. However, when elongating tissue was excised, water was absorbed from the xylem, whose water potential decreased. This collapsed the gradient and prevented further water uptake. Tissue Ψ_p then decreased rapidly (5 min) by about 0.1 MPa in the elongating tissue. The Ψ_p decreased because the cell walls relaxed as extension, caused by Ψ_p, continued briefly without water uptake. The Ψ_p decreased until the minimum for wall extension (Y) was reached, whereupon elongation ceased. This was followed by a slow further decrease in Y but no additional elongation. In elongating tissue excised with mature tissue attached, there was almost no effect on water potential or Ψ_p for several hours. Nevertheless, growth was reduced immediately and continued at a decreasing rate. In this case, the mature tissue supplied water to the elongating tissue and the cell walls did not relax. Based on these measurements, a theory is presented for simultaneously evaluating the effects of water supply and water demand associated with growth. Because wall relaxation measured with the psychrometer provided a new method for determining Y and wall extensibility, all the factors required by the theory could be evaluated for the first time in a single sample. The analysis showed that water uptake and wall extension co-limited elongation in soybean stems under our conditions. This co-limitation explains why elongation responded immediately to a decrease in the water potential of the xylem and why excision with attached mature tissue caused an immediate decrease in growth rate without an immediate change in Ψ_p.

Key words: Cell wall relaxation – Cell elongation – *Glycine* (growth control) – Turgor pressure – Water potential.

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Abbreviations and symbols: L = tissue conductance for water; m = wall extensibility; Y = average yield threshold (MPa); Ψ_o = water potential of the xylem; Ψ_p = turgor pressure; Ψ_s = osmotic potential; Ψ_w = water potential of the elon gating tissue

Introduction

The enlargement of plant cells, which is the primary means by which plants increase in size, is almost

entirely attributable to an increase in cellular water content driven by osmosis. The process depends on the simultaneous uptake of water, extension of the cell walls, and accumulation of solute. Enlargement is initiated when the walls are loosened by metabolic events, and a turgor-driven extension follows (Cleland 1971, 1981; Taiz et al. 1981). As extension occurs, turgor pressure (Ψ_p) decreases and creates a lowered water potential within the cells. This low water potential causes water to enter the cells and occupy the new space created by the extension of the walls (Lockhart 1965; Molz and Boyer 1978; Silk and Wagner 1980; Cosgrove 1981). Solute accumulation maintains the osmotic potential Ψ_s necessary to generate Ψ_p and water potential. During steady enlargement, these processes occur continually and result in lower Ψ_p and water potential than when the walls have not been loosened and growth is not occurring.

In cells enlarging in this way, theory predicts that withholding water will allow turgor-driven extension to continue but no water to enter the cells. This should cause Ψ_p to decrease and the cell walls to relax until Ψ_p reaches a minimum for extending the walls. At this Ψ_p, called the yield threshold (Y), further wall extension would cease. Thus, Ψ_p and water potential should be still lower than when water was supplied and growth was occurring.

Attempts have been made to detect both the lowered water potential of enlarging tissue and the further lowering when water is withheld, for example by excising the tissue from its water supply. Early measurements of the water potential of enlarging tissue (Bennett-Clark 1956; Brouwer 1963; Burström 1953; Burström et al. 1967; Cleland 1959, 1967; Ordin et al. 1956) depended on immersion of tissue segments in concentrated solutions and determination of the solution water potential that prevented enlargement. However, this approach suffers from problems due to long times, solute penetration, unstirred layers of solution, and the need to prevent growth in order to measure water potential. Kinetic measurements of the rate of equilibration of tissue segments with isotopically labelled water (Ordin and Bonner 1956; Ordin et al. 1956; Ray and Ruesink 1963) or with solution were subject to similar problems (Ray and Ruesink 1963). The best early estimates appear to have been obtained from the kinetics of water uptake by previously dehydrated tissue (Ray and Ruesink 1963). These suggested that enlarging tissue required a water potential 0.1 to 0.15 MPa below the water potential of the external solution in order for water to be supplied to the enlarging cells.

Subsequently, isopiestic thermocouple psychrometry was used to measure water potential directly in tissue that remained attached to the plant and grew during the measurement (Boyer 1968; Boyer and Wu 1978; Molz and Boyer 1978; Cavalieri and Boyer 1982; Westgate and Boyer 1984). The water potentials were 0.15 to 0.25 MPa below that of the vascular system and were not observed in mature tissue. However, comparisons of water potentials of intact and excised tissue failed to detect a lowering of water potential after excision (Boyer 1968; Boyer and Wu 1978; Molz and Boyer 1978; Cavalieri and Boyer 1982; Savage and Cass 1984), although Baughn and Tanner (1976) occasionally observed changes in water potential when leaf tissue was excised. The comparisons were made with different instruments that would have detected excision effects only larger than about 0.15 MPa. Therefore, in order to increase the resolution of the measurements, we designed a new guillotine psychrometer that allowed water potential to be measured in intact tissue and to be followed during and after excision of the same tissue. We investigated i) whether there are persistent water potential differences between the intact enlarging tissue and the vacular system, ii) whether Ψ_p decreases when enlarging tissue is separated from its water supply by excision, iii) whether enlargement ceases at the same time, and iv) whether water supply or wall extension are rate-limiting for cell enlargement.

Theory

The enlargement of plant cells requires a high enough Ψ_p to extend the walls and a low enough water potential to provide water for the enlargement process. The Ψ_s must be sufficiently low to allow both these requirements to be met simultaneously. For tissue enlarging at a steady rate, these characteristics of the cells should remain constant but could vary with position because water potential gradients involved in moving water to the enlarging cells imply that water potentials of the outlying cells are inevitably lower than the water potential of the xylem. For many experiments, however, growth rates are measured as average rates for the entire enlarging region. Therefore, average tissue water potential, Ψ_s, and Ψ_p are adequate to describe the enlargement process. As will be shown below, the thermocouple psychrometer provides average water potential, Ψ_s, and Ψ_p in soybean stems. Accordingly, the steady rate of tissue enlargement can be related to the average wall extensibility (m) and average Ψ_p that extends the wall by the equation (Green et al. 1971):

$$\frac{dV}{dt}\frac{1}{V}=G=m(\Psi_p-Y) \qquad \text{eqn. 1}$$

where V is the volume of the enlarging tissue (cm^3), Y is the average yield threshold (MPa), and m has units of $s^{-1}\cdot MPa^{-1}$. This equation represents the demand for water caused by the turgor increment (Ψ_p-Y) that extends the walls.

The steady rate of water uptake necessary to support cell enlargement can be related to the average tissue conductance for water (L) and average water potential difference between the water source and the protoplasts. For most plant tissues, the water source is the xylem and thus:

$$\frac{dV_w}{\mathrm{dt}}\frac{1}{V_w}=G=L(\Psi_o-\Psi_w) \qquad \text{eqn. 2}$$

where V_w is the volume of water in the enlarging tissue (cm^3), Ψ_o is the water potential of the xylem (MPa), Ψ_w is the average water potential of the elongating tissue (MPa) and L has units of $s^{-1}\cdot MPa^{-1}$. The V of eqn. 1 is not identical to V_w of eqn. 2 because V involves intercellular space as well as water volume whereas V_w involves only water volume. The differential forms are equivalent, however, and G of eqn. 1 equals G of eqn. 2. Equation 2 represents the supply of water caused by the growth-induced water potential ($\Psi_o-\Psi_w$) that drives water inflow (Molz and Boyer 1978; Silk and Wagner 1980).

Growth G therefore depends on a water supply function (eqn. 2) as well as on a water demand function (eqn. 1) which act simultaneously during steady tissue enlargement. The magnitude of the coefficients m and L will determine whether the supply or the demand function is rate limiting. Substituting $\Psi_p=\Psi_w-\Psi_s$ in eqn. 1 and combining eqns. 1 and 2 to eliminate Ψ_w gives:

$$G=\frac{mL}{m+L}(\Psi_o-\Psi_s-Y) \qquad \text{eqn. 3}$$

which is the combined rate equation governing tissue enlargement (Lockhart 1965; Boyer and Wu 1978) and showing both the effects of wall extensibility and water conductance. The coefficient $mL\,(m+L)^{-1}$ determines the rate of tissue enlargement at a particular Ψ_o. When m is large, the coefficient approximates L, which then controls growth. When L is large, the coefficient approximates m, which controls growth. The factor ($\Psi_o-\Psi_s-Y$) is the net osmotic force for growth and represents the maximum osmotic force ($\Psi_o-\Psi_s$) diminished by Y.

Figure 1 A, B illustrates the water supply and demand of growing tissues (eqns. 1, 2), and Fig. 1 C shows the combined effects (eqn. 3) in diagrammatic form. Enlarging tissue has a water potential defined by the intersection of the lines formed by eqns. 1 and 2 when growth occurs steadily. This water potential (Ψ_w) can be measured in intact enlarging tissues with the psychrometer. It is obvious that plants may differ with respect to their tissue water potential and growth depending on the water supply and demand. When L is large, Ψ_w is close to Ψ_o and the growth rate is large at a particular m. When m is large, Ψ_w is close to the water potential at Y and the growth rate is large at a particular L. Moreover, the growth-induced water potential ($\Psi_o-\Psi_w$) can be modified either by the activity for growth, which affects Ψ_w, or by conditions in the xylem, which affect Ψ_o. To emphasize this point, we will hereafter refer to the growth-induced water potential as ($\Psi_o-\Psi_w$).

Upon excision, two processes take place. First, the water in the xylem is rapidly depleted and Ψ_o falls until it equilibrates with Ψ_w, preventing water entry into the enlarging cells. Second, since Ψ_p remains large, the walls continue to extend, but as no water can enter, the walls progressively relax, and turgor decreases to Y. Figure 1 D shows that the change resulting from excision should be a movement of Ψ_o and Ψ_w to the right with a decrease of Ψ_p to Y until $\Psi_o=\Psi_w=\Psi_s+Y$, where enlargement ceases. In principle, the turgor increment for wall extension can be measured by this decrease in Ψ_p to Y.

It should be noted that while Ψ_p is moving toward Y, the tissue is not in a steady state and the above equations cannot be applied. However, after Ψ_p reaches Y, the tissue is in water potential equilibrium with its surroundings and growth ceases because (Ψ_p-Y) and ($\Psi_o-\Psi_w$) become zero.

The relative magnitudes of m and L may be compared by setting eqn. 1 equal to eqn. 2:

$$\frac{m}{L}=\frac{(\Psi_o-\Psi_w)}{(\Psi_p-Y)} \qquad \text{eqn. 4}$$

This relationship shows that simple measurements of ($\Psi_o-\Psi_w$) and (Ψ_p-Y) in the intact tissue can indicate whether cell enlargement is limited by water conductance ($mL^{-1}\gg 1$), wall extensibility ($mL^{-1}\ll 1$) or both ($mL^{-1}\simeq 1$). The following paper will give experimental evidence that allows each term of the water supply and demand functions (eqns. 1 to 4) to be determined and the control of growth by m and L to be evaluated.

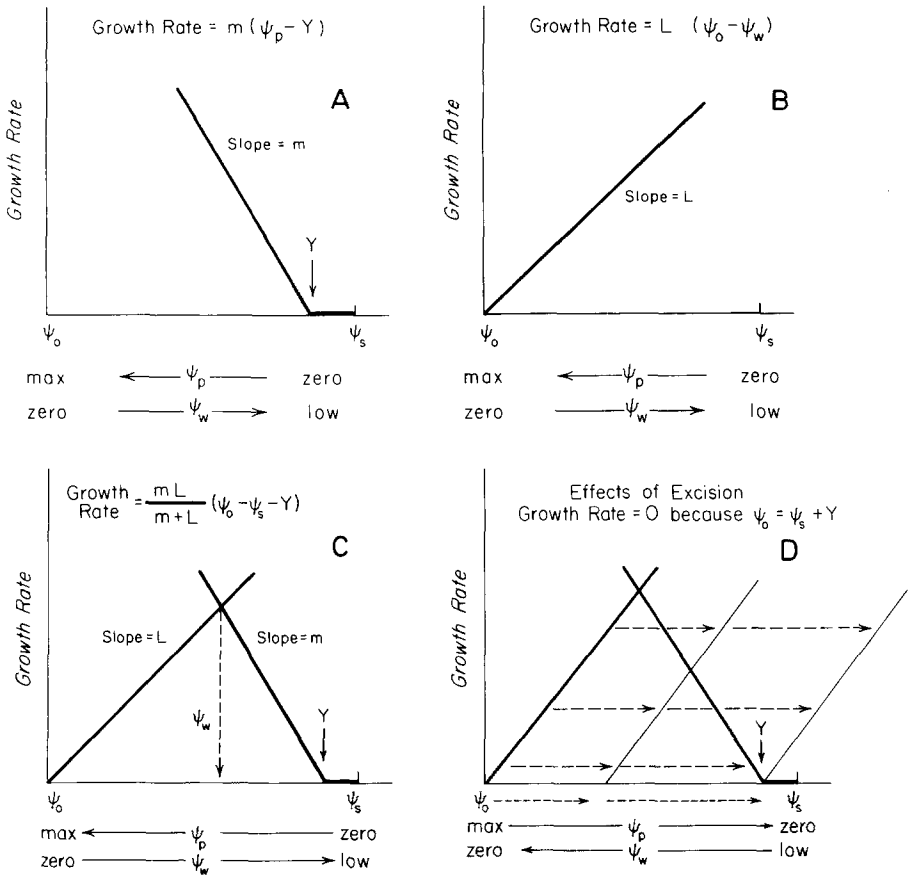

Fig. 1 A–D. Diagrammatic representation of effects of wall extensibility m (**A**), water conductance L (**B**), wall extensibility and water conductance (**C**), and excision on steady enlargement of plant tissue (**D**). **A–C** represent eqns. 1–3 in text. **D** shows decrease in water potential of water source (Ψ_o) as water is depleted after excision. After depletion, $\Psi_o = \Psi_s + Y$. Also, tissue water potential (Ψ_w) decreases from central position in **C** to $\Psi_w = \Psi_s + Y$ in **D**. Growth decreases to zero after excision. The decrease in Ψ_w from **C** to **D** represents the first phase of excision effects and is completed within about 5 min (see below). A further decrease in Ψ_w occurs during the next few hours but appears to involve a slow decrease in Y

Material and methods

Plant material. Soybean (*Glycine max L.* Merr. cv. Wayne; Illinois Foundation Seed, Tolono, Ill., USA) seedlings were grown from seed in vermiculite in the dark at 29±0.5° C for 4 d. The atmosphere was saturated with vapor, and water (8 ml g^{-1} vermiculite) was supplied as $CaCl_2$ solution (0.1 mM). Seed coats were removed after the first 2 d and the seedlings were transplanted to identical vermiculite for the remaining 2 d.

Tissue water status. Measurements of stem (hypocotyl) water potentials were conducted in a specially constructed isopiestic thermocouple psychrometer that allowed four whole seedlings to be sealed into a thermally stable chamber (plant chamber) inside of which was a small vapor pressure chamber for the thermocouple (thermocouple chamber). The thermocouple chamber was enclosed by a tube with a sharpened edge (guillotine) that could be slid along the outside of the chamber until it excised the tissue in the chamber (Fig. 2). No cut surfaces were exposed within the chamber, as the excision was made on the outside. The apparatus was large enough to allow water to be supplied to the seedling roots, which were wrapped in tissue paper soaked in $CaCl_2$ solution (0.1 mM). A thermocouple bearing a sucrose solution of known water potential could be placed in the thermocouple chamber for measurement of tissue water potential. 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 assured that the tissue neither hydrated nor dehydrated during the course of the measurement (Boyer and Knipling 1965).

The water potential was measured at various positions along the stems by sealing the appropriate region (Fig. 2A–D) in the thermocouple chamber with petrolatum (Vaseline). The stems were about 100 mm long (Fig. 3) and consisted of a meristematic region immediately behind the cotyledons, an elongating region extending 35 or 40 mm below the cotyledons, and a basal region that was completely nongrowing (Cavalieri and Boyer 1982). The cortical tissue comprised 75% of the tissue volume, the rest consisting of vascular and pith tissue. The tissue in the thermocouple chamber could be excised with the guillotine and, when portions of the stems extended outside of the apparatus, by hand with a razor blade (Figs. 2, 3). The dimensions of the thermocouple chamber (20 mm diameter) and the guillotine (22 mm diameter) permitted the chamber to be filled solely with elongating tissue or with mature tissue (Fig. 3A, B). Cuts made by hand were usually in the positions shown in Figs. 2C, D, 3C, D.

For water potential measurements in the upper stem, the seedlings were placed with their cotyledons in holes in the plant chamber adjacent to the thermocouple chamber, and the elongating region was positioned in the grooves in the bottom of the thermocouple chamber (Figs. 2A, 3A). The cotyledons were thus immobilized and the elongating tissue remained constantly in position in the thermocouple chamber. New stem produced in the elongating region was continually pushed through the thermocouple chamber in a downward direction (Fig. 2). For water potential measurements in the basal stem, the stems just above the root-stem transition were sealed in the thermocouple chamber (Figs. 2B, 3B).

For some water potential measurements, the stems extended outside the thermocouple and plant chambers as in Figs. 2C, D and 3C, D. In these cases, the stems were sealed not only

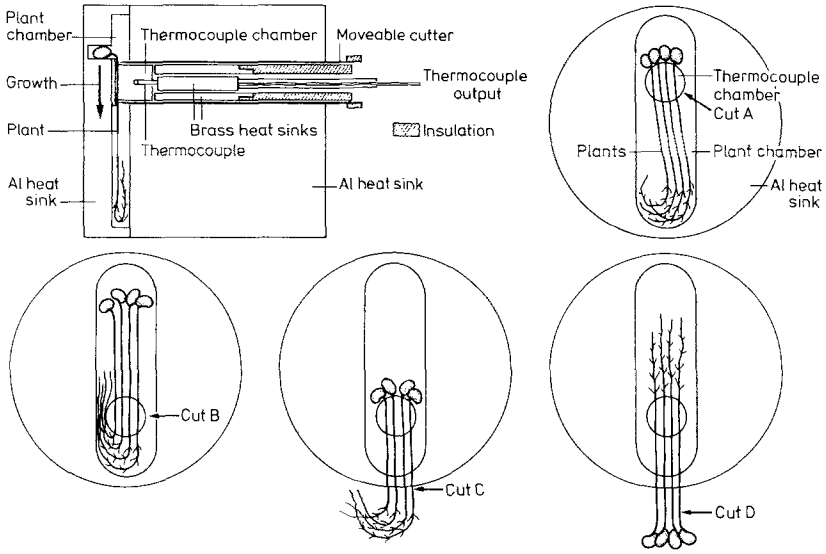

Fig. 2. Design of guillotine thermocouple psychrometer. Instrument consists of a vertical plant chamber inside of which a thermocouple chamber encloses the part of the plant to be measured. A thermocouple is sealed in the thermocouple chamber. Elongation pushes new stem tissue downward through the thermocouple chamber. A supply of water is provided to the roots by wrapping in wet tissue paper. The guillotine (movable cutter) is a tube with a sharp edge that slides between the thermocouple chamber and the heat sinks. The cut is made on the outside of the thermocouple chamber. *Cut A* excises the stem elongating region; *cut B* excises the stem mature region; *cut C* excises the roots at the stem base and can be followed by a cut with the guillotine in the elongating region; *cut D* excises the stem elongating region and can be followed by a cut with the guillotine in the stem mature region. See text for further details and Fig. 3 for exact position of cuts

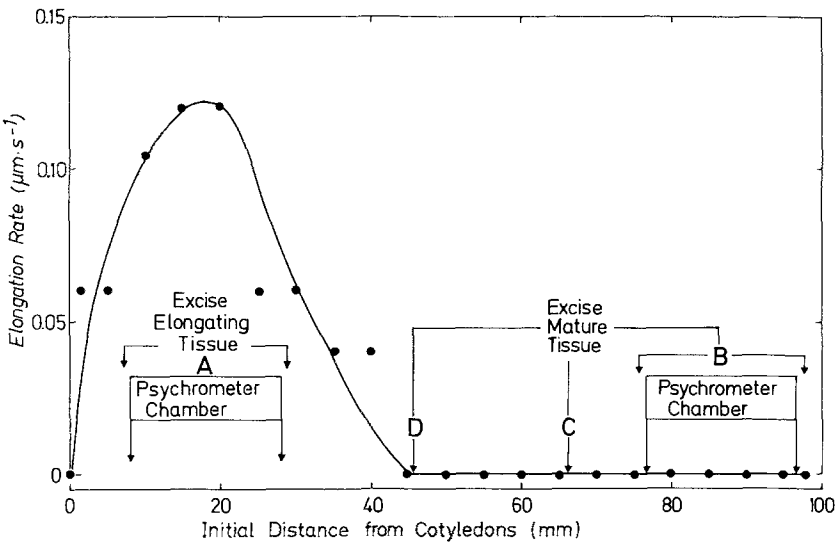

Fig. 3. Profile of elongation growth and position of cuts along stems of soybean seedlings. Elongation rate is shown for a 14 h growth period. *Cuts A–D* are made in guillotine psychrometer as shown in Fig. 2. Initial length of stems was about 100 mm

in the thermocouple chamber but also in grooves in the wall of the plant chamber through which the stems passed to the outside.

The thermocouple chamber consisted of a grooved base to which a top was fitted. A thermocouple could be inserted through a hole in the top (Fig. 2). Both the top and the grooved base were coated with melted and resolidified petrolatum to reduce sorption of water vapor on the walls (Boyer 1967), and the grooves in the base dissipated most of the heat of respiration (Barrs 1965). The thermocouple, the heat sink above the chamber, and the guillotine were insulated to prevent heating by the hand during manipulation of the apparatus.

The entire instrument was placed so that the seedlings grew vertically (Fig. 2). It was covered by an insulated box to further reduce thermal effects within the instrument. The output of the thermocouple was amplified (Model 148 Nanovoltmeter, Keithley Instruments, Cleveland, Ohio, USA) and displayed on a strip chart recorder. The circuitry was all copper except for a thermocouple switch (Model 8248, Leeds and Northrup, Philadelphia, Pennsylvania, USA). All junctions in the circuitry were tested for thermal insensitivity. The measurements were made at 26° C in a constant temperature room.

The osmotic potential (Ψ_s) of the tissue in the thermocouple chamber was measured by excising the sample immediately

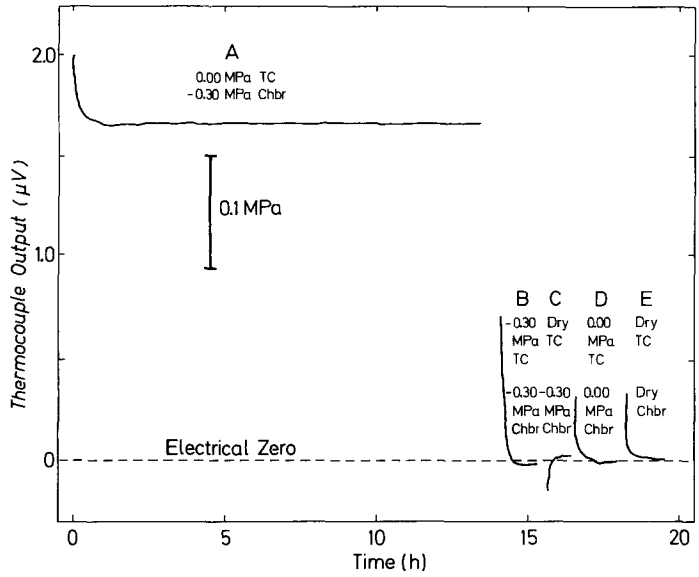

Fig. 4. Performance of guillotine psychrometer when the thermocouple junction (*TC*) contains water and thermocouple chamber (*Chbr*) contains sucrose solution (−0.30 MPa) in place of plant tissue (*A*), when junction and chamber both contain same solution as in *A* (*B*), when junction is dry and chamber contains same solution as in *A* (*C*), when junction and chamber contain water (*D*), and when junction and chamber are dry (*E*). *Tests A–C* represent the measurements usually required for an isopiestic determination with plant tissue. *Test D* represents an isopiestic measurement of a water potential of zero and checks for thermal offsets in the electrical circuitry. *Tests B–E* should give zero output and *test A* should give high stable output when instrument functions properly. Direct traces of recorder chart

after a water potential measurement, placing the segments in a hypodermic syringe, freezing and thawing, and expressing the cell solution under pressure by filtration through filter paper in the syringe. The solution was placed on a thermocouple junction and its Ψ_s was measured above sucrose solutions by isopiestic technique. Tissue Ψ_p was calculated by subtracting Ψ_s from Ψ_w. No correction was made for dilution of the cell solution by apoplast water as the cell walls represented less than 4% of the cell volume (Molz and Boyer 1978).

Growth rates. Stem elongation in the psychrometer was determined by measuring stem length before and after the water potential measurements.

Detailed measurements of the length of soybean stems were made in a dark atmosphere saturated with water vapor at 29° C using a radial displacement transducer. The transducer was clamped to the upper part of the stem, and a rigid bar was clamped to the lower stem. Length was recorded electrically and represented the length between the transducer and rigid bar. The transducer and bar were mounted on a microscope, the fine adjustment of which could be used to calibrate the instrument without disturbing the tissue.

Tissue manipulations. All tissue manipulations were carried out in an atmosphere that was water saturated under a green safelight having spectral characteristics described by Boyer and Wu (1978).

Results

Test of apparatus. Measurements of high resolution and fast response are necessary to measure the water potentials associated with growth because the potentials are small and relaxation could be rapid. Moreover, growth during the measurements was so fast in our experiments that all of the cells in the thermocouple chamber were replaced in 10 to 20 h (20 mm of growth), which represented a possible source of variability. In order to obtain accurate measurements, we required that the guillotine psychrometer provide i) high sensitivity (better than 50 nV), ii) stable outputs for long periods, iii) zero output at zero water potential, iv) isothermal conditions (better than 0.001° C), v) no water potential offsets, vi) rapid response to changes in water potential, and vii) no effect of operating the guillotine. In this way, changes associated with tissue behavior could be distinguished from instrument behavior.

The stability and kinetic behavior of the psychrometer were tested by placing a solution instead of plant tissue on the floor of the thermocouple chamber. A thermocouple bearing water on the junction rapidly stabilized and gave a high constant output for 13 h as water evaporated from the thermocouple and was absorbed by the solution (Fig. 4A). A resolution better than 0.01 MPa was obtained. An isopiestic reading could be produced by placing an identical solution on the thermocouple junction, and thermocouple output fell to within 0.005 MPa of zero within 30 min (Fig. 4B), as expected from the lack of net vapor transfer in the equilibrium condition. Temperatures within the chamber were isothermal, as indicated by the thermocouple when dry (Fig. 4C), and the thermocouple detected water potential of zero when water was present on the chamber floor and on the thermocouple (Fig. 4D). There were no thermal offsets in the apparatus, shown by the zero output of a dry thermocouple in a dry thermocouple chamber (Fig. 4E).

The thermocouple responded rapidly to a change in water potential of the contents of the thermocouple chamber. Water injected isothermally onto the chamber bottom caused an immediate change

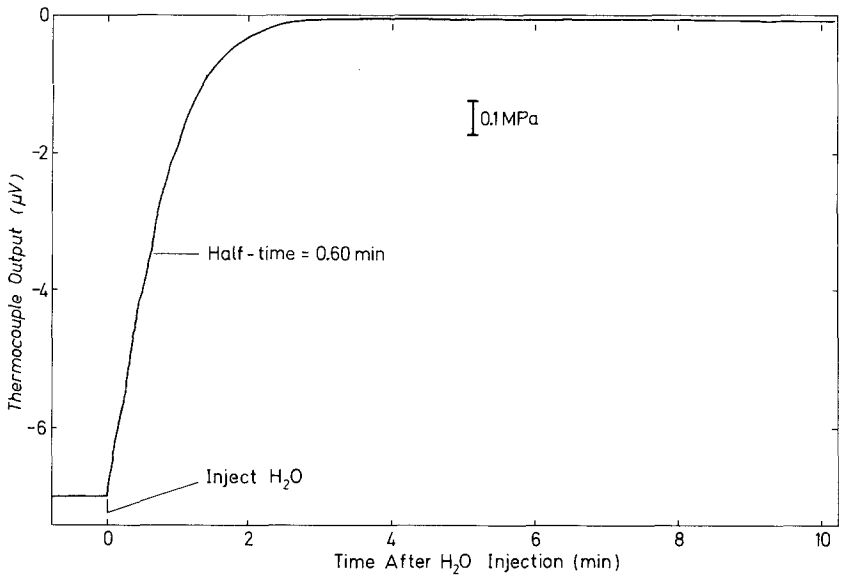

Fig. 5. Kinetics of thermocouple response to a step change in the water potential of filter paper in guillotine psychrometer. Step change was made by injecting isothermal water onto filter paper at zero time. Direct trace of recorder chart

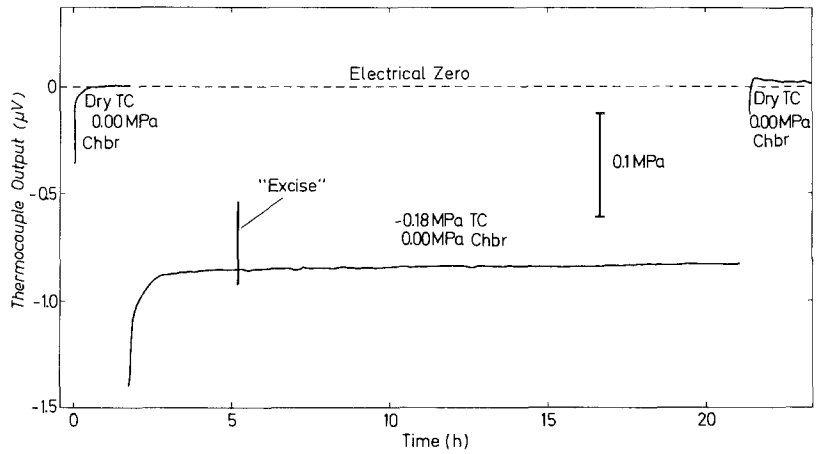

Fig. 6. Effects of operating guillotine in guillotine psychrometer. Thermocouple (*TC*) contained sucrose solution (−18 MPa) and thermocouple chamber (*Chbr*) contained water on bottom instead of plant material. Before and after the determination, the dry thermocouple was inserted into the chamber to test for isothermal conditions. Guillotine was operated at "excise". Direct trace of recorder chart

in thermocouple output (Fig. 5) that stabilized rapidly (half time = 36 s) and was controlled mostly by droplet size on the thermocouple junction. When the guillotine was operated as if to cut plant tissue, the thermocouple did not respond except for a rapid, reversible transient (Fig. 6).

These tests showed that the seven requirements for instrument performance could be met in this study. However, besides the instrument test without tissue, it was essential to test the system with tissue in order to learn where water potential was detected. This would allow the measurements to be interpreted in relation to the theory. For excised tissue that is equilibrated internally, the water potential is the same everywhere and should be an average for the water potential gradients that existed prior to the equilibration. However, intact tissue that is growing is continually absorbing water, and water potential gradients are present. In this situation, the water potential indicated by the psychrometer is not certain and could be that of surface cells or inner cells or an average for all the cells. It is possible to distinguish between these possibilities by comparing the water potential of intact tissue with that of the same tissue after excision and internal equilibration. If the intact tissue contains water potential gradients, the comparison indicates whether the water potential shown by the psychrometer is wetter or drier than the average for the tissue.

To make this test, we allowed mature stem tissue to hydrate and interrupted the hydration by excising the tissue. Because water was absorbed during the hydration, gradients in water potential were present in the attached tissue but not in excised tissue. By using mature tissue, the complicating effects of growth were avoided.

The seedlings were first dehydrated to 80% of their fresh weight by allowing water loss to air of low humidity in the dark. The stem bases were

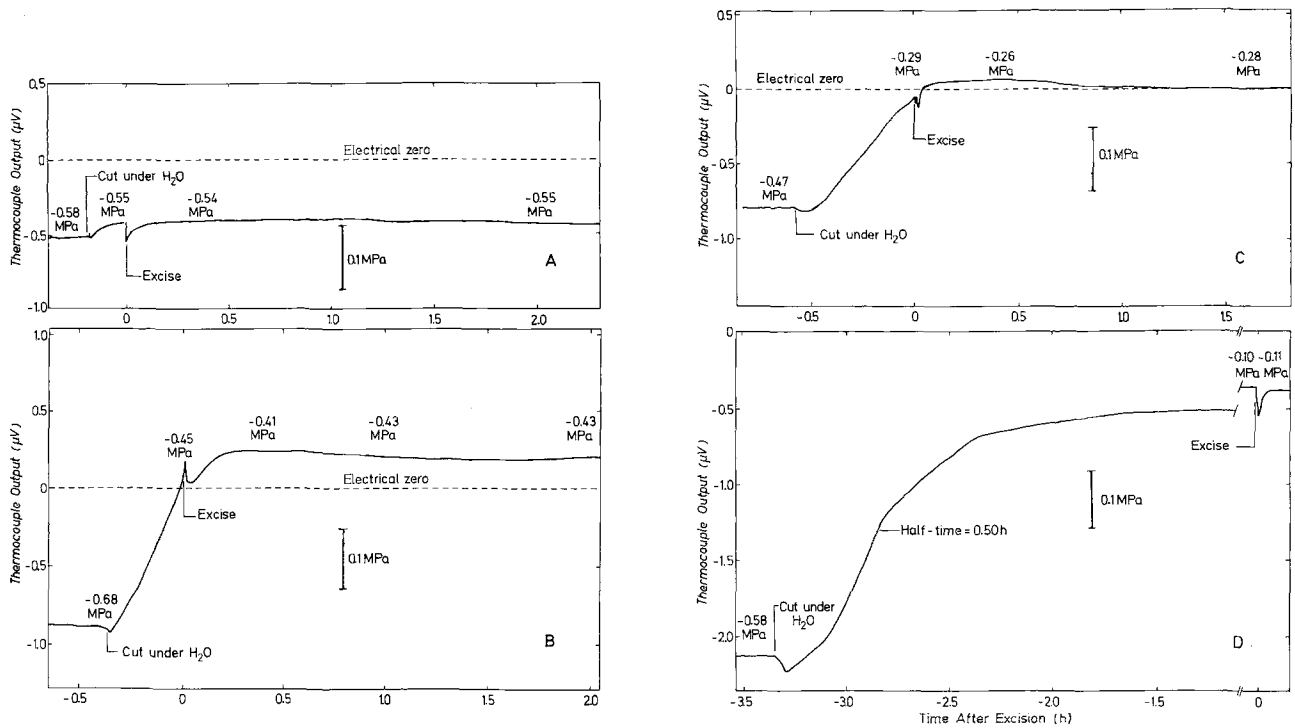

Fig. 7A–D. Effects on thermocouple output of interrupting water flow into mature soybean stem tissue with guillotine. Water entry was initiated by cutting dehydrated seedlings under water at position shown in Figs. 2D and 3D. Water entry was then interrupted by operating the guillotine, excising the mature tissue. Immediately before excision, water potential represents that of intact tissue into which water was moving. After excision, water potential represents that of the same tissue that had no water entering and was equilibrating internally. Tissue was rehydrated to water potentials of **A**, −0.54 MPa; **B**, −0.45 MPa; **C**, −0.29 MPa; and **D**, −0.10 MPa before excision. Water potentials before rehydration were −0.47 to −0.68 MPa. Direct traces of recorder chart

placed in the thermocouple chamber as in Fig. 2D and, after an initial measurement of water potential, the apical portion of the stems was cut under water (Fig. 2, cut D). Rehydration occurred and water potential increased in the mature tissue (Fig. 7). The rehydration was complete in about 3 h (Fig. 7D) and represented an average water flux about twice that occurring during rapid growth (calculated from mass of water gained in 3 h). The mature tissue was excised with the guillotine, interrupting hydration, at various times in the course of rehydration. After rehydrating 15 min, water potential before excision was within 0.01 MPa of that after excision (Fig. 7A, excise). After rehydrating 20–36 min, water potential before excision was within 0.03 to 0.04 MPa of that after excision (Fig. 7B, C at excise) and, after 3.3 h, within 0.01 MPa (Fig. 7D, excise). After excision, the water potential required about 1 h to become stable as water potential gradients equilibrated internally (Fig. 7B, C). However, water potential changed only slightly (maximum of 0.03 MPa) during this equilibration. In each measurement, care was taken that the excision occurred when the thermocouple system was close to the isopiestic condition (within 0.1 MPa of electrical zero).

Figure 8 summarizes all these measurements by comparing water potential after a 2 h equilibration with water potential in the same tissue when attached and rehydrating. In all cases, water potentials were within 0.04 MPa of each other, averaging 0.011 MPa lower in attached than in excised tissue. Therefore, the water potential of the mature tissue was similar whether water potential gradients were present (attached rehydrating) or absent (excised equilibrated). Because the thermocouple psychrometer must indicate an average water potential for excised tissue, this result shows that it also indicates an average for attached tissue into which water is moving. Thus, despite the presence of water potential gradients in the intact tissue, the psychrometer indicates water potentials that are intermediate between the wettest and driest cells in the tissue.

Stem water status. The stability, high resolution, and rapid response of the psychrometer system allowed the apparatus to be used to measure water

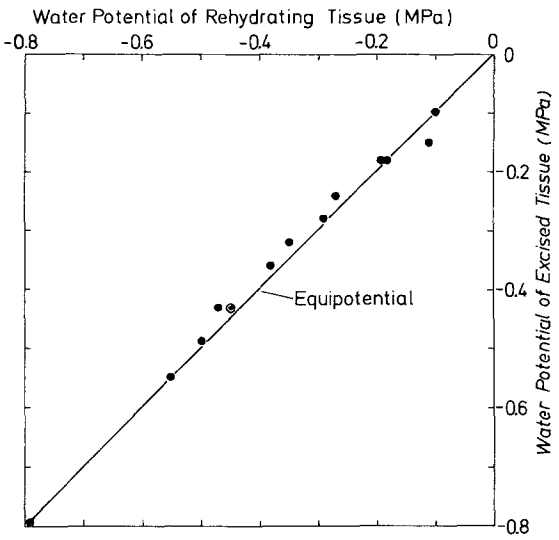

Fig. 8. Comparison of water potentials of intact rehydrating tissue with the same tissue 2 h after excision with the guillotine. The comparisons were made with mature soybean stem tissue as in Fig. 7

potential before and after excision of growing tissue. We began by measuring water potential in intact tissue to test whether there were any differences between mature and elongating regions. The tissue was placed into the thermocouple chamber as in Fig. 2A (elongating) or 2B (mature), and the output was determined for a dry thermocouple and for the same thermocouple bearing water. Figure 9 shows that the thermocouple output was lower when wet than when dry, as expected from the evaporation of water from the thermocouple in response to the vapor pressure of water in the plant tissue. The wet thermocouple always had a lower output with elongating than with mature tissue (Fig. 9), indicating that elongating tissue had a lower water potential than mature tissue. The lower water potential persisted throughout the 21 h experiment although the output of the thermocouple rose (Fig. 9). It should be noted that the dry thermocouple had a slight output indicating that the thermocouple chamber was not completely isothermal. This effect resulted from heat generated by the respiratory activity of the tissue (Barrs 1965) and could be corrected by considering the dry thermocouple to be a baseline against which the output of the wet thermocouple was compared. Since the nonisothermal conditions were generated only by the tissue, this procedure was accurate to outputs of 0.4 μV for the dry thermocouple (Boyer 1972). The output of the dry thermocouple was routinely measured at the beginning and end of the water potential determinations to check for stable temperature conditions.

The continually changing output of the wet thermocouple (Fig. 9) could have resulted from changes in the diffusion characteristics of the tissue as it grew during the experiment. This would alter the rate of evaporation from the thermocouple, a problem always present in dynamic measurements with thermocouple psychrometers. We therefore placed a solution on the thermocouple to give an output similar to that of the dry thermocouple. The identity of dry and wet thermocouple outputs indicated that no vapor exchange was occurring between the thermocouple and the tissue because the thermocouple solution and tissue water were isopiestic (Boyer and Knipling 1965). The diffusion characteristics of the tissue cannot affect isopiestic determinations and, when this approach was used with elongating or mature tissue, thermocouple output was more stable (Fig. 10) than with water, indicating that changes in the dif-

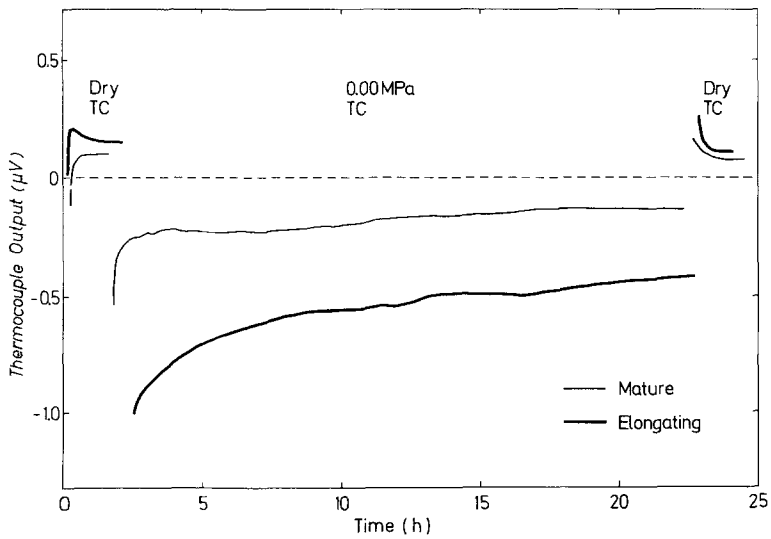

Fig. 9. Output of thermocouple (*TC*) containing water when elongating or mature soybean stem tissue was in thermocouple chamber of guillotine psychrometer. Stems were intact throughout the measurements. Dry thermocouple before and after measurements tested for isothermal conditions. Direct traces of recorder chart. Elongating tissue grew at 0.30 $\mu m \cdot s^{-1}$ in the psychrometer

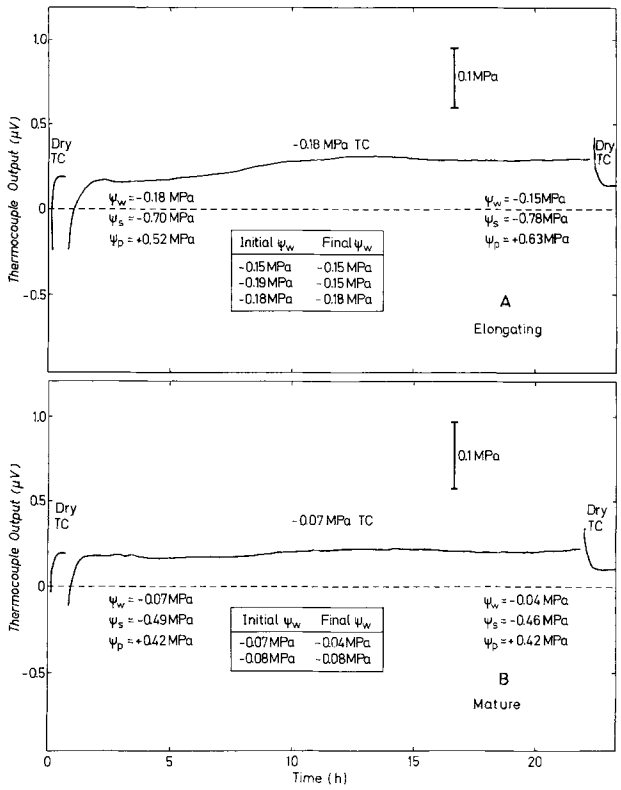

Fig. 10A, B. Water potential (Ψ_w), osmotic potential (Ψ_s), and turgor (Ψ_p) of elongating (**A**) or mature (**B**) soybean stem tissue measured isopiestically in guillotine psychrometer. Stems were intact throughout the 23 h measurements. Dry thermocouple (*TC*) before and after measurements tested for isothermal conditions. Direct traces from recorder chart. Inset tables show result of replicate experiments. The Ψ_w, Ψ_s, and Ψ_p are from the same tissue except for the initial Ψ_s, which was measured on similar plants grown with the experimental plants. The Ψ_p were calculated as ($\Psi_w - \Psi_s$). The elongating tissue grew at 0.32 $\mu m \cdot s^{-1}$ in the psychrometer during the experiment **A** for which the chart tracing is shown

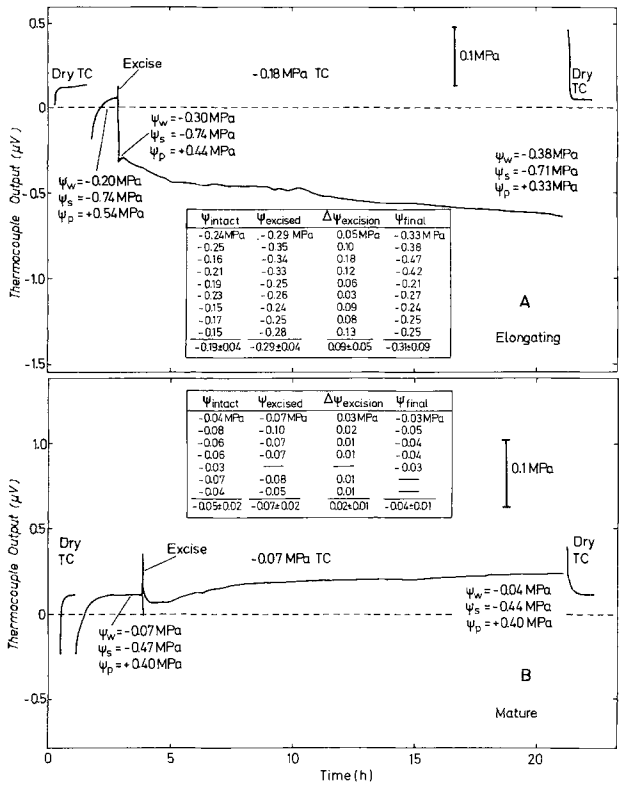

Fig. 11A, B. Excision effect on water potential (Ψ_w), osmotic potential (Ψ_s) and turgor (Ψ_p) of elongating (**A**) or mature (**B**) soybean stem tissue measured isopiestically in guillotine psychrometer. Excision **A** occurred in elongating region in position shown in Figs. 2A and 3A; **B** occurred in mature tissue in position shown in Figs. 2B and 3B. Excision was with guillotine. Direct tracing of recorder chart. The stems grew at 0.28 $\mu m \cdot s^{-1}$ before excision in the experiment **A** for which the tracing is shown. *Inset tables* show replicate experiments. Other conditions were as in Fig. 10

fusional characteristics of the tissue accounted for most of the changes in thermocouple output with water (Fig. 9).

The isopiestic determinations confirmed that the water potential for elongating stem tissue (−0.18 MPa) was lower than for mature stem tissue (−0.07 MPa) and remained lower for the 22 h experiment with only slight changes (Fig. 10). Several replicate experiments (Fig. 10, inset table) confirmed this behavior. It was also possible to measure Ψ_s in these tissues by sampling the tissue in the thermocouple chamber at the end of the water potential measurement. Figure 10 shows that Ψ_s was lower (−0.78 MPa) in the elongating than in the mature tissue (−0.46 MPa), and this difference was greater than the differences in water potential (0.32 versus 0.11 MPa). This results from higher Ψ_p in elongating than in mature tissue. By sampling similar tissue from plants grown with the experimental plants, the initial Ψ_s could also be estimated. This was not significantly different from the final Ψ_s and thus Ψ_p remained constant throughout the measurements.

Growth occurred and, at the end, the stems were 25 mm longer than at the beginning. The psychrometer therefore permitted rapid growth for long times while water potential was determined in completely intact plants. Because the water potential, Ψ_p, and Ψ_s remained stable in the intact plants, any change resulting from excision could be readily detected.

We determined the effects of excision in the elongating region by operating the guillotine as in Fig. 2A. Excision resulted in an immediate decrease of 0.10 MPa in water potential that was complete in about 5 min (Fig. 11A). Besides the

rapid spike due to the handling of the guillotine (see Fig. 6), there occasionally was a slight spike at the end of the 5 min. It was always small (less than 0.01 MPa) and did not originate from the movement of the guillotine. This experiment was repeated several times (Fig. 11 A, inset table) with similar results (average decrease = 0.09 MPa). The initial decrease was followed by a much slower decrease lasting 3 to 5 h and averaging 0.05 MPa. Afterwards, water potential decreased slightly (Fig. 11) or sometimes increased (Fig. 11 A, inset table). The Ψ_s of the tissue remained essentially constant in all replicates. Therefore, Ψ_p decreased in parallel with the decreases in water potential (Fig. 11 A).

In contrast, the water potential of the mature region did not change when excised with the guillotine (as in Fig. 2 B and 3 B) or, at most, it decreased gradually about 0.03 MPa (Fig. 11 B and inset). The Ψ_s remained at -0.44 to -0.47 MPa and Ψ_p at 0.40 MPa throughout the experiment.

The contrasting behavior of elongating and mature tissue indicated that most excision effects were associated with the growth process. Excision disrupted the water supply of the tissue completely. On the other hand, if elongating and mature tissue had been excised together, the mature tissue could act as a water supply because its water potential was higher than in the elongating tissue. This water potential difference would be a driving force for water movement toward the elongating region. We previously showed that the water potential of mature tissue decreases when it is excised together with elongating tissue (Cavalieri and Boyer 1982), which is consistent with the concept that it could act as a water source, dehydrating as water is transferred to the growing cells. We therefore tested the effect of excising the elongating region with mature tissue attached.

A cut was made in the mature region (Fig. 2, cut C) to excise the elongating region together with the cotyledons and part of the mature stem. There was no immediate decrease in water potential of the elongating tissue (Fig. 12 A). A gradual but small decrease (as large as 0.03 MPa) sometimes occurred in replicate experiments but often there was no change (Fig. 12 A and inset). If the elongating region was subsequently excised from the remaining mature tissue with the guillotine (Fig. 12 A), it showed the rapid decrease seen in Fig. 11, and water potential continued to decrease slowly thereafter.

We repeated this experiment but with a long time between the initial and final excision in order to determine the long term response to the first

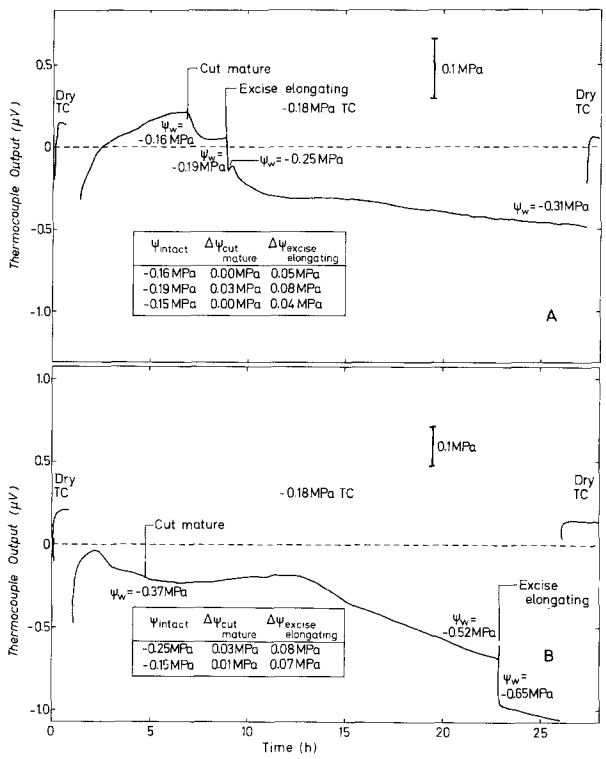

Fig. 12 A, B. Effect of excision on water potential of elongating stem tissue when mature tissue remained attached. Measurements were made in guillotine psychrometer. Initial cut ("cut mature") removed mature and elongating tissue together and was made in position shown in Figs. 2 C and 3 C. Subsequent excision with guillotine removed elongating tissue from mature tissue. **A** Cut in mature region followed after 1.7 h by excision of elongating region with guillotine; **B** same as **A** except excision of elongating region occurred after 18 h. Dry thermocouple (*TC*) before and after measurements tested for isothermal conditions. Direct traces from recorder chart. *Inset tables* show results from replicate experiments

excision. Again, the water potential of the elongating region showed no immediate response after the initial excision, although a gradual decrease (as large as 0.03 MPa) occurred in some experiments (Fig. 12 B and inset). After 8 h, the water potential slowly decreased (Fig. 12 B). When the elongating region was excised with the guillotine at a later time, the usual rapid decrease in water potential occurred (Fig. 12 B).

Stem elongation. Excision of the elongating region should have inhibited cell elongation but the rapid and slow changes in water potential that followed excision raised the possibility that internal exchanges of water might occur and cause additional cell elongation. It was possible to investigate this possibility by measuring stem elongation with a position-sensitive transducer. Seedlings were placed with the elongating region (Fig. 3 A) in the

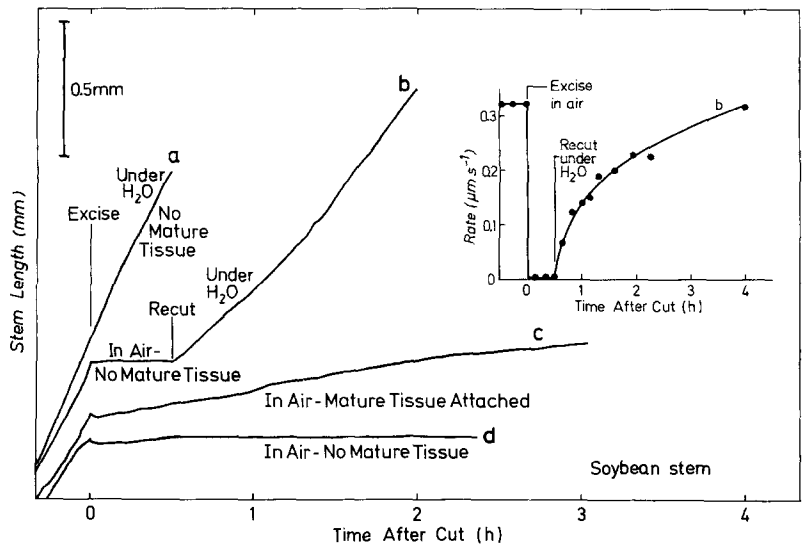

Fig. 13. Growth of the elongating region of soybean stems before and after excision under water (*a*), in air and recut under water (*b*), in air, with mature tissue attached to elongating region (*c*), in air (*d*). Length was measured with a position-sensitive transducer. Only elongating tissue was present between the cuts except in *curve c* where 3 cm of mature tissue was present at the base of the elongating region. About 10 mm of the elongating region was placed in the instrument and the measurements were conducted in the dark in an environment saturated with water vapor to simulate conditions during thermocouple psychrometry. Cut ends in air were blotted with filter paper. Direct traces from recorder chart. Inset shows elongation rates for tissue in *b*

apparatus and were kept in the dark in an atmosphere saturated with water vapor to simulate conditions in the psychrometer chamber.

Elongation was first measured in the intact plant and then an excision was made above and below the elongating region. When the lower end was excised under water and the upper end in air, elongation continued unchanged (Fig. 13a). This indicated that excision itself had no effect on elongation. When both ends were excised in air and quickly blotted with filter paper, however, elongation ceased abruptly (Fig. 13b, d). The effect occurred within 60 s, which was the time resolution of the measurement, and ceased to within 50 μm, which was the length resolution of the measurement. When the lower end of the segment was recut under water (Fig. 13b), elongation immediately resumed but at a slow rate that gradually increased to that of the attached segment (Fig. 13 inset). This indicated that elongation had been fully inhibited throughout the 30 min period in air. There was no change in length if the segment remained in air for several hours (Fig. 13d) and thus the inhibition in air was not temporary. There was no evidence of shortening of the tissue after long times in this condition, indicating that the vapor saturated atmosphere had prevented dehydration of the tissue and that the decrease in Ψ_p did not cause cell shrinkage. On the other hand, if the elongating region was excised in air but with 3 cm of mature tissue attached (Fig. 13c), elongation was immediately inhibited although slow growth continued. This effect of mature tissue on the elongation of stems was also reported by Cavalieri and Boyer (1982).

Discussion

The low water potential and high Ψ_p associated with enlarging tissue require that Ψ_s be low enough to support these potentials. This occurred in the elongating region, which remained below the Ψ_s of the mature tissue and almost constant throughout the 22 h experiment. The constancy of Ψ_s indicates that solutes accumulated fast enough to prevent dilution of the cell contents by the water entering the elongating cells. Excision of the elongating region prevented solute influx and stopped water entry so that Ψ_s remained virtually unchanged, and mechanisms that allow alterations in Ψ_s (such as limited water supplies for intact plants (Meyer and Boyer 1972, 1981) or production of osmotically active substances) did not occur. The experiments were conducted for long times (22–24 h) to assure that all changes in tissue water status would be observed and that all temporary water potential disequilibria would equilibrate. The stability of the data show that these requirements were met and that the water potential of elongating tissue was significantly lower than the water potential of mature tissue and the water potential of elongating tissue decreased abruptly when the water supply was removed.

Water potential difference between xylem and elongating region. The low water potential of elongating tissue relative to mature tissue indicates that a water potential gradient must exist somewhere between the two tissues. The mature tissue and the xylem should have had identical water potential because neither transpiration nor growth oc-

curred in the mature tissue and thus there was no net water exchange. There is evidence that water potential gradients are negligible in the xylem (Boyer and Wu 1978). Therefore, the water potential difference between elongating and mature tissue represented a difference between the elongating tissue and the xylem, and this formed a gradient extending radially from the xylem to the epidermis in the elongating region.

This gradient between elongating tissue and the xylem water supply is a continuous part of the growth process and must be distinguished from the changes that occur after excision. The gradient existed in intact plants that grew during the measurements. It was not transient and was almost constant throughout the 22 h experiments. It could not be attributed to water loss from the elongating tissue because transpiration was prevented by the double seal of the psychrometer apparatus. It probably originates from continuous cell wall extension by Ψ_p that prevents Ψ_p from reaching its maximum (defined by Ψ_s, see Fig. 1). The resultant lowering of cell water potential would create a driving force for water entry from the xylem that sustains the enlargement process, as shown in Fig. 1C (Boyer 1968, 1974; Cosgrove and Steudle 1981). An alternative hypothesis is that the lowering arises from solutes in the cell walls (Cosgrove and Cleland 1983). However, this seems unlikely in view of the significant pressures required to move water out of growing leaves (Boyer 1968, 1974, 1977), which would not be expected if cell wall solutes are the primary reason for low water potentials in the elongating region.

The water potential of the mature tissue was used as a measure of the water potential of the xylem (Ψ_o), and the water potential of the elongating region when intact as a measure of Ψ_w. From these measurements, the Ψ_o was -0.07 MPa and Ψ_w was -0.18 MPa. Accordingly, $(\Psi_o - \Psi_w)$ was 0.11 MPa. It is important to note that, although Ψ_o was determined at equilibrium in the mature tissue, Ψ_w was not because a water potential gradient existed during growth in the psychrometer. The Ψ_w was therefore an average for this gradient that represented an intermediate between the high water potential close to the xylem and the low water potential in the epidermis.

Also note that, because $(\Psi_o - \Psi_w)$ is the basis for moving water into the elongating cells, water is supplied to the cells according to the hydraulic conductance. It must be distinguished from the water demand determined by turgor and cell wall extensibility that exists at the same time (Fig. 1C).

Wall relaxation. In principle, the differences between water potential of attached and excised tissue could be caused by several factors including the release of root pressure or tension in the xylem solution, release of solutes from cut cells, and the deterioration of tissue after excision. Each of these factors would affect elongating and mature tissue alike. The lack of excision effects in mature tissue showed that none of them influenced the results. Nonetheless, intact elongating tissue differed from mature tissue by having water uptake and water potential gradients that were lacking in mature tissue. We simulated this situation by allowing mature tissue to rehydrate to induce water potential gradients, but when we interrupted water uptake by excision, the water potential did not change significantly. Therefore, the interruption of water uptake would not, of itself, alter the water potential of the tissue. In elongating tissue, however, excision interrupted water uptake and water potential decreased. Therefore, the decrease could be attributed only to an additional factor fundamental to the growth process. The remaining possibility is cell wall relaxation as the cell walls were extended by Ψ_p in the absence of a water supply.

The data show that this relaxation occurred in two phases. There was first a rapid phase, completed in about 5 min, when water potential and Ψ_p decreased about 0.09 MPa on average. The rapid decrease of Ψ_p is expected from the inelastic nature of the water in the cells and the extremely small amount of wall relaxation necessary to affect Ψ_p. In theory, the decrease in Ψ_p should stop at Y.

The first phase was so rapid that it approached the kinetic resolution of the psychrometer. Therefore, the 5 min time must be regarded as an upper bound and the actual relaxation may have been even faster. Such a rapid response makes it unlikely that the characteristics of the cell walls in the intact plant could have changed significantly. Thus, the Ψ_p after the first phase must have represented Y in the intact plant just prior to excision.

The measurements of stem elongation support this interpretation. Elongation ceased completely when the elongating region was excised in air. No detectable elongation occurred after 1 min, and no wall extension took place during the following 30 min when measured by subsequently recutting one end of the tissue under water. The lack of detectable elongation and wall extension indicates that the apoplast water was almost instantaneously depleted and wall relaxation occurred immediately during the first phase until Ψ_p decreased to Y.

We did not observe any effects of wall relaxation itself on tissue length (elongation or shrink-

age) probably because the relaxation had too small an effect to detect by our techniques.

The Ψ_p measured before excision and Y measured after excision allowed $(\Psi_p - Y)$ to be determined for the elongating region of the stem and, because of the rapidity of the measurement, for the intact plant. Accordingly, Y was about 0.44 MPa in the elongating regions of soybean stems, and $(\Psi_p - Y)$ averaged 0.09 MPa. It should be noted that, because $(\Psi_p - Y)$ is the turgor increment driving extension of the cell walls, a demand for water is created in the cells and is different from the force supplying water from the xylem $(\Psi_o - \Psi_w)$ that is also present simultaneously in the elongating region (see Fig. 1C).

The first phase of wall relaxation was followed by a second phase in which Ψ_p decreased slowly for several hours. Because cell wall extension had ceased beforehand, Ψ_p must have been continually at the yield threshold. Thus, changes in Ψ_p during the second phase represented changes in Y. Green et al. (1971) observed changes in Y in *Nitella* when growth was inhibited. Also, Cosgrove et al. (1984) observed a decrease in Ψ_p measured directly with a pressure probe (Hüsken et al. 1978) in elongating cells of pea stems after excision of the elongating region. The decrease required 1–5 h, which is suggestive of the second phase of changes in Ψ_p reported here. Thus, pea stems and soybean stems may behave similarly.

The observation of a rapid wall relaxation is consistent with the theory of cell enlargement first described by Lockhart (1965) and subsequently expanded upon (Molz and Boyer 1978; Boyer and Wu 1978; Silk and Wagner 1980; Cosgrove 1981). It is tempting to extrapolate this result to any excision that removes enlarging tissue from the plant. However, the data show that the excision of elongating tissue together with attached mature tissue did not cause wall relaxation for several hours (Fig. 12B).

This result has important implications. The high water potential of the mature tissue caused water to move toward the elongating tissue, preventing wall relaxation. Moreover, growth responded immediately without significant changes in water potential, Ψ_p, or Ψ_s of the tissue. Growth was reduced so rapidly when mature tissue was attached that differences in substrate availability, growth regulators, or altered synthetic activity of the cells could not have been involved (Fig. 13C). This indicates that the presence of mature tissue altered some fundamental tissue character that was not detected by the guillotine psychrometer. The only remaining possibility is Ψ_o. The Ψ_o must have decreased sufficiently to cause water to move out of the mature tissue with the result that $(\Psi_o - \Psi_w)$ became smaller and the water supply to the elongating tissue was less than in the intact plant. This implies that the water potential gradient in the elongating tissue can control the rate of enlargement and moreover that Ψ_o can alter the water potential gradient. Thus, growth can decrease without a significant change in tissue water potential but with variable Ψ_o (refer to Figs. 12B and 13c).

Control of cell elongation. The identification of the factors that are limiting for cell enlargement is of considerable interest. Attempts have been made to measure each of them but methods available in the past have required different techniques, long times (hours or days), or laborious procedures (Cleland 1971, 1981; Taiz et al. 1981). The necessity to use different plants and the possibility of changes in tissue characteristics during the measurements increases the variability and reduces the possiblity of identifying the sources of rate-limitation. Thus far, it has not been possible to rapidly evaluate all the factors that might limit the rate of enlargement of intact tissue.

Equations 1–4 provide a means for evaluating these factors in a single tissue sample enlarging at a steady rate. For elongating regions 35 mm long (Fig. 3), G was $0.86 \times 10^{-5}\ s^{-1}$ during the psychrometer measurements. The psychrometer estimated $(\Psi_o - \Psi_w)$ of 0.11 MPa and $(\Psi_p - Y)$ of 0.09 MPa for the same stems while intact. Accordingly, from eqns. 1 and 2, m was 0.95×10^{-4} and L was $0.78 \times 10^{-4}\ s^{-1} \cdot \mathrm{MPa}^{-1}$. From eqn. 4, $mL^{-1} = 0.11 \times 0.09^{-1}$. Thus, wall extensibility and tissue conductance for water were of similar size, mL^{-1} approximated 1, and m and L were equally rate-limiting for cell enlargement.

The co-limitation by m and L indicates that a disturbance of either $(\Psi_p - Y)$ or $(\Psi_o - \Psi_w)$ would affect growth rate. In our experiments, such a disturbance occurred when elongating tissue was excised. The excision removed the water supply, Ψ_o decreased and wall relaxation followed. When mature tissue remained attached to the elongating tissue, excision caused water to move out of the mature tissue. The smaller $(\Psi_o - \Psi_w)$ in this situation inhibited growth rate immediately but was not expressed in terms of the water potential, Ψ_p or Ψ_s of the elongating tissue because it involved a change in Ψ_o. This transient condition began to disappear after several hours as the water supply was exhausted and the tissue approached water potential equilibrium (Cavalieri and Boyer 1982).

It is important to recognize that during this transient condition eqns. 1–4 cannot be applied because growth was not occurring steadily. The misapplication of eqns. 1–4 to this situation would lead to the conclusion that Ψ_p must decrease when growth decreased, a serious error that is at variance with the constancy of Ψ_p that we observed. Rather, $(\Psi_o - \Psi_w)$ became smaller, and this diminished growth even though $(\Psi_p - Y)$ remained large enough to support rapid growth. This behavior emphasizes the importance of $(\Psi_o - \Psi_w)$ as a factor affecting growth rate.

The co-limitation of growth by water supply and water demand may also apply to other enlarging tissues. Dicotyledonous leaves show $(\Psi_o - \Psi_w)$ of 0.15 to 0.25 MPa, and crude estimates of $(\Psi_p - Y)$ are in the range of 0.2 to 0.4 MPa (Boyer 1968; Matthews et al. 1984, Radin and Boyer 1982). However, as enlarging tissue matures and secondary wall thickening occurs, m is likely to become smaller than observed here, mL^{-1} would probably become less than 1, and growth would be limited by wall extensibility. Alternatively, in tissues enlarging at extremely rapid rates, m could be larger than we observed and growth might be limited by water conductance. These possibilities suggest that the limitation of cell enlargement and the magnitude of wall relaxation after excision will vary with tissue type and conditions. Therefore, an analysis similar to the one presented here will be necessary before the sources of limitation can be identified.

Significance. The data indicate that the primary event inhibiting growth after excision is the decrease in Ψ_o as water is removed by the growing tissue. A similar primary event may apply in a larger context. The removal of the water supply by excision is analogous to the withholding of water from soil except that the supply of water is larger in the soil than in the xylem tissue, and events affecting elongation take longer in the soil-plant system.

Fluctuations of Ψ_o are common in plants because of variations in transpiration and losses in soil water (Kaufmann 1976; Schulze and Hall 1982). If the data from elongating tissue are applied to intact plants growing in soil, growth should respond rapidly. However, if the effects of attached mature tissue are also considered, water potential, Ψ_s, and Ψ_p of elongating tissue should change slowly. Eventually, the water potential of the enlarging tissue would stabilize after forming new water potential gradients, but the kinetics would be slow enough (more than 18 h in Fig. 12 B) that other tissue characteristics such as cell solute content could change.

In intact plants, the Ψ_s of cells often decreases (osmotic adjustment) because of solute accumulation when the soil water supply is limited (Turner and Jones 1980). The decrease in Ψ_s can be large enough to maintain Ψ_p constant in enlarging tissue (Cavalieri and Boyer 1982; Michelena and Boyer 1982). In stems of intact soybean seedlings, the water potential decreases gradually after the water potential around the roots has decreased. The Ψ_s also decreases within 4–12 h because the utilization of organic solute for synthesis of new stem tissue is inhibited and solutes "pile up" in the elongating region (Meyer and Boyer 1981). If this process is prevented (Meyer and Boyer 1972; Michelena and Boyer 1982), growth becomes more sensitive to low water potential. Therefore, these changes in Ψ_s contribute to growth maintenance. The Ψ_s did not change in the present study because low Ψ_o was caused only by excision, which simultaneously prevented solute import.

If so, upon withholding water from the soil, the sequence of events in enlarging regions of stems and probably in leaves would be i) a decrease in water potential between the vascular supply and the enlarging tissue that would cause an immediate growth reduction with little or no decrease in Ψ_p, and ii) an accumulation of solute in the enlarging tissue during the next several hours that would maintain the ability of the cells to remain at high Ψ_p even though the water potential of the vascular supply had decreased. Other factors might also change but it is important to note that, according to this mechanism, Ψ_p of the enlarging tissue does not decrease when the vascular water potential is diminished.

Nevertheless, growth is inhibited, as has been observed (Cavalieri and Boyer 1982; Michelena and Boyer 1982). It is possible that a few cells, particularly those cells adjacent to the vascular supply, would decrease in Ψ_p as the xylem water potential decreased. However, according to the present results, the effects on average water potential and Ψ_p should be small.

The leaves of graminaceous species have frequently been used to investigate the relationship between water potential and growth. They contain a basal elongating region and distal mature tissue in analogy with the same feature of soybean stems. As pointed out by Michelena and Boyer (1982), Ψ_p is usually estimated in the mature region but the correlation between this Ψ_p and growth does not imply that Ψ_p caused the growth effect. Michelena and Boyer (1982) found that the Ψ_p of the

elongating tissue did not change. Although they were unable to identify what inhibited leaf growth, the mechanism of growth inhibition suggested above can explain how the inhibition could have occurred. At the same time, the correlation between elongation and the decreased Ψ_p of the mature tissue is expected because of the lack of complete osmotic adjustment in this tissue (Michelena and Boyer 1982). Therefore, to a considerable extent, changes in Ψ_p of mature tissue indicate changes in Ψ_o. Correlations between leaf elongation and Ψ_p in mature tissue then represent changes in $(\Psi_o - \Psi_w)$ in the elongating region, not an effect of Ψ_p on growth.

Osmotic adjustment is one form of acclimation of plant tissue to limited supplies of water. Another might be the decrease in Y that occurred in the second phase of wall relaxation. Its effect would be to increase the amount of Ψ_p capable of extending the cell walls thus increasing the possibility of cell enlargement at low water potentials. It may be important that leaves of soybean plants grown at low water potential had a lower Y and were able to grow at lower water potentials than leaves grown at high water potential (Bunce 1977), although the reverse was found for sunflower (Matthews et al. 1984). The full extent to which Y can change is unknown but, when coupled with osmotic adjustment, might be considerable.

The effects of excision on the water status of elongating tissue indicate that growth studies with excised segments should be interpreted cautiously. Tissue immersed in solutions is likely to remain out of water potential equilibrium with the solutions as long as growth occurs. In addition, there are problems not only with solute exchange and unstirred layers of solution but also with changes in $(\Psi_o - \Psi_w)$ as growth rates change. Moreover, times are much too long to detect wall relaxation. Psychrometers for excised tissue also can be subject to excision effects. However, as shown here, the effects are often small in soybean stems and may be insignificant if mature tissue is included with the elongating tissue in the psychrometer. The amount of mature tissue present in earlier measurements (Boyer 1968, 1974, 1977; Baughn and Tanner 1976; Boyer and Wu 1978; Cavalieri and Boyer 1982; Westgate and Boyer 1984) is unknown. In the future, the presence of mature tissue will need to be ascertained before the effects of wall relaxation on the measurements can be predicted.

The guillotine psychrometer provides a new method for determining all the parameters of water supply and water demand for growing tissue. Because Y can be determined, a complete analysis of the control of cell enlargement is possible in a single tissue. Under the conditions used here, the co-limitation of enlargement by m and L implies that growth rate will be affected not only by changes in turgor but also by changes in water potential gradients.

We thank Dr. E. Van Volkenburgh for preliminary experiments with an early version of the guillotine psychrometer. We are also indebted to Dr. Champ B. Tanner for suggesting the syringe technique for rapidly extracting cell solution for measuring osmotic potentials, to Dr. E. Brinckmann for help setting up our equipment at Bayreuth, and to Dr. J. Ehleringer for use of his word processor. Thanks are due to the Von Humboldt Foundation, the SFB 137 from the Deutsche Forschungsgemeinschaft at the Universität Bayreuth, FRG and the National Science Foundation for Grant PCM 79-09790 for their support of one of the authors (JSB) for much of this work.

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Received 22 June; accepted 18 October 1984