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Wall relaxation in growing stems: comparison of four species and assessment of measurement techniques

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Abstract. This study was carried out to develop improved methods for measuring in-vivo stress relaxation of growing tissues and to compare relaxation in the stems of four different species. When water uptake by growing tissue is prevented, in-vivo stress relaxation occurs because continued wall loosening reduces wall stress and cell turgor pressure. With this procedure one may measure the yield threshold for growth (Y), the turgor pressure in excess of the yield threshold (P−Y), and the physiological wall extensibility (ϕ). Three relaxation techniques proved useful: "turgor-relaxation", "balance-pressure" and "pressure-block". In the *turgor-relaxation method*, water is withheld from growing tissue and the reduction in turgor is measured directly with the pressure probe. This technique gives absolute values for P and Y, but requires tissue excision. In the *balance-pressure technique*, the excised growing region is sealed in a pressure chamber, and the subsequent reduction in water potential is measured as the applied pressure needed to return xylem sap to the cut surface. This method is simple, but only measures (P−Y), not the individual values of P and Y. In the *pressure-block technique*, the growing tissue is sealed into a pressure chamber, growth is monitored continuously, and just sufficient pressure is applied to the chamber to block growth. The method gives high-resolution kinetics of relaxation and does not require tissue excision, but only measures (P−Y).

The three methods gave similar results when applied to the growing stems of pea (*Pisum sativum* L.), cucumber (*Cucumis sativus* L.), soybean (*Glycine max* (L.) Merr.) and zucchini (*Curcubita pepo* L.) seedlings. Values for (P−Y) averaged between 1.4 and 2.7 bar, depending on species. Yield thresholds averaged between 1.3 and 3.0 bar. Compared with the other methods, relaxation by pressure-block was faster and exhibited dynamic changes in wall-yielding properties. The two pressure-chamber methods were also used to measure the internal water-potential gradient (between the xylem and the epidermis) which drives water uptake for growth. For the four species it was small, between 0.3 and 0.6 bar, and so did not limit growth substantially.

Key words: Cell expansion – Cell wall relaxation – Growth (stems) – Stem growth – Turgor pressure – Wall loosening.

Symbols: L = tissue hydraulic conductance; P = cell turgor pressure; Y = wall yield threshold; ε = volumetric elastic modulus; ϕ = physiological wall extensibility

Introduction

Recent work has demonstrated that wall loosening continues after growing tissues are excised and deprived of a water supply (Cosgrove et al. 1984; Boyer et al. 1985; Cosgrove 1985). Without water to be absorbed, cell-wall loosening reduces the mechanical stress borne by the cell walls and thereby reduces cell turgor, which is the mechanical counterforce to wall stress. This reduction in turgor occurs without water loss by the tissue, and has been termed "in-vivo stress relaxation" to distinguish it from analogous procedures carried out with isolated cell walls under uniaxial tension (Cosgrove 1985). Such relaxation reduces the water potential of growing tissues and has been monitored with various techniques, including the pressure probe (Cosgrove et al. 1984; Cosgrove 1985), psychrometry (Cosgrove et al. 1984; Boyer et al. 1985), a pressure-chamber method (Milburn 1979, p. 150) and the Shardakov technique (Hsiao et al. 1985).

In-vivo stress relaxation may be used to measure the cell-wall properties that govern growth,

namely the yield threshold (Y) and the physiological wall extensibility (ϕ). Cosgrove et al. (1984) demonstrated this possibility by using the pressure-probe method and thermocouple psychrometry to measure the yield threshold of growing pea stems. Wall relaxation was observed to reduce turgor pressure by 2–3 bar over the course of 1–2 h after excision, and finally ceased when turgor reached the yield threshold (about 2.9 bar in pea stems). Subsequent work used the same principle to measure the yield threshold of bean leaves (Van Volkenburgh and Cleland 1986) and soybean stems (Boyer et al. 1985). A theoretical analysis showed that the rate constant for the decay in turgor pressure ideally should be given by $\phi\varepsilon$, that is, by the product of physiological wall extensibility and volumetric elastic modulus (Cosgrove 1985). Knowing ε, one may calculate ϕ from the relaxation time course.

These techniques improve upon earlier measures of wall-yielding properties, but they have some drawbacks. With the pressure-probe technique, it is necessary to work with relatively large growing cells, such as the stem cortical cells of etiolated seedlings. The plasma membrane must seal tightly against the glass capillary to prevent leakage, and the cell must not plug the capillary tip. These practical considerations restrict the range of plant material that can be studied.

With the psychrometer technique, we found that the early time course for stress relaxation could not be followed, probably because of interference from solutes released from damaged cells at the excision site (Cosgrove et al. 1984). Without a cuticle over these cells, the thermocouple communicated most effectively with the cut surfaces of the excised tissue. Boyer et al. (1985) cleverly avoided this problem by using a modified psychrometer chamber in which the cut surfaces were excluded from vapor contact with the thermocouple. However, the psychrometer technique is extremely sensitive to temperature fluctuations and requires a relatively large quantity of tissue. Psychrometry also requires that the tissue sample be in good vapor contact with the thermocouple; the cuticle often obstructs such contact.

A problem common to all in-vivo relaxation methods developed to date is that the growing tissue must be excised from the rest of the plant, to withhold water from it. Such excision can alter the growing tissue because of wound effects, release of root pressure, and isolation from endogenous sources of hormones, carbohydrates and other nutrients essential for growth. Thus, current relaxation methods have technical limitations which restrict the types of experiments that can be carried out and the types of tissues that can be studied.

The aims of this study were to develop and test improved methods for measuring in-vivo stress relaxation and to compare relaxation in the growing stems of four different species. With the new methods it was also possible to measure the internal water-potential gradient which supports water uptake by expanding tissues. In the case of growing stems this gradient develops between the xylem and the epidermis (Molz and Boyer 1978). It arises because cell-wall loosening reduces the turgor pressure of the expanding cells. Because these cells are hydraulically coupled to the surrounding apoplast, the pressure in the apoplast becomes more negative and water is pulled from the xylem. Recent studies have disagreed about the size of this gradient driving water uptake and whether it limits growth (Boyer et al. 1985; Cosgrove 1985; for review see Cosgrove 1986).

Theory

During steady growth, water absorption and irreversible wall yielding take place simultaneously and at equal rates. Wall yielding tends to reduce wall stress and cell turgor, but this effect is normally counterbalanced by water absorption, with the net result that turgor pressure remains constant. When water uptake is prevented, wall yielding results in stress relaxation and, assuming a simple model of cell growth and ideal conditions (Cosgrove 1985, 1986), the time course for the reduction in turgor pressure P(t) from an initial turgor of P_o is given by:

$$P(t) = (P_o - Y)\, e^{-\phi\varepsilon t} + Y \quad (1)$$

According to this equation, an initially high turgor pressure will decay to the yield threshold with a rate constant given by $-\phi\varepsilon$. Equation (1) is based on a model of cell-wall expansion in which the loosened wall yields to the mechanical stress borne by the wall (Cosgrove 1986; Green et al. 1971, 1977), with an expansion rate (r) given by:

$$r = \phi\,(P - Y) \quad (2)$$

It should be noted that in both these equations it is wall stress (tension) that is considered important for wall yielding. However, wall stress is a complex function of cell geometry and wall tickness and is not readily measured. Instead, the equations are written in equivalent terms of turgor pressure.

Measurement of in-vivo stress relaxation requires (i) that cell dimensions be held constant,

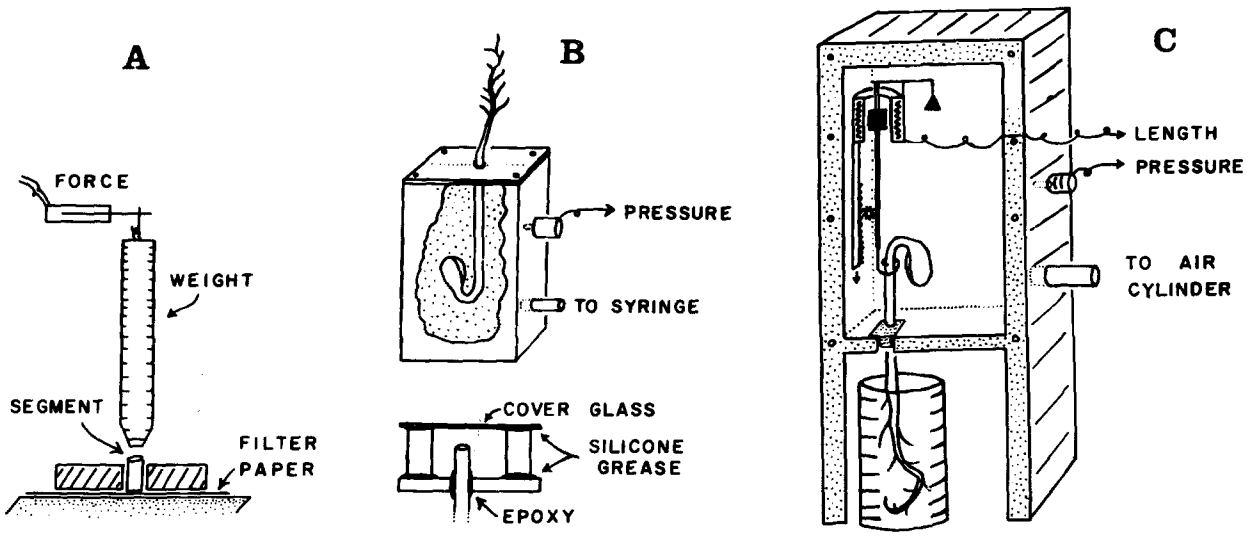

Fig. 1 A–C. Apparatus used to measured stress relaxation of stem tissues. **A** The external-force apparatus consisted of a 51-g brass weight suspended from a force transducer onto a 4-mm stem segment. The segment was in contact with filter paper wetted with water or auxin solution, and was held upright by a Plexiglas plate drilled with holes of diameter slightly larger than that of the segment. **B** The balance-pressure apparatus consisted of an oil-filled pressure chamber (cutaway shows plant and oil) with a built-in electronic pressure sensor. The intact seedling was sealed into the chamber with epoxy, then excised and covered with a plastic ring and glass coverslip, shown at bottom, center. **C** The pressure-block device consisted of a pressure chamber, a position transducer for measuring stem elongation, a built-in pressure sensor, and a port connected to a pressurized air cylinder and manual regulator. The intact seedling was sealed with epoxy into a slot in the base of the pressure chamber. The front face of the pressure chamber was covered with Plexiglas plate (1-cm thickness), held in place with metal bolts. A layer of Neoprene rubber served as a gasket between the plate and the chamber. The displacement transducer could be raised or lowered via a rack and pinion. The pinion extended to the outside of the chamber through a pressure-tight bearing

and (ii) that either wall stress or turgor pressure, or some correlated property such as water potential, be measured as a function of time after cell dimensions are held constant. In principle, numerous ways could be devised to meet these requirements. Four approaches are described below; they work either by withholding water or by applying a hydrostatic pressure or external force to prevent water absorption.

Turgor-relaxation method. In this procedure cell size is held constant by excising the growing tissue and withholding water. Without water, the cells cannot enlarge. As wall loosening proceeds, wall stress and turgor pressure decrease in concert. The process is monitored by direct measurement of turgor pressure with the pressure probe (Cosgrove et al. 1984; Cosgrove 1985). Direct measurement of P, Y, and (P − Y) are possible with this method.

External-force method. Instead of withholding water, cell expansion may be prevented by applying an external force, e.g. with an external brace or clamp. In this situation, the normal mechanical balance between wall stress and turgor is upset. Along the direction in which expansion is prevented, wall stress decreases as the cells grow against the external brace and transmit force to it. Turgor remains high because the cells remain in contact with water. Stress relaxation is monitored as the increase in force exerted by the tissue against the external brace. Using the apparatus illustrated in Fig. 1 A, I experimented with this technique, but found the pattern of force exerted by the tissue to be highly variable. Therefore, this technique was not pursued further.

Balance-pressure method. In this procedure, water is withheld from the excised growing region and relaxation is monitored by the standard pressure-chamber technique. As relaxation progresses, the pressure in the apoplast decreases (becomes more negative) at the same time that cell turgor decreases because the two compartments are closely coupled hydraulically. The reduction in apoplast pressure may be measured as the "balance pressure", the applied pressure needed to raise the apoplast pressure to zero gauge pressure (ambient pressure) and to cause the xylem sap to return to the cut surface of the stem outside the chamber. The balance pressure may be measured at timed intervals to monitor the course of stress relaxation. This technique is capable of measuring (a) changes in P during relaxation and (b) the effective force for wall ex-

pansion (P−Y), but not absolute values for P or Y.

Pressure-block method. This procedure does not require the growing tissue to be excised, and so it has important advantages over the preceding methods. The growing region of an intact plant is sealed into a pressure chamber and its size is monitored by any of several methods, e.g. by electronic position transducer, by video-image analysis, by potometry to measure water uptake (which equals growth, if transpiration is absent) and so on. The growth-measuring technique must have sufficient resolution to assess the growth rate over a short time interval (1–10 s).

To start the relaxation, the chamber pressure is raised to a value just sufficient, to block growth (i.e., stop water influx), but not large enough to cause shrinkage. This added pressure initially raises the water potential of the growing tissue and nullifies the internal water-potential gradient which ordinarily sustains the influx of water for growth. With time wall loosening continues and the chamber pressure required to keep the organ from growing increases further, until wall stress and cell turgor decrease to the yield threshold. It is important to note in this context that cell turgor is the difference between the hydrostatic pressures of the cell and the chamber. As the walls of the growing tissue relax, cell turgor decreases. Water absorption is prevented by the imposed pressure, which raises the cell's water potential to match that of the water source (the roots). Thus, during a pressure-block procedure, the tissue water potential remains high, the *absolute* pressure within the cells stays high, but wall stress and cell turgor fall. The chamber pressure required to block growth is thus used to measure the relaxation of wall stress and cell turgor. As with the balance-pressure method, the change in P during relaxation is measured, not individual values of P and Y.

Materials and methods

Plant materials. Pea (*Pisum sativum* L. cv. Alaska), zucchini (*Curcurbita pepo* L., cv. Fordhook), and cucumber (*Cucumis sativus* L. cv. Burpee's Pickler) were obtained from W. Atlee Burpee Co, Warminster, Pa., USA. Soybean (*Glycine max* (L.) Merr. cv. William) seeds were from Pioneer Hybrid, Johnston, Ia., USA. All seeds were sown in polyethylene vials (50 mm tall, 28 mm diameter) filled with vermiculite, drenched with distilled water, and germinated in the dark at 27–29° C. Unless otherwise noted, seedlings were handled under dim green light obtained from a 40-W cool-white fluorescent lamp (F40CW; Westinghouse Electric Corp., New York, N.Y., USA) filtered with two amber and one green acetate filters (Roscolene No. 813 and No. 874; Rosco, Port Chester, N.Y.). Typically, seedlings were selected for use when their stems were 4–6 cm in length.

Turgor-relaxation method. Details of this method were published previously (Cosgrove et al. 1984; Cosgrove 1985). In brief, the growing stem of an intact seedling was mounted and sealed in a plastic chamber to prevent evaporation. After 30–60 min of equilibration, the turgor pressure of about ten individual cortical cells was measured in the middle of the growing region with the pressure-probe apparatus (Cosgrove and Durachko 1986). In pea seedlings, the measurements were made about 5 mm below the top of the hook, in cucumber about 6 mm below the hook, in soybean and zucchini 8–10 mm below the hook. Immediately after these intact measurements, the chamber was opened, the apical portion of the stem was quickly excised by a single cut 1.2–1.5 cm below the hook, and the chamber was immediately resealed. Turgor pressure was measured in the same regions noted above at timed intervals. As a control, growing tissues were excised as described above, except that a water supply was provided by placing a drop of water on the cut surface of the excised stem, to prevent turgor relaxation. As a second control, basal (non-growing) stem segments were isolated from the rest of the seedling by two cuts made (unless otherwise noted) at about 3 cm and 4.5 cm below the apical hook, and turgor pressure was measured at timed intervals thereafter.

Balance-pressure method. The apical end of an intact soybean seedling (1.5–3 cm of stem, depending on the experiment) was inverted and sealed into a custom-made pressure chamber, as shown in Fig. 1 B, using rapid-set epoxy (Quik Stik; GC Electronics, Rockford, Ill., USA). During the 15–20 min required for hardening of the epoxy, the roots and other parts of the seedling outside the chamber were covered in wet paper towel. After the epoxy hardened, the chamber was filled with well-aerated silicone oil (Fluid 200; 10000 centistoke viscosity; Dow Corning, Midland, Mich., USA) and sealed tight. Pressure was applied to the chamber by compressing an oil-filled syringe and was measured with a pressure transducer (Model 22–100 G; ICSensors, Sunnyvale, Cal., USA) installed in the side of the chamber and connected to a strip-chart recorder. In a few cases, oil was omitted from the chamber and pressure was applied by means of a pressurized air cylinder and a manual pressure regulator. This procedure had the disadvantage that air movement through cortical air channels tended to dehydrate the tissue and caused bubbling and frothing at the cut surface.

To make a measurement, the stem was excised with a new razor blade. The stem external to the chamber (a piece about 4 mm long) was covered with a plastic ring and glass coverslip, sealed with silicone grease to prevent evaporation (see Fig. 1 B, bottom). The cut surface was illuminated with a fiber-optic light source and was observed with a stereomicroscope. Periodically, the chamber was pressurized just enough to bring the xylem sap to the cut surface and to eliminate the curved menisci, but not so much as to cause exudation of xylem sap. These manipulations were done both under green safelights and under room lighting. This technique is a variation of a procedure outlined by Milburn (1979, p. 150).

Pressure-block method. Intact seedlings were mounted into a custom-made pressure chamber, as shown in Fig. 1 C. Only the upper part of the stem (typically, 10–15 mm from the hook) was included within the pressure chamber. The plant was connected to a special lightweight core of a position transducer (model 050HR and 0.5 g core, from Schaevitz Engineering, Pennsauken, N.J., USA) using a 1-mm glass rod and a thin,

malleable wire (0.25 mm diameter, tinned copper). The core assembly was counterweighted, so there was a net upward force on the stem, equivalent to about 0.05 g. For pea seedlings, the wire was attached to the stem at least 3 mm below the hook with the help of a tiny drop (<1 µl) of Quik Stik epoxy. In cucumber seedlings, the epoxy sometimes induced a slight stem curvature, so instead the wire was carefully squeezed with the aid of a fine needle-nosed pliers into a tight fit around the stem. As with pea seedlings, the wire was attached at least 3 mm below the apical hook. For zucchini and soybean seedlings, both of the above methods were used to attach the transducer core. The inside of the chamber was partly lined with wet paper towel to provide a humid atmosphere, although sometimes there were slight air leaks in the chamber which prevented full water-vapor saturation when the chamber was pressurized.

The electrical outputs of both the position transducer and the pressure transducer (ICSensors Model 22-100G) were recorded by a strip-chart recorder. After a plant was sealed into the chamber, the growth rate was monitored until it became stable, typically in 45–60 min. Then pressure was applied to the chamber by means of a manual regulator and an air cylinder so as to prevent the stem from elongating.

Growth. Changes in stem length were measured electronically using position transducers connected to a microcomputer data-acquisition system (Cosgrove 1982). In other cases, the surface of a stem was marked with an oil-base black ink (Speedball; Hunt Manufacturing, Stateville, N.C., USA) and photographed with Kodak Tri-X film (Eastman Kodak, Rochester, N.Y., USA) using a camera mounted on a stereomicroscope. To measure growth, the photographs were projected onto a digitizing tablet (Digipad 5; GTCO, Rockville, Md., USA), the positions of the marks were digitized and recorded by a microcomputer connected to the tablet, and distances were calculated by a custom program. This method proved highly sensitive and reproducible. For example, ten measurements of a 2-mm interval had a typical standard deviation of about 1.5 µm.

Pressure calibration. All pressure sensors were periodically calibrated against a Bourdon Tube Pressure Gauge (Heise model CM-59-497; Dresser Instrument Div., Newtown, Conn., USA).

Osmolality. Seedlings were placed in a humid glovebox and after 60 min the stems were excised and root-pressure exudate was collected in glass capillaries during the subsequent hour. Osmolality was measured with a vapor-pressure osmometer (model 5500; Wescor, Logan, U., USA). In other experiments, nanoliter samples of fluid were collected with a glass capillary pulled to a fine point and immediately measured with a freezing-point osmometer (Clifton Technical Physics, Hartford, N.Y., USA).

Temperature. In some experiments the pressure chamber was partially submerged in ice to cool the growing tissue. To measure the temperature of the tissue inside the chamber, a needle-type temperature microprobe (Bailey Instruments, Saddle Brook, N.J., USA) was inserted down the length of the stem until it reached the tissue within the chamber.

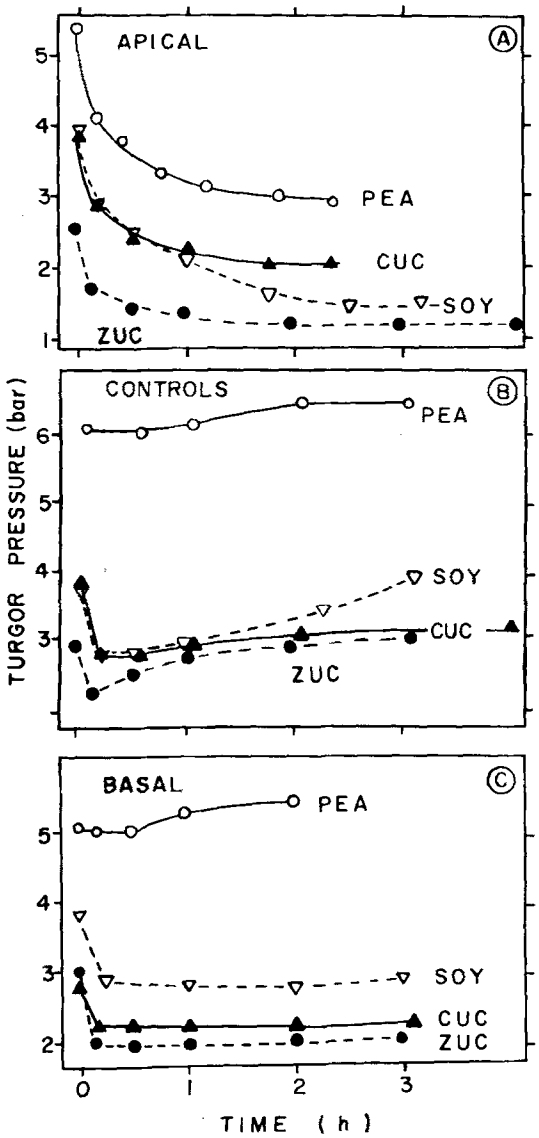

Fig. 2. **A** Time course for turgor relaxation in pea, cucumber (*CUC*), zucchini (*ZUC*) and soybean (*SOY*) stem segments. Turgor pressure was measured in the intact plant, then the apical 1.5 cm of the shoot was excised by a single razor cut. The stem undergoing relaxation included the apical hook and cotyledons, or plumule in pea. **B** Control experiments in which the stem tissue was excised as in **A**, but was supplied with a drop of water on the cut surface. **C** Basal controls, in which nongrowing stem tissue, about 1.5 cm in length, was excised and treated as in **A**. Each point is the average of (usually) 8–12 turgor measurements in cortical cells, with typical SE values of 0.04–0.09 bar. The initial pressure (time zero) is that of the intact plant. Each curve represents the relaxation of a single plant, and the pattern shown is typical of four or more such experiments

Results

Turgor-relaxation method. When the growing stem regions of young seedlings were excised and isolated within the sealed, humid chamber, cell turgor decreased with time, at first rapidly and then more gradually (Fig. 2A). Turgor pressure of nongrowing segments treated in the same fashion decreased immediately upon excision in three of the four species, but did not decline further (Fig. 2C). As indicated below, this immediate drop in turgor is prob-

Table 1. Results of turgor-relaxation procedure.[a] Turgor pressure was measured in about 10 cortical cells in the growing region of the intact plant, then the upper 1.2–1.5 cm of the shoot was excised, isolated from water, and measured with the pressure probe at timed intervals (see also Fig. 2)

Plant	Intact turgor pressure (bar)	Yield threshold[b] (bar)	Mean (P−Y) (bar)	% Reaching stable turgor[c]	No. of plants
Pea	5.7 (0.15)	3.0 (0.24)	2.7	44	9
Soybean	4.8 (0.28)	2.2 (0.3)	2.6	43	7
Cucumber	4.0 (0.08)	1.7 (0.09)	2.3	56	18
Zucchini	2.7 (0.07)	1.3 (0.09)	1.4	60	5

[a] Means followed in parentheses by SE
[b] Turgor pressure when a stable value was reached, or at 120 min after start of procedure, whichever came first
[c] The remainder continued to relax at a slow rate (0.1–0.2 bar·h^{-1}, without reaching a stable pressure during the measuring period

ably caused by release of a positive xylem pressure ("root pressure"). Transpirational loss of water from the tissue was insignificant, as evidenced by the stable turgor pressures in nongrowing segments. Moreover, in many of the apical segments, turgor pressure eventually stabilized at values in the range of 1–3 bar. The *gradual* decline in turgor of growing tissues was prevented by supplying water to the cut surface (Fig. 2B). These results indicate that wall relaxation is responsible for the gradual reduction in turgor which occurs when the growing tissue is isolated from water.

Table 1 summarizes the pertinent pressure-probe data for the four species studied. Relaxation was usually completed in 60–90 min, and resulted in a reduction in cell turgor by 1.4–2.7 bar. Zucchini presented particular difficulties for these measurements because the cells tended to plug the capillary tip with a sticky substance. Similar, but less severe problems with tip occlusions were also encountered with soybean and cucumber tissues.

As Table 1 indicates, turgor pressure did not always stabilize, but sometimes continued to decline slowly, by 0.1–0.2 bar·h^{-1}. This occurred in about half of these trials, in which the growing stem (with apical hook attached) was excised, and the reduction in turgor was similar to observations made previously with pea stems (Cosgrove 1985). Two possible explanations for this slow loss in turgor deserve mention. It might be that the yield threshold is gradually decreasing in these tissues. Alternatively, spatial gradients in osmotic pressure or in yield threshold (e.g., variations along the stem axis) may enable the more apical stem regions to draw water from the lower regions, and thereby

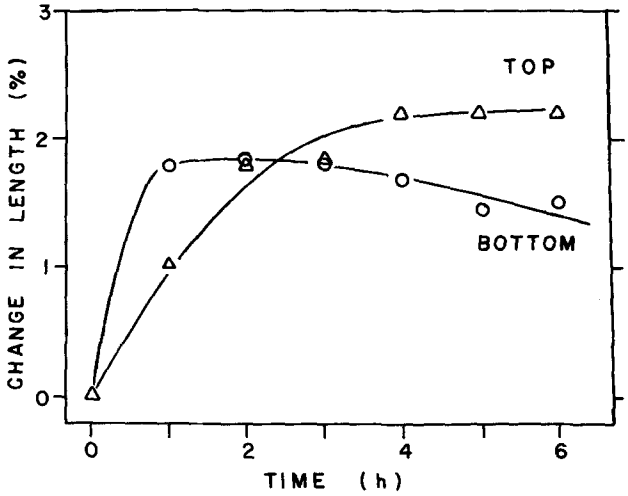

Fig. 3. Length changes in the top and middle of a pea stem segment during a turgor-relaxation procedure. An intact plant was marked with a pair of marks about 1 mm apart at a position about 1 mm below the apical hook ("*TOP*") and at a second position about 10 mm below the hook ("*BOTTOM*"). At time zero, the stem was excised about 15 mm below the apical hook (apical plumule was left intact). This experiment was carried out five times, with results similar to those shown here. Note that the entire excised segment is only part of the growing region, which normally extends about 22 mm down the stem from the hook (Cosgrove 1985)

to reduce the turgor of these lower regions. In such a case, one might expect a biphasic relaxation, rapid at first because of simultaneous relaxation of all the cells, and slower late in the time course because only the most apical cells continue to relax and thereby withdraw water from lower cells.

To examine this second possibility, I marked 1-mm regions at the top and in the middle of the growing region of pea stems undergoing turgor relaxation, and photographed the stems at intervals during the turgor relaxation period. Figure 3 demonstrates that both marked regions expanded slightly during the first hour, probably by absorption of free water from the apoplast in the first 15 min after excision (Cosgrove 1985). Subsequently, the upper region continued to elongate slowly, whereas the lower region shrank slightly. These data show that water was internally redistributed in the tissue during turgor relaxation. Such movement may account at least partly for the prolonged but slow relaxation seen in some of the tissues.

Another characteristic of the curves for zucchini, cucumber and soybean deserves comment: In these plants (but not in pea), there was an immediate loss of turgor upon excision, and such loss was not prevented by supplying water to the cut surface (Fig. 2B). This rapid drop in turgor varied between 0.3 and 1.5 bar in magnitude, and appar-

Fig. 4. Exudation from zucchini (*left*), soybean (*center*), and cucumber (*right*) stumps. Photograph was taken about 30 min after excision of the zucchini and soybean stems and about 15 min after excision of the cucumber stem

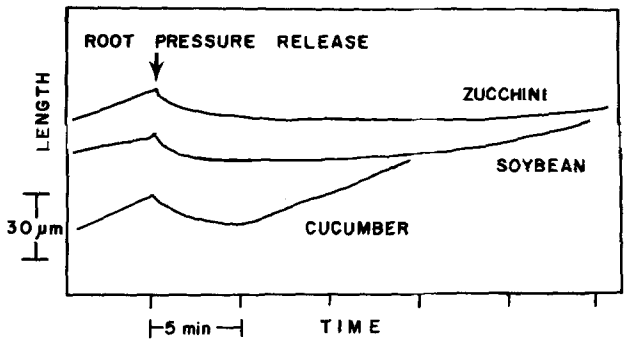

Fig. 5. Shrinkage of zucchini, cucumber and soybean stems after root excision. Seedlings were gently uprooted and mounted in a holder which permitted the roots to be suspended in a 50-ml beaker of water. The stems were supported by a small plastic clamp which fastened the plant in the middle of the growing region and held the plant upright. After the stems were attached to the displacement transducers and steady growth rates were attained, the basal ends of the stems were excised under water (time indicated by *arrow*), and the subsequent changes in length were monitored. In more than six trials with each of these three species, the rate and magnitude of the initial shrinkage was consistent from one trial to the next, but the recovery in growth rate was more variable. The shrinkage was more easily observed in slowly-growing plants than in rapidly-growing individuals

ently was a consequence of release of "root pressure" upon excision. Cucumber, zucchini and soybean stems exhibited substantial exudation from both cut surfaces immediately after excision. When kept in a humid atmosphere, the stumps exuded copiously (Fig. 4), particularly in the case of cucumber seedlings. The exudate contained a low concentration of solutes, indicative of xylem exudation. The osmotic pressures of these exudates were measured by vapor-pressure osmometry and averaged 0.5 bar for cucumber, 1.4 bar for soybean, and 1.7 bar for zucchini (mean of three or four samples). With time a gummy layer developed on the surface of the exudate and the stumps ceased exuding. These observations support the idea that the rapid loss of turgor pressure upon excision is caused by release of root pressure.

As further evidence for root pressure, when the stems of cucumber, zucchini or soybean seedlings were excised under water, they shrank transiently, then began growing again (Fig. 5). Such transient shrinkage would be expected if excision released the positive pressure in the xylem, and thereby transiently reversed the normal water-potential gradient.

Balance-pressure method. When soybean stems were mounted in the chamber (Fig. 1 B) and excised with a single cut, both cut surfaces exuded fluid, apparently because of the release of "root pressure" (see above). The cut surface attached to the growing stem ceased exuding within 0.5 min. The exuded fluid was reabsorbed by the tissue in 4–10 min (6 min; average of 19 trials); thereafter a positive pressure inside the chamber was required to bring the xylem fluid back to the cut surface, and this pressure gradually increased with time, as shown in Fig. 6. In 17 of 25 trials, the balance pressure reached a plateau typically between 60 and 90 min after excision. This plateau averaged 3.1 bar (16 trials, SE = 0.14 bar). In the remainder of the trials, the balance pressure continued to increase after this period, although at a slower rate.

Because Boyer et al. (1985) reported that water-potential equilibration was very slow in soybean seedlings, it was important to determine whether the balance pressure recorded in these experiments measured (1) real changes in the tissue water potential due to wall relaxation, or (2) merely the time required for the tissue to reabsorb water released upon excision (without a major change in the average water potential of the tissue). To answer this question, I recut the stem surface after a balance pressure of 1–2 bar had developed. The fluid released from the cut cells was reabsorbed quickly (Fig. 6, arrow), and the balance pressure returned

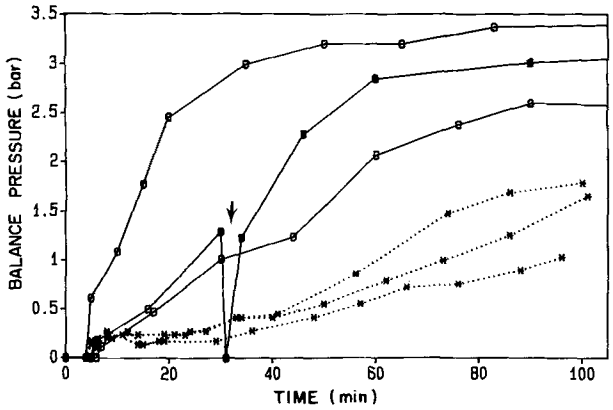

Fig. 6. Changes in the balance pressure of soybean stems after excision and isolation from a water supply. *Circles* (o—o) show three representive curves for relaxation time courses at 28° C. In one of the trials (*solid circles*), the stem was recut during the middle of the relaxation (denoted by *arrow*) to test for the time needed for resorption of fluid released upon cutting. This recut experiment was performed four more times with similar results. *Dotted lines* (* ····· *) show three representative time courses for relaxation when the tissue was excised at 28° C, then immediately iced to reduce the temperature of the tissue and thereby to inhibit relaxation after excision

within 3–5 min to the value extant before the cutting operation. This experiment was repeated four more times with similar results and shows that water absorption was rapid and confirms that the tissue was indeed undergoing relaxation after excision.

To test this idea further, wall relaxation after excision was inhibited by cold temperature. An intact seedling was sealed into the chamber (temperature was 28° C), then the stem was cut in the usual fashion and *after excision* the chamber was iced to inhibit subsequent wall relaxation in the tissue. In these experiments the increase in balance pressure was greatly retarded (Fig. 6, broken lines). The surface liquid was absorbed as usual in 4–8 min, but the balance pressure after 0.5 h averaged only 0.3 bar (mean of six trials). The temperature of the tissue within the chamber ranged from 7 to 10° C in these experiments. These results indicate that the internal water-potential gradient which drives water uptake for growth is about 0.3 bar. In still other experiments, the entire procedure was carried out at a low room temperature (11.5 or 14° C). The increase in balance pressure was much slower than at 28° C (data not shown), as expected if the balance-pressure corresponded to wall relaxation.

Two complications arose in these experiments with soybeans. First, the xylem pathway for exudation often appeared to become partially or completely blocked after 20–40 min. This was noticeable as a change in the location of exudation. At the start of the balance-pressure procedure, fluid first appeared at multiple points at the xylem surface and, if the tissue was overpressurized, the fluid would spread from those sources. Later in the time course the first fluid often appeared in regions within the cortex. In microscopic examination of free-hand sections of the stem, no obvious occlusions in the xylem were seen, so the nature of the apparent blockage remains unknown.

A second anomaly was that the balance pressure, after reaching values of 2–3 bar, sometimes fell drastically to values of 0.2–1 bar. This occurred in about one third of the cases and usually 60–90 min after the start of the procedure. Such decrease in balance pressure was always accompanied by relatively massive exudation of fluid from the cortical region (not the xylem region), and this exudate would not be reabsorbed when the chamber pressure was decreased to ambient. The osmotic pressure of the exudate was measured in two cases with the nanoliter osmometer and found to be high, in the range of 5.8–6.8 bar. Previous aeration of the oil with pure oxygen did not prevent such exudation. The reason for this leakage of cell sap is not known, but may arise from cell death because of mechanical crushing of the tissue at high pressures, anoxia, or toxicity of the silicone oil. It appeared to be irreversible.

Pressure-block method. Figure 7 shows the early dynamics of two experiments and Fig. 8 shows complete pressure-block time courses for the four species studied. In Fig. 7A, stem length was controlled in large, exagerated steps to illustrate the dynamic shrinkage and recovery occurring after small pressure steps are applied. Figure 7B shows the more usual, smoother control of stem length.

Three phases may be discerned in these early time courses. In the initial 1–2 min the pressure increases rapidly. Although one can momentarily halt stem elongation or even induce shrinkage by applying 0.1 bar pressure, within 5–10 s the stem resumes elongation and requires an additional pressure increment to block growth. The rate of pressure increase falls off quickly, however, and after 1–2 min becomes nearly constant for the next 5–10 min. This first phase is believed to represent the collapse of the internal water-potential gradient which drives water uptake and which is established by wall yielding (Cosgrove 1985; Molz and Boyer (1978). The second phase is the period in which the rate of pressure increase is nearly constant. This is thought to be caused by continued wall relaxation and at any point should be represented

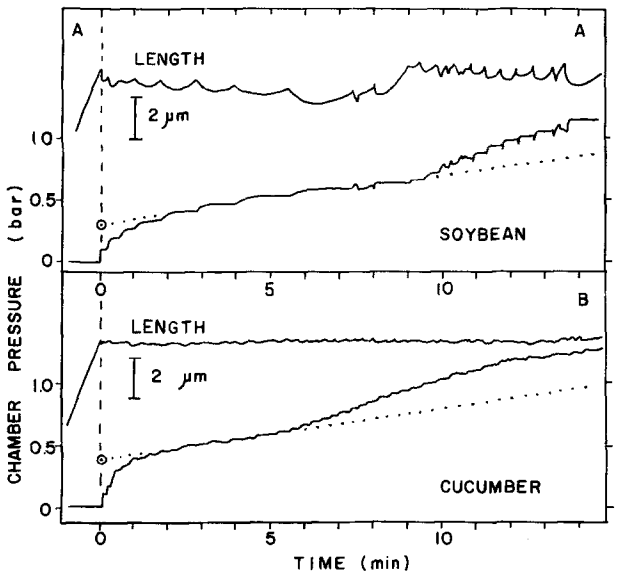

Fig. 7 A, B. Chart-recorder tracings of length and pressure changes at the start of the pressure-block procedure. At time zero the pressure was regulated to hold stem length constant. The *dotted lines* indicate the initial rate of wall relaxation after the collapse of the internal water-potential gradient during the first 2 min. Extrapolation to time zero provides an estimate of the size of this gradient. **A** Stem length of a soybean seedling was purposely controlled coarsely to accentuate the changes in stem length that occur after a pressure step-up. More typical control of stem length is shown in **B**

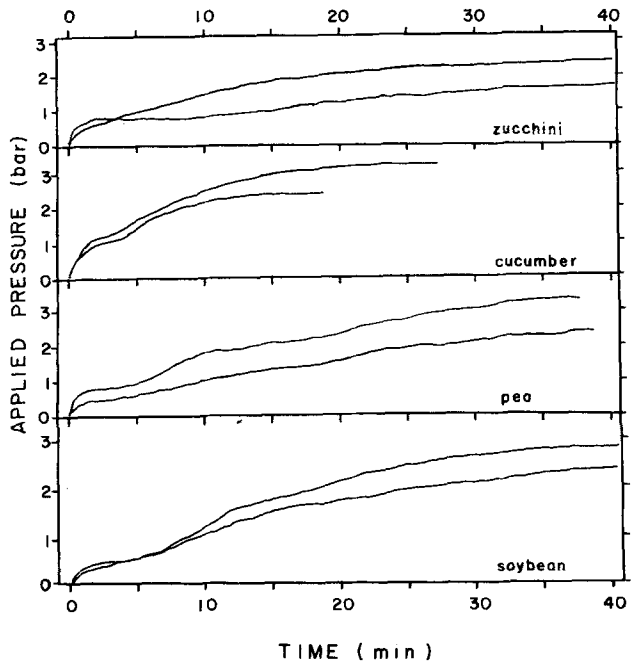

Fig. 8. Chart-recorder tracing of complete stress relaxations measured by the pressure-block technique on intact zucchini, cucumber, pea and soybean stems. Two representative curves are shown for each of the species, but at least ten such measurements were made on each. Note the difference in the axis scale of the soybean tracings

Table 2. Results of the pressure-block procedure[a]

Plant	Initial pressure[b] required to block growth (bar)	Final pressure required to block growth (bar)	(P−Y)[c] (bar)	Time to reach final pressure (min)	*n*
Pea	0.41 (0.06)	3.0 (0.135)	2.6	50 (7.3)	14
Soybean	0.35 (0.04)	2.3 (0.17)	2.0	51 (8.7)	10
Cucumber	0.56 (0.05)	2.3 (0.18)	1.7	17 (1.7)	16
Zucchini	0.51 (0.07)	2.2 (0.18)	1.7	41 (8.6)	10

[a] Means followed (in parentheses) by SE
[b] This was the pressure extrapolated to time zero from the slope of the lines at 3–6 min after the start of the procedure
[c] Effective turgor for growth, calculated as Final pressure (column 2) minus Initial pressure (column 1)

by the equation $dP/dt = -\varepsilon\phi (P - Y)$. See Cosgrove (1985). The third phase begins when the relaxation rate accelerates, some 5–10 min after the start of the pressure block. This phase also represents wall relaxation, but the acceleration is unexpected and appears to be a response to the blockage of growth (see below).

The pressure change during the first phase of the measurement may be used to estimate the internal water-potential gradient (from xylem to epidermis) which drives water absorption. A problem arises, however, because wall relaxation occurs at the same time that the gradient collapses. To take this into account, I estimated the initial rate of wall relaxation from the slope of the line between 3 and 6 min after the start of the pressure block and extrapolated back to time zero (see Fig. 7, dotted lines). This time-zero extrapolation was taken as a measure of the water-potential disequilibrium required for growth. These values, termed "initial pressure required to block growth" in Table 2, averaged between 0.3 and 0.6 bar for the four species.

Figure 8 shows complete pressure-block curves for intact pea, soybean, cucumber and zucchini seedlings. The final pressures required to block growth ranged from 1.5 to 3.5 bar, with the averages ranging between 2.2 and 3.0 bar (Table 2). At this pressure, cell turgor has presumably been reduced to the yield threshold (Y), although the method does not permit Y itself to be measured directly. The average time required to reach these pressures varied with species, about 15–20 min for cucumber, 35–45 min for zucchini, and about 50 min for soybean and pea. In the last three species, there was considerable variability among specimens in the time course for wall relaxation, and the variability did not appear to correlate with

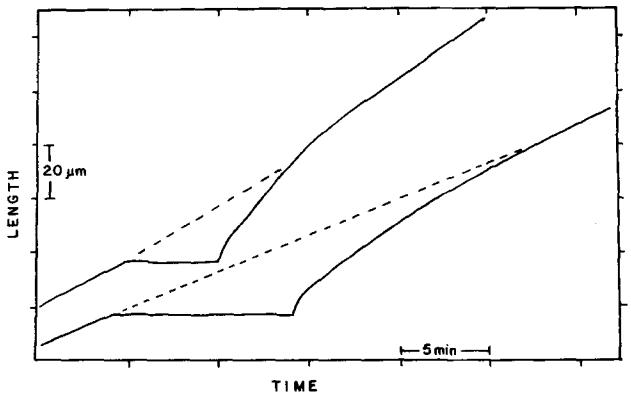

Fig. 9. Recovery of stem length in pea stems after elongation was suppressed for 5 min or for 10 min by the pressure-block method. The results shown here are typical of ten separate trials with three plants

the growth rate of the seedling before the start of the pressure-block procedure.

The turgor pressure in excess of the yield threshold (P−Y) was estimated as the difference between the final, stable pressure reached with the pressure-block procedure and the initial pressure required to block growth, as measured above. The initial pressure is subtracted from the final pressure to take into account the endogenous reduction of cell turgor by the steady growth process. The average values for (P−Y) were found by this method to range from 2.6 bar for pea to 1.7 bar for zucchini (Table 2).

As mentioned above, the rate of relaxation often increased 5–10 min after the start of the procedure. The rate increased transiently by 50–500%. This enhanced relaxation rate is not predicted by simple stress-relaxation theory in which ϕ and Y are constant throughout the relaxation (Cosgrove 1985; Ortega 1985), and indicates that additional wall loosening occurs in response to the growth blockage.

In addition to an enhanced rate of wall relaxation, there was evidence in these experiments of "stored growth" (Green et al. 1977). For example, when the pressure in the chamber was reduced to ambient after a short period of growth blockage, the growth rate of pea stems was greatly enhanced until most or all of the lost length was recovered (Fig. 9). Similar growth accelerations were observed for soybean, cucumber and zucchini hypocotyls.

Discussion

This study shows that the etiolated stems of pea, soybean, cucumber and zucchini seedlings exhibit similar wall-relaxation behaviour. As measured by the turgor-relaxation method, all or most of the wall relaxation is complete in 1–2 h, and the total reduction in turgor pressure for all four species is in the range of 1.4–3 bar. The reduction in turgor pressure gives the value for the effective turgor pressure for growth (P−Y), which was greatest in pea seedlings (average of 2.7 bar) and least for zucchini (average of 1.4 bar). The yield threshold (Y) was also greatest for pea (3.0 bar) and least for zucchini (1.3 bar).

The results obtained with the balance-pressure and the pressure-block techniques were similar to those obtained by turgor relaxation, but the relaxation rate was somewhat faster in many cases by the pressure-block procedure. As discussed below, the pressure-block technique offers major advantages over the other methods.

Root pressure. In zucchini, soybean and cucumber seedlings, excision of the growing region resulted in a rapid reduction in turgor which was not caused by wall relaxation, but rather by release of the positive pressure in the xylem of these seedlings. Evidence for root pressure includes the following observations:

(a) upon excision seedlings copiously exuded a fluid of low osmolality;

(b) turgor pressure fell rapidly in basal tissues upon excision;

(c) turgor pressure likewise fell rapidly in apical tissues which were cut, washed, and left in contact with a water droplet, thus precluding stress relaxation; and

(d) stem tissues shrank transiently when they were excised under water.

Evidently, release of xylem pressure reversed the usual radial water potential gradient (from the xylem to the epidermis), and so induced a transient water efflux and shrinkage of the tissue. As a consequence, turgor pressure of the cortical cells was reduced until a new equilibrium was attained. Such equilibration occurred within 5 min, as measured by stem length changes. Thereafter, if the growing tissue was deprived of water it underwent a further but slower reduction in turgor pressure as a result of wall relaxation. On the other hand, if the tissue was supplied with water, growth restarted as wall loosening reestablished the internal gradient in water potential necessary for water absorption. In excised apical tissues supplied with water, turgor increased gradually with time, probably because of removal of apoplastic solutes (Cosgrove and Cleland 1983).

In experiments with a "guillotine psychrometer", Boyer et al. (1985) reported a rapid reduction

in water potential when soybean seedlings were excised. The water potential fell by about 1 bar in 5 min, which is similar to the rapid drop in turgor found with soybeans in the present study. Boyer et al. (1985) attributed this turgor loss to relaxation to the yield threshold, but Cosgrove (1986, p. 395) calculated that such turgor loss was too rapid to be caused by wall relaxation. It seems likely that Boyer et al. were observing release of root pressure, as in the present study.

Internal water-potential gradients. The rapid but transient shrinkage that occurred when soybean, zucchini and cucumber stems were excised shows that water can move rapidly between the xylem and the rest of the stem. This, in turn, implies that the radial resistance to water flow is small and that the radial gradient in water potential needed to sustain stem growth is also small.

Three results from the current study support this interpretation, most strongly in the case of soybean. (1) The initial pressure required to block growth of soybean hypocotyls was small, about 0.35 bar (Table 2). (2) When soybean hypocotyls were excised and then immediately cooled to inhibit subsequent wall relaxation, the balance pressure was 0.2–0.3 bar (Fig. 7). This experiment should estimate the average water-potential disequilibrium in the intact stem. (3) Shrinkage of the soybean hypocotyl upon excision was complete in less than 5 min, whereas stress relaxation required on average about 50 min for completion in soybean. As shown by Cosgrove (1985), the ratio of these two times can be used to estimate the relative values for wall extensibility (ϕ) and hydraulic conductance (L). In this case the approximation is given by $5/50 = \phi/(L+\phi)$. From this relation, it appears that the hydraulic conductance of the soybean hypocotyl is about nine times greater than its wall extensibility. Similar, though somewhat smaller, values may be calculated for cucumber and zucchini hypocotyls, and these values are close to those found for pea seedlings (Cosgrove 1985).

These results indicate that the internal water-potential gradient which sustains stem growth in these seedlings is small (0.3–0.6 bar), and the limitation of growth by water transport is much less than reported previously for soybeans by Boyer et al. (1985). In calculating a value for hydraulic conductance, these workers did not take into account apoplastic solutes found in the growing region of young stems (Cosgrove and Cleland 1983). Solutes in the apoplast depress the water potential but are ineffective for driving water movement because the reflection coefficient of the wall is low.

Table 3. Advantages and disadvantages of three major techniques for measurement of in-vivo stress relaxation

Characteristic	Technique		
	Turgor-relaxation	Balance-pressure	Pressure-block
Tissue excision necessary?	Yes	Yes	No
Parameters measured[a]	P, Y	ΔP, (P−Y)	ΔP, (P−Y)
Time resolution	Low	Medium	High
Technical difficulty	High	Low	Moderate
Cell size important?	Yes	No	No
Direct control over enlargement	No	No	Yes

[a] ΔP is the change in turgor pressure during relaxation

By ignoring wall solutes, an erroneously low value for hydraulic conductance will be calculated (for discussion of this point see Cosgrove and Cleland 1983).

In the study by Boyer et al. (1985) and in previous work cited therein, soybeans hypocotyls were shown to rehydrate very slowly, in some cases requiring many hours to equilibrate. For example, a seedling which was dehydrated to 80% of its fresh weight required more than 12 h to fully rehydrate (see Fig. 7D in Boyer et al. 1985). In contrast, hydraulic changes caused by release of the xylem pressure were found in the present study to equilibrate within 5 min. Likewise, if we interpret the rapid decrease in water potential upon excision of soybean stems reported by Boyer et al. (1985) to be the consequence of root-pressure release, then it appears that soybeans are capable of rapid propagation of water-potential changes. Steudle and Boyer (1985) also observed very rapid changes in the hydration state of soybean hypocotyls when the xylem pressure was altered with a pump. The reason for the slow rehydration of dehydrated seedlings is not well understood, but it is possible that large dehydrations reduce the conductance of the water-transport pathway. The apparent plugging of the soybean xylem during the balance-pressure experiments may be one symptom of such a change. Further work is needed to examine the changes in the water-transport pathway which may occur when a tissue is dehydrated by 15% or 20%, as in the resorption experiments of Boyer et al. (1985). Such physical changes may be of particular significance in field plants undergoing mild dehydrations and water stress.

Assessment of the methods. Table 3 summarizes the strengths and weaknesses of the relaxation techniques examined in this study. The *pressure-block*

technique seems to have the greatest potential for widespead exploitation. Unlike previous methods, the growing tissue need not be excised to carry out an in-vivo stress relaxation. The time course for relaxation in individual tissues may be followed with high temporal resolution, limited only by the technique used to monitor growth. The internal water-potential gradient which sustains growth may be measured rapidly by this technique. With the pressure-block procedure, enlargement can be stopped immediately and completely, as required for an ideal stress relaxation. Such fine control is not feasible in the other methods because some water may be absorbed from the apoplast or may become redistributed from one part of the stem to another (Fig. 3). Both of these forms of internal water redistribution may retard relaxation (Cosgrove 1985), and may be circumvented with the pressure-block method. Because small regions of growing tissue can be examined with the pressure-block method, this technique may be useful for studying the spatial gradients in growth rate along elongating stems, roots and leaves (Silk 1984; Hsiao et al. 1985).

The pressure-block method should be adaptable for study of a wide range of tissues. The technical skill required is far less than that needed for the turgor-relaxation technique, and cell size and membrane stability do not restrict the useable plant material, as is case with the pressure probe. On the other hand, the pressure-block method does not directly measure turgor pressure or the yield threshold (Y), but only indicates the change in pressure (ΔP) during relaxation and the difference between normal turgor and the yield threshold (P − Y).

The *balance pressure method* proved to be useful for measuring the time course of relaxation, and technically was relatively simple. By cutting the tissue and immediately cooling it to prevent further relaxation, it was possible to estimate the water-potential gradient that drives water uptake for growth. The values obtained for soybean were identical to those obtained by the pressure-block method. However, anomalies which developed late in the measurement period limited the usefulness of the technique as a measure of wall relaxation.

In all four species, it was notable that stress relaxations measured by pressure-block frequently went to completion faster than in the *turgor-relaxation technique*. The magnitude of relaxation was similar with the two procedures. What accounts for the slower relaxations with the turgor-relaxation technique? One possibility is that excision of the growing region at the start of the turgor-relaxation procedure wounded the tissue and slowed the wall-loosening process. It is not unusual to find that intact tissues grow faster than excised ones, even when the hook and cotyledons remain attached to the excised, growing tissue. Another contributing factor might be that in the pressure-block technique the tissue was held to constant length and so did not absorb free-space water, but in the turgor-relaxation method the cells could absorb available water from the apoplast. Such absorption will tend to slow the rate of relaxation (Cosgrove 1985).

Kinetics of wall relaxation. If growing tissues behaved ideally and had constant values for wall extensibility (ϕ), volumetric elastic modulus (ε), and yield threshold (Y), then wall relaxation would cause an exponential decay in cell turgor to the yield threshold, with a rate constant given by $-\phi\varepsilon$. In a previous study of pea stems, I employed the turgor-relaxation technique and observed relaxation kinetics consistent with this prediction (Cosgrove 1985). However, only a few time points were taken for the entire relaxation period, so the detailed kinetics of relaxation, particularly of the early phase of relaxation, was not ascertained.

With the pressure-block method, continuous time courses for wall relaxation were obtained, and in many of the trials the relaxations were more dynamic than predicted by current theory (Cosgrove 1986). After a lag period which was characteristic for each species, the rate of relaxation often increased, apparently in response to the growth blockage. Furthermore, when the chamber pressure was released, the expansion rate exceeded the previous growth rate, often by two- to fourfold, and remained high until most or all of the lost growth was recovered. Thus, these tissues appeared to compensate for the lack of growth by increasing the rate of wall relaxation.

Such changes are probably related to the alterations in wall properties reported by Green and coworkers (Green et al. 1977; 1971). Using osmotica, they induced abrupt changes in the turgor pressure of *Nitella* and of rye coleoptiles, and found that changes in the wall properties at least partially compensated for the changes in turgor. The results from the pressure-block experiments in this study provide new evidence that the wall-yielding properties may indeed be rapidly adjusted so as to compensate for reductions in growth rate imposed by changes in turgor. This interpretation implies that the growing cells may have a "growth-rate sensor" and a rapid feedback system with which they adjust their wall-yielding properties so

as to maintain growth at or near some predetermined setpoint. The nature of this growth sensor and feedback system remains unknown, but the in-vivo stress-relaxation methods described here provide new tools with which to characterize and study this aspect of plant growth.

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References

Boyer, J.S., Cavalieri, A.J., Schulze, E. (1985) Control of the rate of cell enlargement: Excision, wall relaxation, and growth-induced water potentials. Planta **164**, 527–543

Cosgrove, D. (1982) Rapid inhibition of hypocotyl growth by blue light in *Sinapis alba* L. Plant Sci. Lett. **25**, 305–312

Cosgrove, D.J. (1985) Cell wall yield properties of growing tissues. Evaluation by in-vivo stress relaxation. Plant Physiol. **78**, 347–356

Cosgrove, D.J. (1986) Biophysical control of plant cell growth. Annu. Rev. Plant Physiol. **37**, 377–405

Cosgrove, D.J., Cleland, R.E. (1983) Solutes in the free space of growing stem tissues. Plant Physiol. **72**, 326–331

Cosgrove, D.J., Durachko, D.M. (1986) Automated pressure probe for measurement of water transport properties of higher plant cells. Rev. Sci. Instrum. **57**, 2614–2619

Cosgrove, D.J., Van Volkenburgh, E., Cleland, R.E. (1984) Stress relaxation of cell walls and the yield threshold for growth: Demonstration and measurement by micro-pressure probe and psychrometer techniques. Planta **162**, 46–52

Green, P.B., Bauer, K., Cummins, W.R. (1977) Biophysical model for plant cell growth: auxin effects. In: Water relations in membrane transport in plants, pp 30–45, Jungreis, A.M., Hodges, T.K., Kleinzeller, A., Schultz, S.G., eds. Academic Press, New York

Green, P.B., Erickson, R.O., Buggy, J. (1971) Metabolic and physical control of cell elongation rate. In-vivo studies in *Nitella*. Plant Physiol. **47**, 423–430

Hsiao, T.C., Silk, W.K., Jing, J. (1985) Leaf growth and water deficits: biophysical effects. Control of Leaf Growth (Soc. Exp. Biol. Seminar Ser. 27), pp. 239–266, Baker, N.R., Davis, W.D., Ong, C., eds. Cambridge University Press, Cambridge

Milburn, J.A. (1979) Water flow in plants. Longman, London, New York

Molz, F.M., Boyer, J.S. (1978) Growth-induced water potentials in plant cells and tissues. Plant Physiol. **62**, 423–429

Ortega, J.K.E. (1985) Augmented growth equation for cell wall expansion. Plant Physiol. **78**, 318–320

Silk, W.K. (1984) Quantitative descriptions of development. Annu. Rev. Plant Physiol. **35**, 479–518

Steudle, E., Boyer, J.S. (1985) Hydraulic resistance to radial water flow in growing hypocotyl of soybean measured by a new pressure-perfusion technique. Planta **164**, 189–200

Van Volkenburgh, E., Cleland, R.E. (1986) Wall yield threshold and effective turgor in growing bean leaves. Planta **167**, 37–43

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