Rapid Wall Relaxation in Elongating Tissues[1]

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ABSTRACT

Reported differences in the relaxation of cell walls in enlarging stem tissues of soybean (*Glycine max* [L.] Merr.) and pea (*Pisum sativum* L.) cause measurements of the yield threshold turgor, an important growth parameter, to be in doubt. Using the pressure probe and guillotine psychrometer, we investigated wall relaxation in these species by excising the elongating tissue in air to remove the water supply. We found that the rapid kinetics usually exhibited by soybean could be delayed and made similar to the slow kinetics previously reported for pea if slowly growing or mature tissue was left attached to the rapidly growing tissue when relaxation was initiated. The greater the amount of attached tissue, the slower the relaxation, suggesting that slowly growing tissue acted as a water source. Consistent with this concept was a lower water potential in the rapidly elongating tissue than in the slowly growing tissue. Previous reports of wall relaxation in pea included slowly growing tissue. If this tissue was removed from pea, relaxation became as rapid as usually exhibited by soybean. It is concluded that the true relaxation of cell walls to the yield threshold requires only a few minutes and that the yield threshold should be constant during so short a time, thus reflecting the yield threshold in the intact plant before excision. Under these conditions, the yield threshold was close to the turgor in the intact plant regardless of the species. The presence of slowly growing or mature tissue delays wall relaxation and should be avoided during such measurements. However, this delay can be used to advantage when turgor of intact growing tissues is being measured using excised tissues because turgor does not change for a considerable time after excision.

Plants increase in size mostly because cell water content increases. The effect results from the enlargement of individual cells as water enters, driven by osmotic forces. For continued enlargement, a supply of enlargeable cells must be produced by the meristem and solute must be accumulated by the cells. With solute accumulation, turgor (P) develops inside the cells and, if the turgor is above the yield threshold (Y), the resulting hydrostatic pressure makes the walls yield irreversibly, enlarging the cells (3, 8, 20).

To evaluate the forces controlling this kind of growth, one must know P and Y for the cells. Turgor can be measured with a pressure probe (13) or psychrometer (5) but Y is difficult to evaluate because it can change rapidly (5, 12) and may be altered during the measurement. Therefore, methods must be of short duration, preferably no longer than a few minutes.

Most methods of evaluating Y require hours (7, 9, 11) or days (14) and some do not measure turgor directly (7). Only one method (5) determines Y rapidly. It uses a thermocouple psychrometer to continuously monitor the change in water potential caused by P when elongating tissue in soybean stems is excised, depriving the tissue of its water supply. Because no water enters, growth ceases immediately and the cell walls relax until P reaches Y. Wall relaxation was complete in about 5 min and Y was 0.09 MPa below P in soybean stems (5). On the other hand, Cosgrove et al. (9, 11) similarly excised pea stems but they continued to grow for 0.5 to 1 h (9) and the walls required 3 to 5 h to relax when P was measured with a pressure probe (9, 11). The Y estimated by this method averaged 0.3 MPa below P, which was considerably lower than in soybean.

Because of these discrepancies, our knowledge about the yield threshold remains uncertain in higher plants. It is possible that growth is regulated differently in different species or, because a psychrometer was used for one experiment (5) and a pressure probe for the others (9, 11), it is possible that some feature of the methods caused the differences.

The magnitudes of the forces causing cells to enlarge indicate how growth rates are controlled and are necessary to obtain estimates of the extensibility of the cell walls. Without an understanding of Y, it is not possible to obtain this information. Because the reasons for variations in the previous measurements are not known, we investigated Y using both the pressure probe and the psychrometer. The experiments were conducted in a water-saturated atmosphere to ensure that transpiration did not complicate the measurements and all P determinations were affected only be growth.

MATERIALS AND METHODS

Growth Conditions. Seedlings of soybean (*Glycine max* L. Merr. cv Williams; Illinois Foundation Seed, Tolono IL) and pea (*Pisum sativum* L. cv Alaska; Atlee Burpee Co., Warminster PA) were grown in the dark at 29 to 30°C from seeds that had been disinfected in a 1% solution of NaOCl for 1 to 3 min, rinsed with flowing water for 2 to 4 h, and sown in vermiculite. Water was supplied to the vermiculite as a dilute $CaCl_2$ solution (0.1 mM, approximately 5 ml/g of vermiculite, water potential = −0.01 MPa) and the atmosphere was saturated with water vapor.

After 2 d for soybean and 3 d for pea, seed coats were removed and the seedlings were transplanted to identical vermiculite (water potential = −0.01 MPa). The experiments were conducted the next day in soybean and 2 or 3 d later in pea.

Tissue Manipulation. All plant transfers, tissue manipulations, and experiments were conducted in the growth environment under

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a dim green safe light as in Boyer and Wu (4). The local illumination required by the pressure probe was also green (maximum transmission at 525 nm and negligible radiation below 475 nm and above 575 nm). Transpiration under the local illuminator was prevented by coating the shoots with a thick layer of petrolatum (Vaseline) (16). The petrolatum did not limit seedling growth, as coated plants displayed rates similar to those in humid air.

Determination of Growth Rates. Growth profiles along the seedling stems (soybean hypocotyls, pea epicotyls) were obtained by marking at 4 mm intervals with a liquid ink ballpen (Uni-ball, Faber-Castell) and measuring the position of the marks several hours later (6 h, soybean; 16 h, pea). No damage was caused by the marks.

Turgor Measurements. The P of the cortical cells in the stems was measured with a pressure probe (13). An oil-filled glass microcapillary with a tip diameter of 2 to 5 μm was inserted in the stem. The tips were opened with a 'capillary grinder' similar to that used by Shackel et al. (18) or with scissor blades against which the tips were touched. At the position of capillary insertion, the petrolatum covering the stem surface was locally reduced to a thin film to allow the probe to be operated without blocking the glass tip while still preventing seedling transpiration.

Although the tip of the probe was not visible after entering the stem, the entrance of cell solution into the capillary was instantaneous and diagnostic for the penetration of a cell (16). The position of the resulting solution/oil boundary inside the capillary was observed with a stereo microscope ($\times 40$ magnification) while the boundary was moved back toward the stem surface by means of a metal rod pushed into the oil reservoir inside the probe. The pressure necessary to hold the boundary at the original position (*i.e.* close to the stem surface) corresponded to the cell P, as the volume displaced in the capillary by the cell solution was negligibly small (3–4 pl) relative to the total volume of the sampled cell (500–1000 pl, [16]).

In some experiments, P was measured in noncoated seedlings that were sealed in a Plexiglas box (7 × 7 × 14 cm³) containing a water-saturated atmosphere to reproduce conditions used by Cosgrove (9). The intensity of the green light at the measured stem position was approximately 10 μmol photons · m⁻² · s⁻¹ inside the box as well as in the experiments with coated plants.

The P also was measured with an isopiestic guillotine psychrometer according to Boyer et al. (5). Four soybean seedlings were placed in the psychrometer with their stems in the thermocouple chamber and the roots covered in wet tissue paper in the outer plant chamber (5). The water potential was measured isopiestically and continuously in the intact stems and after excision of the stem tissue. The excision was carried out with the guillotine, which cut the tissue immediately outside of the thermocouple chamber. At the end of the measurement, the excised tissue was frozen and thawed, the cell solution was extracted under pressure in the barrel of a syringe, and the osmotic potential was immediately determined. The P was calculated as the difference between the water potential and the osmotic potential. Because the osmotic potential remained constant over the time of the experiments (5), the output of the psychrometer monitored the changes in water potential caused by P. The design of the psychrometer allowed measurements to be conducted with intact seedlings until the excision, and the seedlings grew rapidly in the psychrometer. The atmosphere in the outer plant chamber was saturated.

Excision Experiments. All the P measurements were made between the excisions shown in Figure 1. In both species, the uppermost cuts were made 2 to 3 mm below the stem hook (Fig. 1, position 1). For the pressure probe measurements in pea, the second cut was usually performed 1 cm below the upper one (Fig. 1, position 2) to duplicate the positions given by Cosgrove
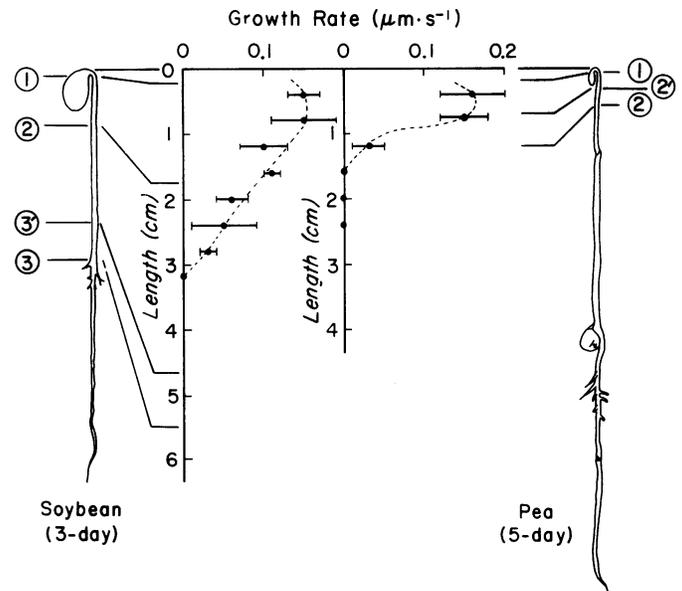

FIG. 1. Distribution of growth along stems of soybean (left) and pea (right). Growth rates of each stem increment (± 1 SD) were obtained by marking the stems and remeasuring 6 h (soybean) or 16 h (pea) later. Encircled numbers refer to positions of cuts made during excision experiments (see text). Total stem growth was 0.64 $\pm$ 0.03 μm · s⁻¹ in soybean and 0.33 $\pm$ 0.04 μm · s⁻¹ in pea when intact.

(9) for pea. For the pressure probe measurements in soybean, the second cut was made 1.5 cm below the upper one (Fig. 1, position 2) to duplicate the positions given by Boyer et al. (5). In pea, some cuts also were made only 0.5 cm below the upper one (Fig. 1, position 2'). In soybean, cuts were sometimes made in the stem base (Fig. 1, position 3 or 3').

While performing these cuts with a razor blade, the cut surface areas were open to saturated air for less than 5 to 10 s before they were completely sealed with petrolatum. The process of sealing displaced the cell fluid from the stem wound. During the experiments with the Plexiglas box (see above), the box was opened in a room with saturated air, while the cut was made within a few seconds.

For the psychrometer, the guillotine made cuts in soybean stems at positions 1 and 2 in the enlarging tissue (Fig. 1), duplicating the cuts made by Boyer et al. (5). In the stem base, the guillotine excised a complete section of tissue having the same dimensions as in the enlarging tissue (approximately position 3 and 3'). When mature tissue remained attached to the stem after excision, the roots covered in wet tissue paper extended to the outside of the apparatus and were excised without disturbing the rest of the shoot inside (5). The cut surface of the shoot was immediately coated with petrolatum to prevent evaporation.

All experiments were replicated at least three times with similar results.

RESULTS

Plant Growth. The plants developed rapidly under the growth conditions and the stems enlongated at 0.64 $\pm$ 0.03 μm · s⁻¹ in soybean and 0.33 $\pm$ 0.04 μm · s⁻¹ in pea (Fig. 1). The profile of stem growth differed in the two species. In soybean, the maximum rate of stem elongation occurred between 3 and 7 mm below the stem hook and declined gradually over the next 3 cm (plants 3 d old, Fig. 1, left). In pea, the elongating region was shorter and declined from the maximum at 3 mm below the hook to zero in the next 1 cm (plants 5 d old, Fig. 1, right).

Pressure Probe Measurements. The probe tip was inserted 150 to 170 μm into the soybean and pea stems where it penetrated the large cortical cells. In the elongating region, the mean P ± SD was 0.45 ± 0.06 MPa in soybean (Fig. 2A) and 0.45 ± 0.03 MPa in pea (Fig. 2C). When the uppermost 15 mm of the soybean stem was excised (positions 1 and 2, Fig. 1), there was a rapid drop in P (0.08 MPa, Fig. 2A). A new stable P was reached within about 6 min, which was the time needed to perform the first measurement after the cuts. The new P did not change during the following 6 h. This excision immediately reduced growth to 5% or less of the initial rate (data not shown, but see Boyer *et al.* [5] and Cavalieri and Boyer [6] for data in identical conditions). Thus, the new P should have been Y. The sudden drop in P upon excision and its following constancy also were seen when the above experiment was repeated on noncoated plants inside a Plexiglas box that was saturated with water vapor as used by Cosgrove (9) (data not shown).

In the basal tissue of the soybean stem, the P was 0.35 ± 0.03 MPa. When the basal tissue was excised, P did not change (Fig. 2B). The tissue was not elongating, although some radial growth may have occurred at a very low rate (*cf.* plants 3 d old in Cavalieri and Boyer [6]).

In contrast, pea tissue excised from the upper portion of the stem (positions 1 and 2, Fig. 1) showed no change in P for about ½ h. This was followed by a gradual decrease (Fig. 2C) for the next 2 to 3 h. Turgor began to stabilize at a new level 0.25 MPa lower than in the intact seedling after this time. The long time and large relaxation are similar to those reported by Cosgrove *et al.* (11) and Cosgrove (9, 10) in segments excised identically.

This slow response could be induced in soybean when mature or at least further differentiated tissue remained attached to the intensively growing stem part after excision. If only the root system was excised (Fig. 3A), no change in P occurred for more
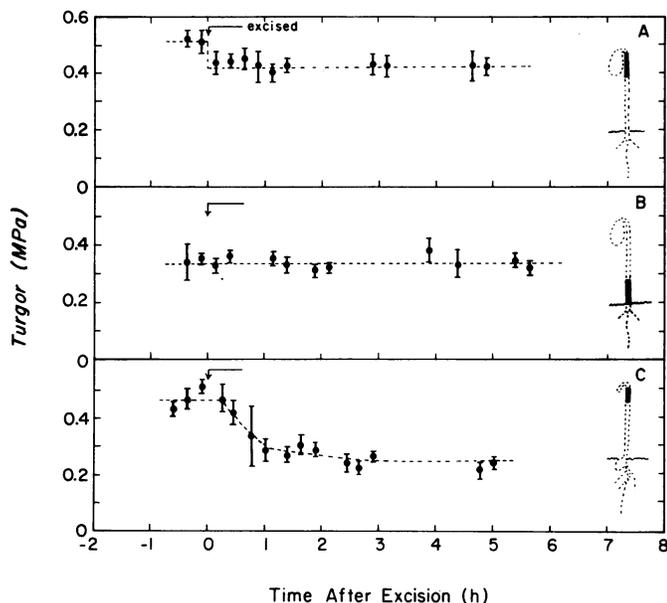

FIG. 2. Cell turgor (pressure probe) before and after excision of soybean (A, B) or pea (C) stem tissue. Excision was in air. Turgor was measured in darkened regions in diagrams at right of figure. Removed parts are shown as dashed lines. Growth rates of the intact stems are shown in Figure 1. A, Measured in elongating region of soybean stem, cuts were at positions 1 and 2, Figure 1; B, measured in basal region of soybean stem, cuts were at positions 3 and 3′, Figure 1; C, measured in elongating region of pea stem, cuts were at positions 1 and 2, Figure 1. Data in each figure are from one plant and show means and standard deviations for 3 to 10 cells.
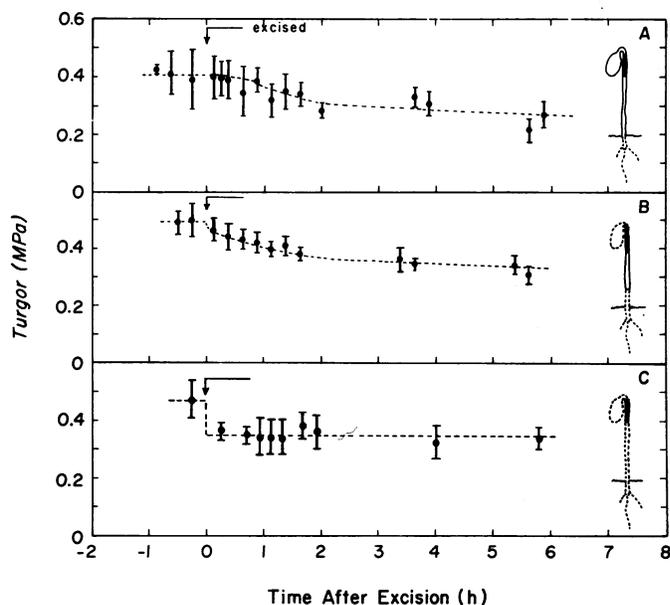

FIG. 3. Cell turgor (pressure probe) of the elongating region of soybean stems before and after excision with various amounts of surrounding tissues remaining attached. Excision was in air. Turgor was measured in darkened regions in diagrams at right of figure. Excised tissue is represented by dashed lines, attached tissue by solid lines. Growth rates before excision are shown in Figure 1. A, Excised only at position 3, Figure 1 (cotyledons and lower stem remain attached, total stem length was 5.5 cm); B, excised at positions 1 and 3′, Figure 1 (basal stem attached, cotyledons and hook removed, total stem length 4.5 cm); C, excised at positions 1 and 2, Figure 1 (elongating region only, no other tissue attached). Data in each figure are from one plant and show means and standard deviations for 3 to 10 cells.

than an hour after which a very slow decline occurred. The P did not stabilize by the end of the experiment. If a shorter length of stem was used (Fig. 3B) and the cotyledons were excised, the drop in P began earlier but remained slow. Only if all the nonelongating tissue was excised did the P drop abruptly after which it remained constant (Fig. 3C).

These results suggested that the elongating tissue of pea used in Figure 2C may have been attached to slowly elongating tissue. This concept is strengthened by comparing the positions of the cuts in the growth profiles of the stems of pea and soybean (Fig. 1). The lower cut for pea is in a more slowly growing part of the profile than for soybean (*cf.* position 2 for soybean and pea, Fig. 1). Therefore, we repeated the excision experiment for pea but made the cuts at comparable positions in the profile (cuts in positions 1 and 2′ in pea but the original positions 1 and 2 in soybean, Fig. 1). Figure 4 shows that pea exhibited an immediate drop in P (0.14 MPa) similar to that in soybean (Figs. 2A and 3C) when the short segment was used. The new P remained stable for at least 2 h (Fig. 4). As the stem section was so short, it was not possible to continue this experiment for as long a time as in soybean (number of measurable cells smaller than in soybean).

Psychrometer Measurements. These experiments with the pressure probe were repeated with the guillotine psychrometer in soybean. In the psychrometer, the osmotic potential of the elongating tissue was −0.70 MPa, the water potential was −0.19 MPa, and P was 0.51 MPa (Fig. 5A) in the elongating region. The standard deviation of each of these measurements was 0.03 MPa or less in repeated experiments. As with the pressure probe, excision caused P to decrease in about 5 min to 0.41 MPa (Fig. 5A) where it changed only slightly in the next 5 h. If only the
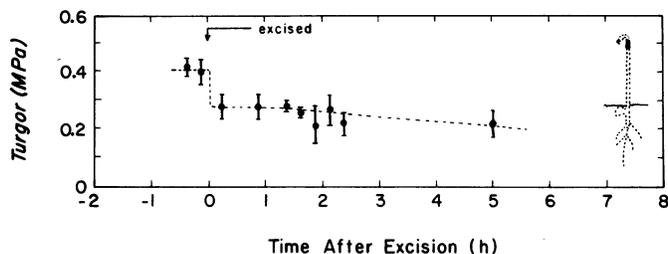
FIG. 4. Cell turgor (pressure probe) before and after excision of short segment in elongating region of the pea stem. Excision was in air. Cuts were made as in Figure 2C except the lower cut was at position 2', Figure 1, to give shorter length of elongating tissue. The cuts were chosen to be at a position in growth profile equivalent to that of soybean (soybean positions 1 and 2, Fig. 1). Turgor was measured in the darkened region shown in the diagram at right of figure. Removed parts are shown as dashed lines. Growth rates before excision are shown in Figure 1. Data are from one plant and show means and standard deviations for 3 to 10 cells.
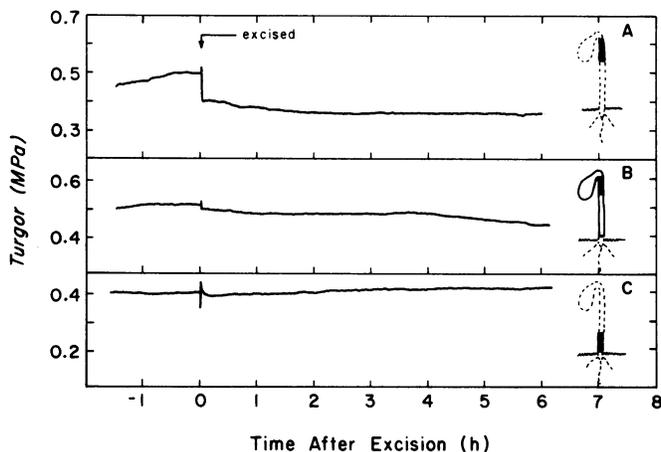

FIG. 5. Turgor (psychrometer) measured in stem tissues of soybean before and after excision. Excision was in air. Turgor was measured in darkened regions shown at right of figure. Excised tissue is represented by dashed lines, attached tissue by solid lines. A, Excised at positions 1 and 2, Figure 1; osmotic potential was −0.70 MPa and water potential was −0.19 MPa before excision. B, Excised only at position 3', Figure 1, total stem length 5.5 cm; osmotic potential was −0.74 MPa and water potential was −0.24 MPa before excision. C, Excised at positions 3 and 3', Figure 1; osmotic potential was −0.45 MPa and water potential was −0.05 MPa before excision. Growth rates before excision in psychrometer are shown in Figure 1.

root system was excised and the cotyledons and basal stem remained attached, there was no initial decrease in P in the elongating tissue (Fig. 5B). After 4 h, a very slow decrease began (Fig. 5B).

In the basal tissue of the soybean stem, the osmotic potential was −0.45 MPa, the water potential was −0.05 MPa, and P was 0.40 MPa when intact in the psychrometer (Fig. 5C). The standard deviation for these measurements was 0.02 MPa or less in repeated experiments. As was observed with the pressure probe, P measured in the psychrometer did not change when the mature tissue was excised (Fig. 5C). The high water potential in this tissue compared to that of the elongating tissue indicates that the mature tissue could act as a water source when it was attached to elongating tissue.

These data indicate not only that the mature tissue had a high and stable P when it was excised but that the excision of the xylem, which disrupted root pressure, had no effect on the be-

havior of the mature tissue. Likewise, as the xylem also supplies water to the elongating tissue, there should have been no effect of root pressure on this tissue either. However, to check this possibility further, we measured P in the elongating region when the roots were removed from the stem under water. Although the excision of the roots should have removed any root pressure, water continued to enter and there was no change in P of the elongating tissue (Fig. 6). Growth continued at near-intact rates (Fig. 1) after excision of the roots because of the continued presence of the water supply (also see Refs. [4] and [5] for examples of these rates).

DISCUSSION

The data show that the kinetics of wall relaxation depended on the amount of slowly growing tissue that was attached to the rapidly growing tissue after excision. When no slowly growing tissue was present and measurements were made in comparable rapidly growing regions of the elongating profile, wall relaxation was very rapid in both pea and soybean. The results were similar with the pressure probe and the psychrometer. Therefore, previously reported differences in these kinetics (5, 9–11) can be ascribed to differences in the position of excision and not to differences in growth regulation or measurement techniques or root pressures (11).

It is likely that the attached tissue acted as a water source. Mature tissue has a higher water potential than elongating tissue (5, 6) and water can move from mature tissue to elongating tissue (5, 6). Consistent with this idea are the longer relaxation kinetics when the entire mature stem plus the cotyledons were attached than when a small amount of stem tissue was attached. In other words, the larger the reservoir of water, the longer the delay in relaxation. In the most extreme case, when the roots were excised under water, water was continuously provided to the elongating tissue and the walls did not relax.

Turgor measured with the pressure probe and psychrometer were similar in all tissues, regardless of whether the tissue was elongating or mature, intact or excised. This confirms the correspondence between the two techniques that has been reported (16). The pressure probe had a time constant of about 1 s (13) and the psychrometer about 45 s (5), which permitted both instruments to follow very rapid changes in P.

The rapid relaxation observed in the absence of the surrounding tissues results from the yielding of the wall and the incompressible nature of liquid water. In the intact plant, the yielding is likely to prevent P from building up to the extent possible if the walls were nonyielding (1). In accord with this idea, the intact elongating tissue of soybean had a P about 0.2 MPa lower than the maximum expected from the osmotic potential. The water
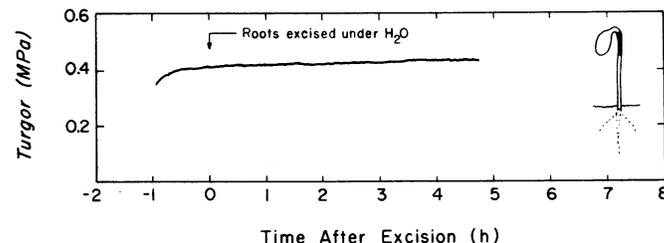

FIG. 6. Cell turgor (psychrometer) measured in elongating region of soybean stem before and after excising the roots under water. Turgor was measured in darkened region at right of figure. Excised tissue is represented by dashed lines, attached tissue by solid lines, and water by wavy lines. Growth rates before excision in psychrometer are shown in Figure 1. Growth after excision continued at rates similar to those in Figure 1.

potential generated by this effect, *i.e.* a growth-induced water potential (15), was thus about 0.2 MPa and probably acted to bring water into the cells to balance the rate of yielding. In contrast, the basal tissue with its nonyielding cells walls had P virtually at its maximum and displayed no growth-induced water potential.

The incompressible nature of water allowed the walls to relax without a detectable change in tissue dimensions, as noted recently (5). Wall relaxation occurred throughout the cortex of the stem and was virtually simultaneous in all the cells of the elongating region. Water would not be redistributed during this relaxation because the kinetics were determined by the walls of each cell acting individually without a need for water movement between cells. Moreover, the kinetics of water redistribution are slow and require several hours for completion (4, 5, 19). This would preclude a contribution from water redistribution.

However, if mature or slowly growing tissue was attached, redistribution would be possible. The redistribution would supply water to the elongating cells and the kinetics would represent the combination of those for water redistribution and wall relaxation. Because water redistribution would be the slowest, it would control the overall kinetics. Thus, the change in P would represent the kinetics of water redistribution and not wall relaxation. The kinetics of wall relaxation can provide information about cell enlargement, and theoretical treatments show that among the properties of enlarging cells that can be inferred, a particularly valuable one is the yielding behavior of the wall (9, 17). However, because the calculations so far (9) are based on pea stem sections with slowly growing tissue attached, the slow kinetics were likely to have been the kinetics of water redistribution.

The presence of mature or slowly growing tissue after excision allows slow growth to occur (5, 6) and P does not indicate Y until the water supply is exhausted, often after many hours. This delay provides time during which Y might change. Such changes have been noted in *Nitella* (12) and soybean stems (5) and are usually in a direction to decrease Y. This would tend to make Y farther below P than is actually the case in the intact plant. Such a behavior may explain why wall relaxation delayed by the presence of slowly growing tissue often shows a lower Y (9, 11) than rapid measurements without these tissues (5). Thus, the true Y can be measured only in the intensively growing tissue in the absence of slowly growing tissue.

The effect of the surrounding tissues in delaying wall relaxation caused P to remain constant for some time after excision in the elongating cells. This behavior can be of considerable importance. Because P remained constant, the P of the intact tissue can be inferred after excision. This probably accounts for several instances in which intact and excised tissues had similar turgor or water potential even though the tissues were enlarging (1, 2, 4, 6, 15, 16, 21).

From these experiments, one may conclude that in rapidly growing stems, Y is close to P. For the two species, Y was only about 0.1 MPa below P, which implies that the cell walls were very extensible. At the same time, the growth-induced water potential (induced by wall yielding) was about 0.2 MPa in soybean. The similar magnitude of these forces in rapidly growing tissue indicates that water uptake and wall extension were co-limiting the rate of enlargement in the intact plant and that decreases in either process would slow the rate of growth.

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