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PROGRESS REPORT

Role of Turgor Pressure and Solute Transport in Plant Cell Growth

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Past Year`s Activities. We have worked on three major areas, as outlined in the major grant proposal of last year:

(4.3) Measurement of Wall Relaxation by Pressurization
(4.6) Coupling between Cell Expansion and Solute Uptake
(4.7) Experiments with Severed Aphids

We have advanced very rapidly in the first area (faster than anticipated), reasonably well in the second area, and have been somewhat stymied in the third area. Our results are outlined briefly below. In addition, reprints and preprints of work attributable to DOE support are attached to the end of this report. (Removed.)

Measurement of Wall Relaxation by Pressurization.

A new method, termed the pressure-block technique, was developed this past year to measure the yielding characteristics of growing plant cell walls. The way it works is as follows. The elongating part of a stem is sealed into a pressure chamber. We monitor stem elongation continuously with a position transducer mounted inside in the pressure chamber. To begin wall relaxation, the chamber is pressurized so as to block water uptake (elongation), but not so much as to cause shrinkage. Wall relaxation then ensues because water uptake is physically prevented; the pressure in the chamber is both the means to keep the cell from expanding and the measure of the relaxation rate.

Our initial experiments showed there was reasonable correspondence between relaxation measured with the pressure-block technique and with the pressure probe (see attached reprint). One difference was that the rate of relaxation was generally faster in the pressure-block technique. This difference was probably due to wounding of excised segments used in the pressure probe technique; such wounding would likely slow relaxation. Preliminary experiments just completed indicate this is the case.

The pressure-block technique provides very high resolution measurements of relaxation in intact plants. This was not possible heretofore, and some interesting observations have been

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made. For example, intact plants have proved to be much more reactive than excised material. This shows up as a complicated relaxation time course (not simple exponential decay) and results in a faster rate of relaxation than observed by the pressure probe procedure.

Furthermore, pea stems show signs of "stored growth"; i.e., when elongation is blocked for a short period (5-15 min) via the pressure-block technique, then released, most or all of the lost growth is recovered in the ensuing period of very high growth rate. We hope to explore these observations in greater detail in the coming year.

Our first measurements with the pressure-block technique were carried out manually. That is, a person observed the stem length tracing and manually adjusted the pressure regulator to keep stem length constant. We have now automated the apparatus so that a microcomputer replaces the person; Under favorable conditions the computer can keep stem length constant within one micrometer, and record on disk the relaxation time course. This makes for more efficient experimentation.

Coupling between Cell Expansion and Solute Uptake.

Our first approach in studying the relationship between solute uptake and growth was to reduce the solute supply to elongating pea stems of etiolated seedlings by excising the cotyledons. Solute import was effectively stopped over the 5 h period after excision; no net change in dry weight of a 0.5 cm segment occurred 5 h after cotyledon removal, compared to an increase of 0.3 mg in intact seedlings.

Stem elongation rate was inhibited by cotyledon removal. The rate showed a steady decline, between 2.5 and 4 h after removal, to 60 % of the control rate (Fig 1).

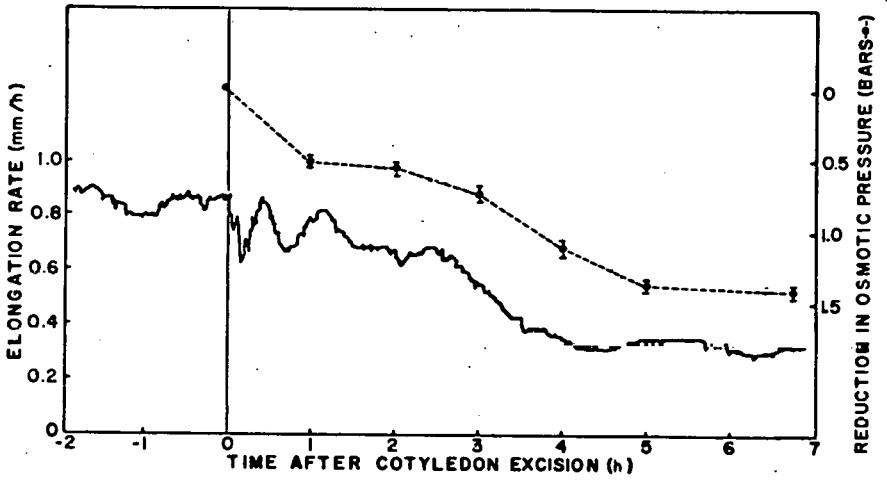

Figure 1: Elongation rate (solid line) and reduction in osmotic pressure (--o--) with time after removal of cotyledons at time zero (vertical line). Elongation rate is average of 15 stems; osmotic pressure is mean of 5 to 8 experiments .

The osmotic pressure of expressed cell sap from the elongating region declined by 0.5 bars within the first h after excision and by 1.4 bars by 5 h (Fig 1). This reduction in bulk osmotic pressure was due to dilution of cell solutes (i.e., solute import was prevented, but water uptake and expansion continued, thus diluting the existing solutes.

The inhibition of elongation rate might possibly be explained by a drop in turgor pressure in response to the decrease in osmotic pressure. However, turgor pressure was measured in individual cells with the pressure probe and was found to remain high despite a reduction in osmotic pressure of 1.4 bars.

The reduction in osmotic pressure with no change in turgor pressure means that the tissue water potential has increased by at least 1.4 bars. There was also an increase in the water potential of the free space solution. Using a pressure perfusion method, we found that the osmolality of the free space solution decrease by half after cotyledon excission. However, it is difficult to estimate absolute concentrations of free space solutes because of the large amount of dilution that inevitably occurs during the perfusion procedure.

These expriments show that the coupling between solute import and growth was not mediated via loss of osmotic pressure and turgor pressure. Rather, growth seemed to depend on one or more solutes needed for metabolic or synthetic processes, eg, sugars for wall synthesis, amino acids for protein synthesis, or perhaps growth hormones. Moreover, turgor pressure was highly regulated, so that under conditions were osmotic pressure was substantially reduced, turgor remained unaffected. Might this be a case of turgor regulation?

Experiments with Severed Aphids.

We constructed a radio frequency microcautery device, and succeeded in excising the stylets from pea aphids feeding on pea epicotyls. However, exudation quickly ceased. The aim of these manipulations was to provide a convenient way for the pressure probe to tap into the phloem, for measurements of phloem transport. So far, we have not had success, but it may prove more practical to probe into the phloem directly with the pressure probe, without using the aphid stylet. This possibility will be examined in the future.

Visiting Scientists.

Two visitors have come to my lab this year to use our advanced pressure probe. Dr. Jaime Kigel is on sabbatical from the Hebrew University (Rehovot, Israel); he plans to use the pressure probe to examine the physical basis for light and water stress effect on growth. Dr. Randall Weisz, an NSF Postdoctural Fellow, will use the pressure probe in studies of the mechanism by which legume root nodules regulate rates of nitrogen fixation.

Research Plan for Next Year.

We plan to focus on three areas in the upcoming year. First, we will continue to develop the pressure-block technique as a method for studying wall yielding. Second, we will resume our experiments on drought stress by rehydrating the water stressed plants and measuring the turgor and wall properties during recovery of growth. Third, we will continue examining the relation between solute import and cell expansion in pea stems.

These areas were outlined in detail in the orginal proposal, and our plans remain mostly intact. One addition is that we will use the pressure-block device to impose rapid step changes in turgor pressure on the growing tissue, and follow the dynamics of the growth response. This, combined with relaxation techniques, will provide a more thorough way to examine the turgor relations of growth, and to study the phenomenon of stored growth.

Reprints and Preprints Attached to this Report:

1) DJ Cosgrove, JKE Ortega, W Shropshire Jr. 1987. Pressure probe study of the water relations of Phycomyces blakesleeanus sporangiophores. Biophysical J. 51:413-423

2) RE Cleland, D Cosgrove, M Tepfer. 1987. Long-term acid-induced wall extension in an in-vitro system. Planta 170:379-385

3) DJ Cosgrove. 1987. Wall relaxation in growing stems: comparison of four species and assessment of measurement techniques. Planta 171: 266-278

4) DJ Cosgrove. 1987. Wall relaxation and the driving forces for cell expansive growth. Plant Physiology 84: 561-564

5) JG Schmalstig, DJ Cosgrove. 1987. Solute supply and stem elongation: effect of cotyledon removal on growth, solute and turgor pressures. In: DJ Cosgrove, DP Knievel, eds, Physiology of Cell Expansion during Plant Growth, Amer. Soc. Plant Physiol., pp. 252-254

6) C Margritz, DJ Cosgrove. 1987. The biophysical mechanism of reduced growth in Pisum sativum seedlings under saline conditions. In: DJ Cosgrove, DP Knievel, eds, Physiology of Cell Expansion during Plant Growth, Amer. Soc. Plant Physiol., pp. 255-257

7) DJ Cosgrove. 1987. Linkage of wall extension with water and solute uptake. In: DJ Cosgrove, DP Knievel, eds, Physiology of Cell Expansion during Plant Growth, Amer. Soc. Plant Physiol., pp. 88-100

8) DJ Cosgrove. 1988. In defence of the cell volumetric elastic modulus. Plant Cell & Environ (in press)

PROJECT SUMMARY FOR ANNUAL REPORT

PENNSYLVANIA STATE UNIVERSITY - University Park, PA 16802

The Role of Turgor Pressure and Solute Uptake in Plant Cell Growth

D.J. Cosgrove, Department of Biology $84,000

Plant cell expansion requires coordination of three distinct processes: wall relaxation and synthesis, water uptake, and solute uptake (or synthesis). Wall relaxation reduces cell turgor pressure and thereby generates the reduced water potential needed for water uptake. Our studies with pea (Pisum sativum L.) and soybean (Glycine max Merr.) seedlings have shown that water uptake is rapid and is not ia major control point for growth. Our current focus is on the processes of wall relaxation and solute transport, and how they are influenced by water stress. One major goal of this project is to examine in detail the dependence of wall yielding on turgor pressure. This is being done by detailed measurements of wall relaxation in living cells, using a computer-assisted pressure microprobe and the new pressure-block technique. Our pressure-block results indicate that wall relaxation is more dynamic than expected. Rapid changes in wall yielding appear to compensate for minor fluctuations in cell turgor pressure, thus maintaining stable growth rates. A second major goal of this project is to determine the interrelationship between cell expansion and solute transport into expanding cells. We will selectively block either cell expansion or solute transport, and measure the effect of such blockage on the unblocked process. A third goal is to examine the basis for reduced cell expansion when plants are water stressed. Our results indicate that growth is retarded in part because of reduced turgor pressure, and in part because of reduced cell wall relaxation. The alteration in wall relaxation will be examined by in-vivo relaxation methods. Thes studies will provide insight into the basic cellular and physical processes controlling plant growth, and how they are perturbed by water stress.

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