Plant Physiol. (1988) 88, 1240–1245
0032-0889/88/88/1240/06/$01.00/0

Growth Inhibition, Turgor Maintenance, and Changes in Yield Threshold after Cessation of Solute Import in Pea Epicotyls[1]

Received for publication March 24, 1988 and in revised form July 27, 1988

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ABSTRACT

The dependence of stem elongation on solute import was investigated in etiolated pea seedlings (*Pisum sativum* L. var Alaska) by excising the cotyledons. Stem elongation was inhibited by 60% within 5 hours of excision. Dry weight accumulation into the growing region stopped and osmotic pressure of the cell sap declined by 0.14 megapascal over 5 hours. Attempts to assay phloem transport via ethylenediaminetetraacetate-enhanced exudation from cut stems revealed no effect of cotyledon excision, indicating that the technique measured artifactual leakage from cells. Despite the drop in cell osmotic pressure, turgor pressure (measured directly via a pressure probe) did not decline. Turgor maintenance is postulated to occur via uptake of solutes from the free space, thereby maintaining the osmotic pressure difference across the cell membrane. Cell wall properties were measured by the pressure-block stress relaxation technique. Results indicate that growth inhibition after cotyledon excision was mediated primarily via an increase in the wall yield threshold.

Continued transport of solutes into growing regions is needed during cell expansion to maintain cell osmotic pressure against the diluting effect of water uptake (1, 4, 17). Water entry creates and maintains turgor pressure, which provides the internal wall stress necessary for cell wall yielding, the irreversible extension of plastic elements of the wall (1, 2, 4). A continued supply of solutes during growth is also needed to provide substrates for respiration and synthetic reactions, *e.g.* wall synthesis.

The coupling of solute and water uptake during cell expansion is not well understood (4, 12). Organic solutes, mainly sugars, are supplied to growing regions via the phloem and inorganic solutes are supplied via both the xylem and phloem. In etiolated seedlings, the cotyledons provide the only source of organic solutes. Cotyledon removal in seedlings of soybean (15) and mung bean (18) decreases stem elongation. In etiolated peas, cotyledon excision results in lower cell sap osmotic pressure (7) and free space solute concentration (6) two days after excision. In this study, we have used the reduction in solute supply resulting from cotyledon excision to examine the nature of the dependence of growth on solute uptake. Specifically, we asked how quickly and by what magnitude does growth respond to a decrease in osmotic pressure and how is the growth reduction mediated. Possible mechanisms include turgor pressure reduction, reduced hydraulic conductance and altered cell wall properties.

[1] Supported by Department of Energy grant DE-FG01-84ER13179.

MATERIALS AND METHODS

Plant Growth Measurement. Seedlings of *Pisum sativum* L. (var Alaska) were grown for 4 d in the dark at 25 to 28°C and 75 to 80% RH in vermiculite soaked with half-strength Hoagland's solution containing macronutrients only (11). Elongation of the epicotyl was measured with a linear displacement transducer as previously described (7). A rod holding the transducer core was attached 3 mm below the plumule and the stem was clamped 2.3 cm below the plumule. Cotyledons were carefully excised with razor blade fragments after a steady growth rate was obtained. All experiments were performed under dim green light (5) and at the same temperature and RH conditions used for growth, unless otherwise specified.

Stems were photographed in the dark using a green flash to determine increases in stem width. The elongating region was marked with oil-base ink at 2 mm intervals, cotyledons were removed from half the seedlings over the following 10 min, and the stems were photographed at 0, 3, 5, 10, and 20 h. Slides of the marked stems were enlarged with a projector onto a digitizing tablet and the width was measured at the marked intervals.

The FW[2] and DW measurements were made of segments cut from the growing region of seedlings with or without cotyledons. The apical 5 mm of the growing region was marked with oil-base ink at time zero on three sets of 10 plants each. The marked lengths were cut from the intact seedling either immediately or after 5 h, with or without cotyledons. Segments were weighed, dried and reweighed using a microbalance.

Osmotic and Turgor Pressure Measurements. Osmolality of cell sap expressed from apical 8-mm segments was measured with a vapor pressure osmometer (model 5500, Wescor, Logan, Utah). Comparison of osmolality from fresh tissue or frozen-thawed tissue revealed no differences, as reported previously (6). Osmolality (Osm kg^{-1}) was converted to MPa using the equivalence 2.48 MPa per (Osm kg^{-1}). The contribution of hexoses to osmolality was measured for cell sap collected from 10 frozen-thawed, 8-mm apical segments. Cell sap was collected by centrifugation of thawed stem sections at 1400*g* at 0°C for 10 min in a Centrex 0.8 μm cellulose acetate microfilter (Schleicher & Schuell, Keene, NH). Osmolality of cell sap was determined by vapor pressure osmometry and compared to the concentration of hexoses measured enzymatically using the method of Outlaw *et al.* (13), except that the buffer used was 200 mM Hepes (pH 7.0).

Samples of the free space solution for osmolality measurements were collected by two techniques: (a) centrifugation of vacuum infiltrated tissue segments (6); and (b) pressurization of the roots and lower stem of intact seedlings to force water through

[2] Abbreviations: FW, fresh weight; DW, dry weight; *L*, growth-specific hydraulic conductance; $\Delta\Psi_g$, radial water potential difference supporting growth; *P*, turgor pressure; *Y*, yield threshold; ε, volumetric elastic modulus; ϕ, wall yield coefficient; P_c, chamber pressure.

the cortex and out stomata on the stem (6). Collection by vacuum infiltration and centrifugation was done as described (6) with the following modification. The free space volume was measured in the same tissue used to collect free space solutes by including a nonbinding dye, indigo carmine, 5 mg mL^{-1}, in the infiltrating solution. The concentration of the dye in the collected solution was measured spectrophotometrically by absorption at 650 nm and converted to osmolality via a standard curve. The osmolality of the collected solution was measured using a freezing-point osmometer (Clifton Technical Physics, Hartford, NY), and corrected for osmolality due to the dye. The free space volume was calculated using the following equivalence:

$$\frac{[\text{original dye}] \times \text{infiltrated volume}}{\text{air space} + \text{free space volume}} = [\text{final dye}].$$

The infiltrated volume was measured by weight increase, the dye concentrations were measured spectrophotometrically, and the air space volume was measured in parallel experiments by weight increase after vacuum infiltration with silicone oil, corrected for oil density (6). The correction factor for dilution of free space solutes was calculated as follows: correction factor = (free space + air space volume)/free space volume. Growth rate was minimized by infiltration at 9°C. Each sample included 15 to 20 apical segments 1.5 cm in length cut directly below the plumule. Plants were kept at 100% RH for 24 h prior to measurements.

In the second technique of collecting free space solution, the root and all but the apical 2 to 3 cm of the stem were submerged in water and slowly pressurized to 0.08 MPa over 1 min. When exudate first appeared on the exposed stem (about 1–2 min), the pressure was released, and the submerged tissues were removed from water and repressurized in air at 0.08 MPa (total manipulation time was about 4 min). Initial exudate during the second pressurization was blotted, and subsequent exudate was collected from the apical 1.5 cm. A droplet several hundred nanoliters in volume was collected with a glass capillary drawn to a fine tip and osmolalities of two samples, each several nanoliters, were measured with a freezing-point osmometer. The 4-min interval between initial pressurization and collection of exudate was considered sufficient to allow equilibration of solutes in the free space with the water forced into the intercellular spaces since exudates collected from the same plant at 4, 8, and 12 min were of the same osmolality. Throughout the collection of exudate, the seedlings were kept at 100% RH to prevent evaporation of exudate.

To estimate the dilution of free space solutes during the procedure, a nonbinding dye, indigo carmine (6), at the concentration of 0.5 mg ml^{-1} was used in the pressure chamber. Concentration of the dye in 1 μl of exudate was measured spectrophotometrically.

Turgor pressure was measured as previously described (8) with a computer-assisted pressure-probe. Six to 12 cortical cells were measured approximately 7 mm from the base of the plumule. Plants were measured at time zero, cotyledons were then removed or left intact and measurements were repeated at 0.5, 3, or 5 h on any one plant.

EDTA Exudation. EDTA-enhanced exudation from the cut stem was measured as described by Hanson and Cohen (10) for etiolated pea seedlings. Ten seedlings were fastened together, cut, and inverted with 1 cm of cut stem in 10 ml of exudation solution (0.05–0.5 mM EDTA in 10 mM Hepes, pH 7.5). Samples of the exudation solution were analyzed enzymatically for sugars as described above and by ninhydrin reaction for amino acids (10).

Pressure-Block Relaxations. Pressure-block relaxations were performed as previously described for peas (5). The apical 10 mm of the stem was sealed in a pressure chamber and elongation was monitored with a position transducer within the chamber. Sufficient pressure was applied to stop growth without causing shrinkage of the stem. Relaxation was gauged as the pressure needed to keep stem length constant.

RESULTS

Elongation. The rate of stem elongation was inhibited by cotyledon excision (Fig. 1). The elongation rate oscillated during the first 1.5 h after cotyledon removal, reached a plateau from 1.5 to 2.5 h, and from 2.5 to 4 h steadily declined to 40% of the initial rate and remained at this rate for the following 13 h. The increase in width of the growing region was not affected until 10 h after cotyledon removal, several hours after the time frame considered here (data not shown). The growth inhibition after cotyledon excision was not a general wounding response because removal of one cotyledon did not inhibit growth. Average growth rates of eight seedlings were 0.91 mm h^{-1} (SE = 0.11) before and 0.95 mm h^{-1} (SE = 0.21) 5 h after excision of one cotyledon.

Solute Import. Cotyledon excision stopped solute influx into the elongating region. No DW accumulation had occurred 5 h after cotyledon excision, compared to an increase of 23% over 5 h in controls (Table I). The osmotic pressure of the cell sap declined by 0.05 MPa within the first h and by 0.14 MPa 5 h after cotyledon excision (Fig. 1). Hexoses contributed 21% and 17% of the osmotic pressure in seedlings with and without cotyledons respectively (Table I).

Two types of calculations indicate that the reduction in osmotic pressure can be accounted for by cessation of solute import followed by dilution of existing solutes due to continued growth. First, accumulation of osmotic solutes into marked growing regions was reduced by 86% at 5 h after cotyledon excision (Table I). Second, actual reductions in cell sap osmolality with time corresponded closely with calculated reductions assuming that no further increase in osmotically active solutes occurred after cotyledon excision (Table II).

We attempted to measure the solute transport directly by using EDTA-exudation from the cut stem as a measure of phloem transport (10). However, there was no decline in amounts or rates of sugar (Fig. 2) or amino acid (data not shown) exudation up to 9 h after cotyledon excision, despite the demonstrated lack of DW increase in stems without cotyledons (Table I). The results were similar whether growing or nongrowing regions were used for exudation. To eliminate the possibility that 0.5 mM EDTA was causing cellular damage, and that leakage from cells swamped out exudation from the phloem, we used 0.1 and 0.05 mM EDTA. Exudates with both lower concentrations contained

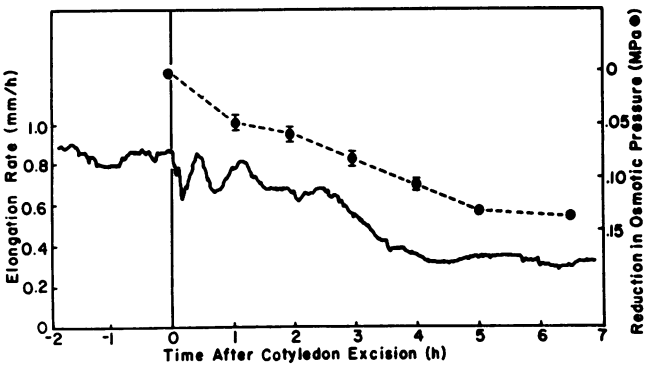

FIG. 1. Elongation rate and reduction in osmotic pressure after excision of cotyledons as time 0. Elongation rate (——) is the tracing of an average of 15 plants. Reduction in osmotic pressure (●- - - -●) was calculated as the difference from osmotic pressure at time zero. Points are means of 4 to 7 experiments, each with 12 samples per time. Error bars show SE of mean differences where larger than symbols. Osmotic pressures were tabulated in Table II.

Table I. *Effect of Cotyledon Excision on FW, DW, and Solute Accumulation and Osmolality*

The apical 5 mm of the epicotyl was marked at 0 h and cut and weighed at 0 h or 5 h. FW and DW are means (SE) of 40 plants per treatment. Osmolalities of apical 8-mm segments are means (SE) of 36 plants per treatment. Total solutes (μOsm) were calculated from (FW − DW) times osmolality. The percentages of osmolality contributed by glucose and fructose (% hexoses) are means (SE) of 3 sets of 20 plants each.

Measurement	0 h	5 h	
		−Cotyledons	+Cotyledons
DW (mg)	1.22[a] (0.0246)	1.24[b] (0.0306)	1.50[a,b] (0.0312)
FW (mg)	11.4[c] (0.556)	13.9[c] (0.762)	16.7[c] (1.098)
Osmolality (mOsm kg^{-1})	356[d] (4.16)	306[d,e] (3.52)	357[e] (4.22)
% Hexoses		17 (0.58)	21 (1.25)
Total solutes (μOsmol)	3.62	3.87	5.43
Solute accumulation (μOsmol)		0.25	1.81

[a-e] Significant differences at 5% significance level between like groupings by analysis of variance.

Table II. *Comparison of Actual and Calculated Reduction in Cell Sap Osmolality following Cotyledon Excision*

Measured osmolalities are means (SE) of 48 to 96 plants. Final osmolalities are referenced to zero time osmolalities from the same batch of plants. Column B shows calculated reduction in osmolality assuming solute import ceased after cotyledon excision. It was derived using osmolality at time zero and volume increases calculated from elongation rate (Fig. 1) and stem widths.

Final Time	Osmolality at Time 0	Osmolality Final Time	*A* Measured Reduction	*B* Calculated Reduction	*A*−*B*
h		*mOsm/kg*			
1	351 (0.85)	331 (1.55)	20	14	6
2	362 (1.05)	341 (0.95)	21	26	−5
3	354 (1.96)	326 (1.76)	29	36	−7
4	353 (2.09)	309 (2.79)	44	43	1
5	365 (1.59)	311 (2.22)	54	48	6
6.5	358 (1.07)	301 (2.67)	57	56	1

lower amounts of sugars but there was no effect of cotyledon excision. We conclude that this technique for measuring phloem transport in etiolated seedlings is not valid and suggest that the small amounts of sugars and amino acids collected were due to leakage from cells. Other evidence that 0.5 mM EDTA may be causing general cell leakage from the stem tissues comes from work with root segments. Rickauer and Tanner (16) reported that bathing root segments in 0.7 mM EDTA decreased the membrane potential within minutes and concluded that it led to general membrane leakiness.

Turgor Pressure. In contrast to the substantial (0.14 MPa) reduction in osmotic pressure, turgor pressure was not reduced during 5 h after cotyledon excision (Table III). Turgor pressure was measured at 3 times after excision at RH used for growth experiments (80%). Turgor was unaffected at 0.5 and 3 h (Table III). At 5 h after excision, there was a small increase in turgor in both control seedlings and those without cotyledons. The increase in turgor 5 h after the initial turgor pressure measurement may have been due to reduced transpiration caused by decreased stomatal conductance. To test this possibility the experiment was repeated several times in nontranspirating conditions (100% RH). Turgor pressure was unchanged over a 5 h period in both controls and seedlings without cotyledons at 100% RH (Table III).

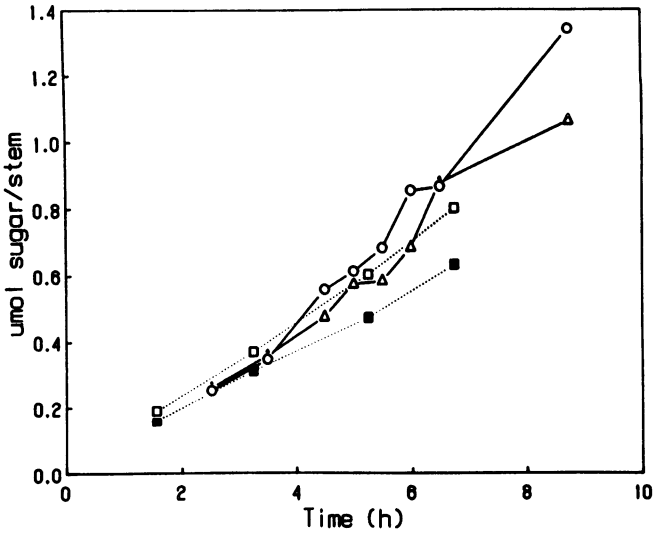

FIG. 2. Accumulation of sugars in collection solution (0.5 mM EDTA, 10 mM Hepes, pH 7.5) from cut ends of 10 seedlings. Data are from two experiments shown as dotted and solid lines with cotyledons intact (■, △) or cotyledons removed (□, ○).

Free Space Solutes. Free space solution was collected by centrifugation of vacuum-infiltrated segments. Osmolality of the extracted solution was 43 mOsm kg^{-1} or 0.11 MPa lower after cotyledon excision (Table IV). When corrected for dilution, the decrease after cotyledon excision was 0.10 MPa (Table IV). Osmotic pressure measured for control plants (0.28 MPa) was the same as that reported previously for pea using this technique (0.29 MPa) (6).

One potential problem with the technique of vacuum infiltration and centrifugation is leakage of solutes from the cut ends of the segments or from the cells during the vacuum infiltration. To eliminate the leakage problem, we used a second technique in which intact seedlings were used and the free space solution was sampled by pressurizing the root and lower stem and collecting exudate which appeared on the surface on the stem. Osmolality of the exudate 5 h after cotyledon removal (11 mOsm kg^{-1}, $n = 10$, SE = 1.04) was approximately half of that from intact plants (25 mOsm kg^{-1}, $n = 9$, SE = 2.32). However, these osmolality measurements are not the actual free space concentrations because of the substantial dilution which occurred as water was forced into the air and cell wall spaces. Attempts to measure the dilution with indigo carmine in the pressurizing solution were unsuccessful because the exudate had the same dye concentration as that of the pressurizing solution in both controls and seedlings without cotyledons. The results do confirm that seedlings without cotyledons had a lower free space osmotic pressure under conditions in which leakage of solutes into the free space solution was minimized.

Cell Wall Yielding Properties. Cell wall properties were measured in intact seedlings by preventing water entry by applying to the growing epicotyl the minimum external pressure necessary to prevent its elongation. The processes in the wall which lead to growth, *i.e.* breakage of load-bearing bonds and wall yielding, continue despite the lack of cell expansion. Instead of wall extension, wall relaxation occurs and higher pressures are needed to prevent water uptake. The kinetics of the pressure increase provides a measure of wall relaxation. Three parameters of wall relaxation were altered by cotyledon excision: the initial pressure

Table III. *Effect of Cotyledon Excision on Cell Turgor Pressure*

Pressures are means (SE) of 6 to 10 plants each measured at time zero and final time as indicated. For −cotyledon treatments, cotyledons were excised immediately after the initial turgor measurements.

Expt. No.	Treatment		Final Time	Turgor at Time 0	Turgor at Final Time	Difference in Turgor
	Cotyledon	RH				
		%	*h*	*MPa*		
1	−	100	5	0.54 (0.011)	0.51 (0.006)	−0.03
1	+	100	5	0.53 (0.012)	0.54 (0.011)	0.01
2	−	100	5	0.49 (0.013)	0.48 (0.009)	−0.01
3	+	100	5	0.57 (0.030)	0.54 (0.020)	−0.03
4	−	80	0.5	0.48 (0.021)	0.49 (0.018)	0.01
4	−	80	3	0.47 (0.007)	0.50 (0.022)	0.03
4	−	80	5	0.51 (0.022)	0.56 (0.020)	0.05*
4	+	80	5	0.53 (0.013)	0.58 (0.010)	0.05*

* Significant increase at 5% significance level.

Table IV. *Effect of Cotyledon Excision on Osmotic Pressure of Free Space Solution Collected by Centrifugation of Apical Segments Vacuum Infiltrated with Indigo Carmine*

Cotyledons were excised 5 h prior to measurements. Osmolalities and free space volumes are means (SE) of five experiments. Air space volumes are means of three experiments. The correction factor for dilution of free space solutes was calculated as the total volume occupied by the infiltration solution (air space + free space water) divided by the free space water volume.

Measurement	+ Cotyledons	− Cotyledons
	mOsm kg^{-1}	
Extracted solution	92 (15.2)	53 (2.3)
Dye in solution	12 (0.93)	16 (2.3)
Free space solutes	80 (15.8)	37 (3.7)
	% tissue volume	
Air space	2.7	3.2
Free space water	6.8	3.2
Correction factor	1.39	2.00
	MPa	
Free space solutes corrected	0.28	0.18

required to stop growth, the final pressure reached and the rate of relaxation in the first 15 min (Table V).

Initially, a rapid increase in pressure is required to stop growth (Fig. 3A). The pressure is needed to collapse the water potential difference ($\Delta\Psi_g$) across the stem which results from water uptake during growth (1, 4, 5). However, at the same time, wall relaxation continues and adds to the pressure needed to stop growth. As an estimate of $\Delta\Psi_g$, the initial pressure required to stop growth was calculated by extrapolating the slope between 5 and 10 min to time zero (5). The average value for intact seedlings was 0.056 MPa compared to 0.036 MPa for seedlings without cotyledons (Table V). The initial pressure was greater for more rapidly growing seedlings (Fig. 4). The value of $\Delta\Psi_g$ is related to the growth rate according to the following equation: growth rate $= L(\Delta\Psi_g)$, where L is the growth-specific conductance for water movement radially across the stem (5). Calculated values for the conductance were not statistically different for seedlings with (0.99 MPa^{-1} h^{-1}) or without (0.82 MPa^{-1} h^{-1}) cotyledons (Table V) and were smaller than the value of 2.0 MPa^{-1} h^{-1} previously calculated for peas by measuring the half-time of tissue swelling upon addition of water after *in vivo* stress relaxation (3).

In theory, the end point of the pressure-block relaxation is reached when cell wall relaxation has resulted in a reduction of cell turgor to the yield threshold. The final chamber pressure was measured at the time when no further relaxation occurred or when the slope of the relaxation fell below about 0.1 MPa h^{-1}.

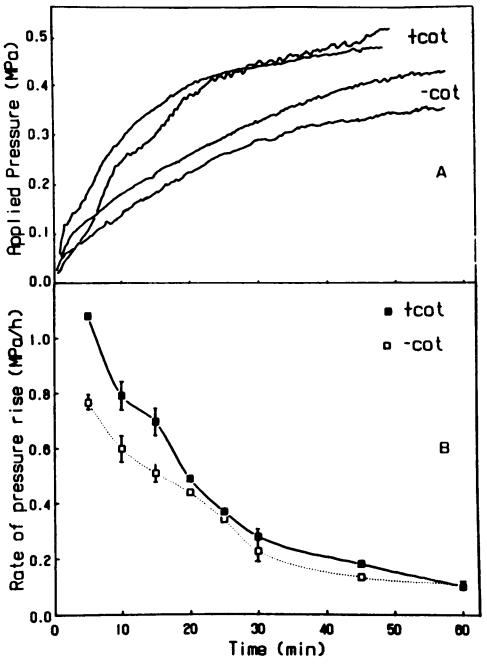

FIG. 3. A, Representative kinetics of pressure-block relaxation of seedlings, either with or without cotyledons. Growth rate was maintained at zero by increasing chamber pressure. Each tracing is of one plant. B, The rate of stress relaxation versus time. Rates were calculated as differentials of polynomial fits to selected regions and averaged for at least 10 relaxations. Vertical bars show SE of the means where larger than symbols.

The final chamber pressure was lower by 25% or 0.13 MPa for seedlings without cotyledons compared to controls (Table V). The final pressure minus the initial pressure to stop growth is a measure of $(P - Y)$, where P is turgor pressure and Y is yield threshold (5). $(P - Y)$ was also 24% lower in seedlings without cotyledons (Table V). Lower $(P - Y)$ without a change in turgor pressure (Table III) implies that the yield threshold was increased after cotyledon removal.

The rate of relaxation in seedlings without cotyledons was 24% to 30% lower than that of intact seedlings during the first 15 min of relaxation (Fig. 3, Table V). For both treatments, the initial 15 min of relaxation accounted for 50% of the total relaxation as measured as the percentage of the final pressure. During the remainder of the relaxation, rates were similar (Fig. 3B). During the first 15 min, the change in pressure may be described by the equation $dP_c/dt = -\epsilon\phi\ (P - Y)$, where ϵ is volumetric elastic

Table V. *Effect of Cotyledon Excision on Pressure-Block Stress Relaxation Parameters*

Cotyledons were excised 5 h prior to measurements. Initial P_c is the chamber pressure required to stop growth, an estimate of $\Delta\Psi g$, obtained by extrapolating the pressure curves between 5 and 10 min to time zero. Final P_c is the chamber pressure at the time when no further relaxation occurred or when the slope of the relaxation fell below 0.1 MPa h^{-1}. Final P_c − initial P_c is an estimate of $(P-Y)$. Growth-specific hydraulic conductance is calculated as growth rate/initial P_c for each seedling. Initial rates of relaxation are given as the slopes at 5, 10 and 15 min. Values are means (SE) of 9 to 14 seedlings.

Parameter	+ Cotyledons	− Cotyledons	Percent Control
	MPa		
Initial P_c	0.056 (0.0060)[a]	0.036 (0.0063)[a]	64
Final P_c	0.51 (0.017)[b]	0.38 (0.018)[b]	75
Final P_c − initial P_c	0.45 (0.015)[c]	0.34 (0.016)[c]	76
	MPa h^{-1}		
Slope at 5 min	1.08 (0.092)[e]	0.76 (0.094)[a]	70
Slope at 10 min	0.79 (0.059)[f]	0.60 (0.070)[f]	76
Slope at 15 min	0.68 (0.054)[g]	0.51 (0.066)[g]	75
	% h^{-1}		
Growth rate	4.9 (0.53)[d]	3.1 (0.61)[d]	63
	MPa^{-1} h^{-1}		
Hydraulic conductance	0.99 (0.138)	0.82 (0.08)	

[a-g] Significant differences at 5% significance level.

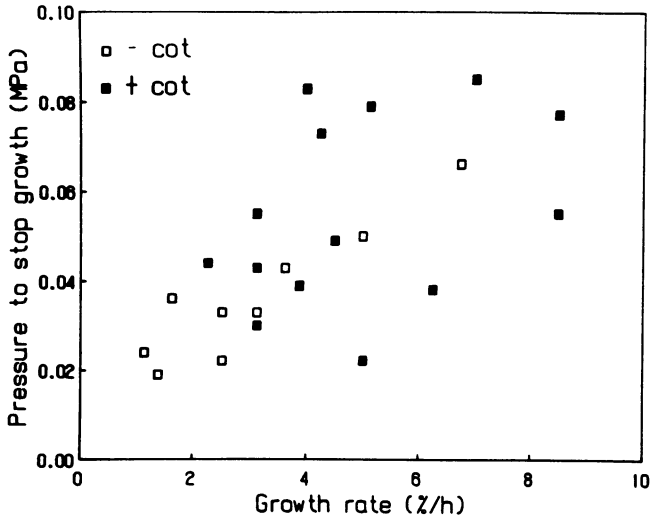

FIG. 4. Initial pressure required to stop growth ($\Delta\Psi_g$) *versus* initial growth rate, either with (■) or without (□) cotyledons.

modulus, and ϕ is the wall yield coefficient (5). The 25% reduction in $(P - Y)$ appears to be able to account for 24% to 30% reduction in the rate of relaxation. This suggests that within the limits of our measurements, the wall yield coefficient was not altered by cotyledon excision.

DISCUSSION

Removal of cotyledons stopped solute import into the growing region, as evidenced by the lack of accumulation of DW or solutes. As elongation continued, albeit at a lower rate, osmotic pressure decreased as expected simply by dilution. Turgor pressure did not decline despite a 0.14 MPa drop in osmotic pressure and, therefore was not the vehicle for the growth rate reduction. The inconsistency between osmotic and turgor pressures emphasizes the need to measure turgor pressure directly and to not assume that turgor follows the osmotic pressure.

For growing tissue studied here, where transpiration is negligible, turgor pressure is given by the difference in intracellular and extracellular osmotic pressures, minus $\Delta\Psi_g$. Because intracellular osmotic pressure decreased by 0.14 MPa after cotyledon removal, it follows that extracellular osmotic pressure or $\Delta\Psi_g$ must likewise have decreased, if turgor is to remain constant. Our measurements indicate that the decrease in extracellular osmotic pressure contributed most (0.10 MPa) to turgor maintenance, with a minor contribution from the reduction in $\Delta\Psi_g$ of 0.02 MPa.

We propose that the growing cells regulate extracellular solutes so as to maintain turgor pressure. Membrane transport of solutes from the free space can have a significant impact on the osmotic pressure in the free space with little effect on that of the cell, thus maintaining the difference between the two after solute supply is reduced. This can be illustrated if we assume that the free space water and the cell sap occupy 6% and 90% of the tissue volume respectively, that free space osmotic pressure is 0.28 MPa (Table IV) and that cell sap osmotic pressure is 0.88 MPa (Table I). A drop of 0.1 MPa in free space osmotic pressure due to solute uptake would result in an increase of only 0.007 MPa in osmotic pressure of the cell sap.

The drop in cell osmotic pressure after cotyledon excision may have been kept within the buffering capacity of the free space solutes by the reduction in growth rate. The growth rate reduction could have resulted from decreased hydraulic conductance for water movement radially across the stem or from altered cell wall yielding properties such as a decrease in wall yield coefficient or an increase in yield threshold.

The growth-specific conductance for water movement radially across the stem did not change after cotyledon removal (Table V). The reduction in growth rate observed within hours after cotyledon excision appears to be mediated via changes in cell wall yielding, primarily via a higher yield threshold. After cotyledon removal, the internal force on the wall, *i.e.* wall stress, remained constant (as measured by constant turgor). A decreased rate of growth at constant wall stress suggests that the ability of the cell wall to yield under constant force was lowered, as predicted by the biophysical model of growth; growth rate = $\phi(P - Y)$ (1, 4, 9). That wall yielding was reduced after cotyledon removal was directly demonstrated by pressure-block experiments. We found a reduced initial rate of stress relaxation (Fig. 3) and a lower chamber pressure at the end of relaxation in seedlings without cotyledons (Table IV). The reduction in rate of relaxation was of the same magnitude as the reduction in $(P - Y)$ and could be accounted for by the reduction in $(P - Y)$ (Table V). Given that turgor was not affected by cotyledon removal, these results indicate that the yield threshold was increased after cotyledon excision.

Our results suggest that the increase in yield threshold may be related to the decrease in osmotic pressure. Simple substrate-limitation for wall synthesis seems unlikely because the cells contained at least a 50-fold higher amount of hexoses than that used for synthesis of macromolecules over a 5 h period. We measured the contribution of hexoses to osmolality to be 20%. The rate of incorporation of hexoses into wall material can be estimated using data of Maclachlan *et al.* (14). They measured incorporation of radioactive sugars into solubles and insolubles, which were mainly polysaccharides, in pea stem sections. Their measurements of 0.1 μg/mg FW incorporated into insolubles in 2 h, converts to 0.015 μmol hexoses per 0.5 cm segment over 5 h in our system, or only 2% of the hexoses present. Although hexose supply is sufficient for wall synthesis, the lowering of a particular substrate may serve as a sensor for the decreased solute supply and trigger an increase in the yield threshold. Alternatively, altered wall properties might result from lack of a hormonal growth factor normally supplied from the cotyledons.

In conclusion, our study shows that stem elongation in etiolated peas is apparently linked to solute supply from the cotyledons, not through effects on turgor pressure or other hydraulic properties, but via affects on the cell wall yield threshold.

LITERATURE CITED

1. BOYER JS 1985 Water transport. Annu Rev Plant Physiol 36: 473–516
2. CLELAND R 1967 A dual role of turgor pressure in auxin-induced cell elongation in *Avena* coleoptiles. Planta 77: 182–191
3. COSGROVE DJ 1985 Cell wall yield properties of growing tissue. Evaluation by *in vivo* stress relaxation. Plant Physiol 78: 347–356
4. COSGROVE DJ 1986 Biophysical control of plant cell growth. Annu Rev of Plant Physiol 37: 377–405
5. COSGROVE DJ 1987 Wall relaxation in growing stems: comparison of four species and assessment of measurement techniques. Planta 171: 266–278
6. COSGROVE DJ, RE CLELAND 1983 Solutes in the free space of growing stem tissues. Plant Physiol 72: 326–331
7. COSGROVE DJ, RE CLELAND 1983 Osmotic properties of pea internodes in relation to growth and auxin action. Plant Physiol 72: 332–338
8. COSGROVE DJ, DM DURACHKO 1986 Automated pressure probe for measurement of water transport properties of higher plant cells. Rev Sci Instrum 57: 2614–2619
9. GREEN PB, K BAUR, WR CUMMINS 1977 Biophysical model for plant growth: auxin effects. *In* AW Jungreis, TK Hodges, A Kleinzeller, SG Schultz, eds, Water Relations in Membrane Transport in Plants and Animals. Academic Press, New York, pp 30–45
10. HANSON SD, JD COHEN 1985 A technique for collection of exudate from pea seedlings. Plant Physiol 78: 734–738
11. HOAGLAND DR, DI ARNON 1950 The water-culture method for growing plants without soil. Calif Agric Exp Stn, Bull 347: 1–32
12. HSIAO TC, J JING 1987 Leaf and root expansive growth in response to water deficits. *In* DJ Cosgrove, DP Knievel eds, Physiology of Cell Expansion during Plant Growth, American Society of Plant Physiologists, Rockville, MD, pp 180–192
13. JONES MG, WH OUTLAW, OH LOWRY 1977 Enzymic assay of 10^{-7} to 10^{-14} moles of sucrose in plant tissues. Plant Physiol 60: 379–383
14. MACLACHLAN GA, AH DATKO, J ROLLIT, E STOKES 1970 Sugar levels in the pea epicotyl: regulations by invertase and sucrose synthetase. Phytochemistry 9: 1023–1030
15. MEYER RF, JS BOYER 1981 Osmoregulation, solute distribution, and growth in soybean seedlings having low water potentials. Planta 151: 482–489
16. RICKAUER M, W TANNER 1986 Effects of Ca^{+2} on amino acid transport and accumulation in roots of *Phaseolus vulgaris*. Plant Physiol 82: 41–46
17. STEUDLE E 1985 Water transport as a limiting factor in extension growth. *In* NR Baker, WJ Davies, CK Ong, eds, Control of Leaf Growth. Society of Experimental Biology Seminar 27, Cambridge University Press, Cambridge, pp 35–55
18. ZHAO YJ, S KAMISADA, Y MASUDA 1985 Quantitative relationships between osmotic potential and epicotyl growth in *Vigna radiata* as affected by osmotic stress and cotyledon excision. Physiol Plant 64: 431–437