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Ethylene-Induced Lateral Expansion in Etiolated Pea Stems[1]

THE ROLE OF ACID SECRETION

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ABSTRACT

Ethylene-induced inhibition of elongation and promotion of lateral expansion in the stems of etiolated pea (*Pisum sativum* L. var Alaska) seedlings is not associated with any alteration of auxin-stimulated proton extrusion. Indeed, lateral expansion in response to ethylene apparently requires an acidified wall since it is prevented by strong neutral buffers and by the ATPase inhibitor orthovanadate. Ethylene treatment reduces the capacity of live and frozen-thawed sections to extend in the longitudinal direction in response to acid. The effect of ethylene on lateral acid growth capacity is more complicated. Ethylene-treated internodes do not exhibit acid-induced lateral expansion. Ethylene-treated segments which have been frozen-thawed do show an enhanced capacity to extend in the transverse direction at acid pH, but only when the inner tissues have been removed by coring. We conclude that two of the factors which control the directionality of expansion during ethylene treatment are a decrease in the sensitivity of the walls to acid longitudinally and an increase in the sensitivity of the outer cortical parenchyma walls to acid in the transverse direction.

Although the effect of ethylene on the reorientation of the cellulose microfibrils in pea stems has been well established (1, 3, 9, 13, 17, 22, 27), there is scant and contradictory information available on the changes in wall extension capacity associated with the shift to longitudinal microfibrils. Ridge (22) using a plasmometric method demonstrated a 50% reduction in the longitudinal extensibility of pea stem sections which had been previously treated 3 h with ethylene. In contrast, two extensibility studies on methanol-extracted walls using an Instron (16, 18) failed to detect a significant difference between ethylene-treated and control stems after 3 h, although Osborne (18) detected a 32% decrease in longitudinal plastic extensibility after 18 h of ethylene treatment. Sadava and Chrispeels (25) reported a 20% decrease in the extensibility of methanol-extracted sections after 3 h of ethylene treatment.

The lack of correspondence between the plasmometric studies and the short term Instron determinations prompted us to begin a series of experiments probing the physical and physiological consequences of ethylene-induced reorientation of cellulose microfibrils in the cell wall. In this paper, we focus on changes in the directionality of acid-induced extension in ethylene-treated and control tissues.

The possible importance of wall acidification and acid-induced wall loosening for ethylene-promoted lateral growth is suggested by the data of Demichelis and Lado (6). These authors present data showing that ethylene does not block auxin and fusicoccin-initiated medium acidification and that both agents enhance transverse growth in the presence of ethylene. A plausible explanation of these data is that the relevant cell wall property in determining the directionality of growth (*i.e.* longitudinal extension *versus* lateral expansion) is the capacity of the wall to respond to acid.

MATERIALS AND METHODS

Pisum sativum L. (var Alaska) seeds were soaked for 6 h in tap water and planted in moist vermiculite. Seedlings were grown for 7 d at 20°C in darkness at which time the third internode was 1 to 2 cm in length. Harvested third internodes were manually abraded, to facilitate the measurement of proton extrusion, with a slurry of emery powder (Emery No. 305, Edmund Scientific Co.), cut to 1 cm, and incubated in media as previously described (7). Control experiments demonstrated that abrasion itself did not stimulate ethylene production above nonabraded levels. Continuous growth measurements were made with a laser optical lever auxanometer device, following the method of Taiz and Metraux (28). Proton secretion experiments were carried out according to the procedure of Jacobs and Taiz (11). Ethylene (100 μl/l) was bubbled into the medium as indicated. This concentration was previously shown to inhibit elongation and promote lateral expansion (7). The acidification and growth curves represent the average of three or more experiments.

Longitudinal extensibility experiments with frozen-thawed sections were performed as described by Rayle *et al.* (19). Average creep rates were calculated over specific time intervals preceding the addition of acid buffer or following the addition of acid buffer. The specimen was extended using a force of 7.5 g for 15 min in K-phosphate (5 mM, pH 6.8) and the rate was determined between 10 and 15 min. The buffer solution was then changed to 5 mM citrate-phosphate (pH 4.8), and the new rate was determined between 5 and 15 min after the addition of acid.

Transverse extension experiments were carried out on 2-mm sections excised from the apical end of the subhook region. Sensitivity to acid was improved by coring out the central tissue using glass capillaries (Microcaps, Drummond Scientific Co.). Control and short term ethylene-treated stems were cored using a small capillary (approximately 0.8 mm o.d., 10 μl) while the wide 5- to 18-h ethylene-treated stems were cored with a slightly larger capillary (approximately 1.2 mm o.d., 25 μl). The cored sections were glued with a fast-drying cyanoacrylate glue (Duro

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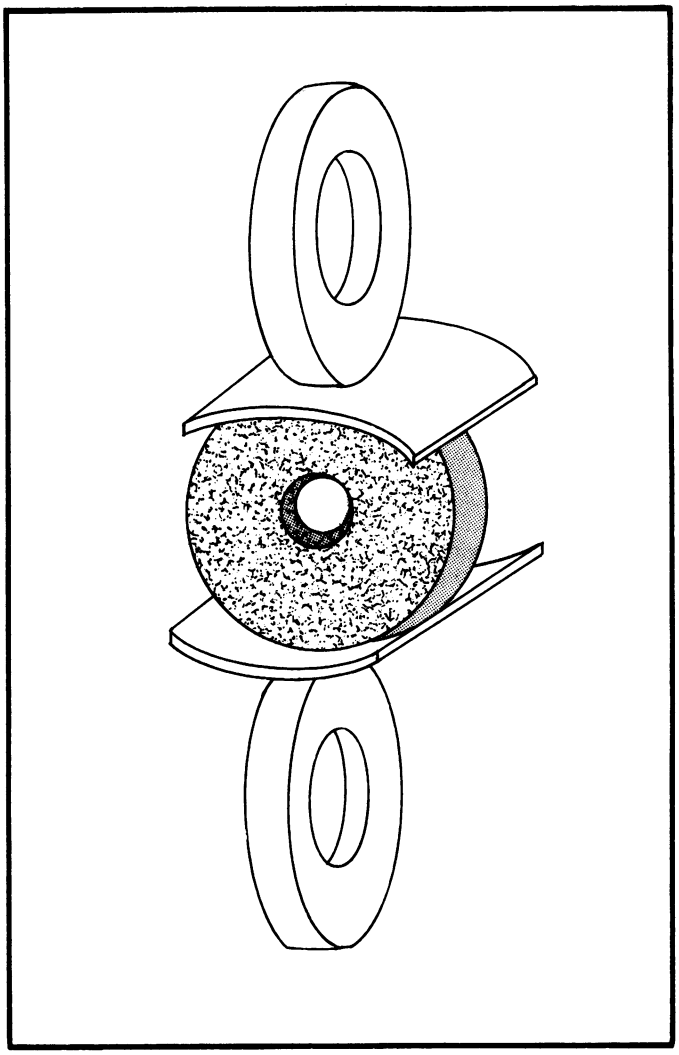

FIG. 1. Method for gluing 2-mm pea stem section to plastic holders for transverse extension studies. After removal of the central tissues by coring with a capillary, the preparation was frozen-thawed twice and hooked into the extension apparatus by means of the two opposite rings.

Superglue) to two small holders constructed of plastic (Fig. 1). The glued preparation was twice frozen and thawed. Next the specimen was hooked into a single pan balance-type extensometer, as described by Metraux and Taiz (14), except that the tissue was enveloped in a hanging droplet of 50 mM Tris-HCl (pH 6.8) or 50 mM Na-acetate (pH 4.8), rather than being submerged in a perfusion chamber. Control experiments substituting plastic tubing for the stem section indicated that the glue itself was not weakened by this pH shift. Creep rates were determined between 5 and 15 min after each buffer treatment began. The applied force was 2.5 g for control and short term ethylene-treated sections, and 5 g for 5- to 18-h ethylene-treated sections. Although a greater force was applied in the latter cases to compensate for differences in tissue thickness, it was found that the percentage of acid-stimulated extension over the pre-acid rate was independent of the applied force.

RESULTS

The effect of ethylene on medium acidification by abraded pea stem segments is shown in Figure 2. Ethylene did not inhibit acidification during a 30-min preincubation period. At time zero, the solution was changed to one containing 1 μM IAA. After a 15-min lag period, the rate of acidification, when measured over

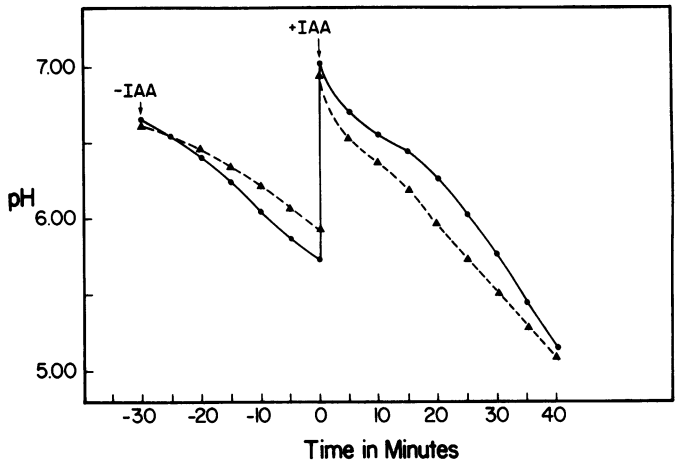

FIG. 2. Effects of ethylene on auxin-induced proton secretion. Fifty abraded 1-cm sections were incubated as described by Jacobs and Taiz (11). After a 30-min preincubation, the old medium was removed by suction and fresh medium containing auxin (1 μM IAA) was added. (●——●), Control; (▲- - -▲), 100 μl/l ethylene.

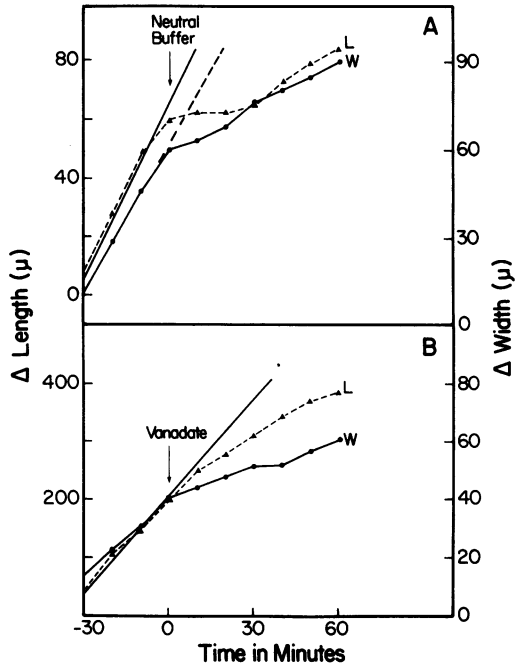

FIG. 3. Effects of neutral buffer and vanadate on ethylene-induced stem expansion. One cm abraded stem sections obtained from 4-h ethylene-treated intact seedlings were monitored continuously by the method of Taiz and Metraux (28). At time zero, either 20 mM K-phosphate (pH 6.8) or 1 mM sodium vanadate was added. A, Phosphate buffer; B, vanadate. (▲- - -▲), Length; (●——●), width.

the same pH range, was twice the control rate, and ethylene had no inhibitory effect. Even an 18-h pretreatment of stems with ethylene did not alter the subsequent proton extrusion rates of the sections (data not shown).

Auxin-induced elongation is blocked by neutral buffers and by orthovanadate (4, 11). As shown in Figure 3A, 20 mM phosphate buffer (pH 7) inhibits both the elongation and lateral expansion of ethylene-treated sections. Similar results were obtained with orthovanadate (Fig. 3B). Thus, transverse expansion appears to require an acidified wall and is decreased by an inhibitor of proton extrusion.

The response of untreated, abraded pea stem sections to exogenous acid is illustrated in Figure 4A. Control segments exhibit

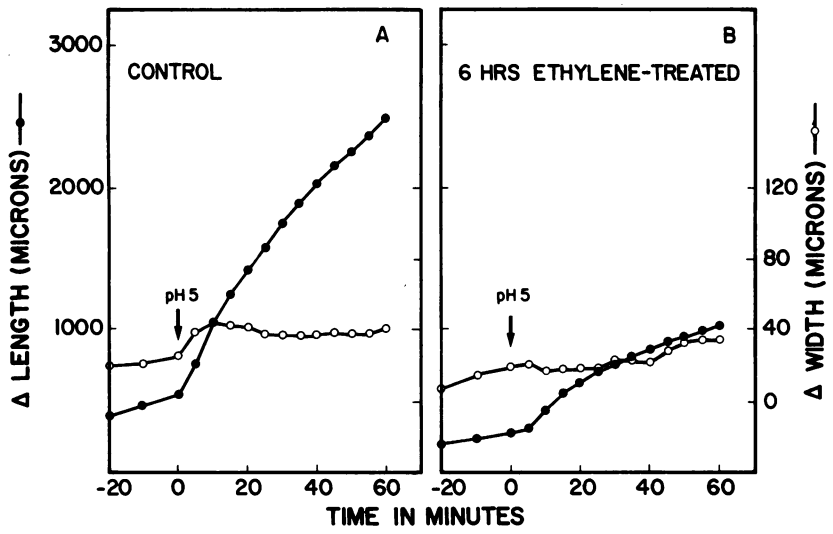

FIG. 4. Effects of ethylene on acid-induced elongation and lateral expansion. A, Control stem sections; B, stem section excised from seedling which was pretreated with 100 μl/l ethylene for 6 h. (●), Length; (○), width.

a typical rapid elongation rate in the presence of pH 5 buffer, and the diameter is stable after a transient increase. The acid response of a 6-h ethylene-treated stem section is shown in Figure 4B. Ethylene-treated stems have a markedly reduced elongation rate in response to acid, but the rate of lateral expansion is unaffected. Similar results were obtained with segments given an 18-h ethylene pretreatment. Thus, ethylene inhibits acid-induced elongation, but does not promote acid-induced lateral expansion.

The effect of ethylene on acid-sensitive longitudinal wall creep was also investigated using frozen-thawed sections (Table I). No significant inhibition of creep rate by ethylene is observed at pH 6.8, whether ethylene was given as a pretreatment to intact seedlings or as a treatment to isolated sections. In contrast, ethylene significantly inhibited creep in response to acid pH, particularly in the case of the pretreated intact plants where acid-induced extension was reduced by about 40%. Isolated control sections also lost much of their sensitivity to acid 5 h after excision (Table I).

In preliminary experiments, it was observed that while frozen-thawed 2-mm-long segments exhibited typical viscoelastic behavior when extended transversely, they were apparently insensitive to changes in pH. However, when the central tissues were cored out, ethylene-pretreated tissue consistently responded to acid pH. A typical transverse extension curve for an 18-h ethylene-treated stem is shown in the insert of Figure 5. Extension increases immediately upon the addition of pH 4.8 buffer, and is reversed by pH 6.5 buffer. Acid-stimulated transverse extension increases proportionally to the duration of ethylene treatment (Fig. 5), whereas the controls show no significant response to acid. After 5 h of ethylene treatment, acid causes a 3-fold increase in the rate of transverse extension, while after 18 h there is a 10-fold increase in acid-induced extension caused by acid.

DISCUSSION

It is well established that ethylene alters the microfibrils in pea stems from a transverse to a longitudinal orientation (1, 3, 9, 13, 17, 22, 27). However, there is little information available on the changes in wall extensibility associated with the shift to longitudinal microfibrils, and the evidence so far has been contradictory (16, 18, 22, 25). Our investigation has focused on the effect of ethylene on wall acidification and acid-induced wall extension. The possible role of wall acidification for ethylene-promoted lateral growth is suggested by the observations of Demichelis and Lado (6) that both auxin and fusicoccin enhance transverse growth in the presence of ethylene. We have shown that ethylene does not interfere with auxin-induced medium acidification by isolated stem sections, in agreement with the results of Demichelis and Lado (6). These data suggest that the free space pH is maintained in the acid range by auxin during ethylene-induced transverse growth. Furthermore, the relevant cell wall mechanical property which determines the directionality of growth may be the response to acid pH. A precedent for this notion can be found in *Nitella* (25). The walls of isopropyl *N*-phenylcarbamate-treated cells exhibit an increase in acid-induced transverse extensibility compared to control cell walls. The increased acid-induced transverse extension is correlated with the deposition of randomly oriented cellulose microfibrils on the inner quarter of the wall (24, 25).

Table I. *Effect of Ethylene on the Longitudinal Extensibility of Frozen-Thawed Pea Stem Sections at Neutral and Acid pH*

Sections were prepared and extension rates were calculated as described in the text. Values are the averages of 8 to 10 experiments ± SD. T_0 is the initial value.

Treatment	Extension Rate at pH 6.8	Extension Rate at pH 4.5	Stimulation by Acid	Inhibition by Ethylene
	μm/min		%	
T_0, intact	1.5 ± 0.7	7.26 ± 2.0	484	
5 h Ethylene, intact	1.3 ± 0.2	3.72 ± 1.7	290	40
5 h Control, section	1.2 ± 0.3	3.92 ± 0.9	325	
5 h Ethylene, section	1.3 ± 0.8	3.33 ± 0.8	256	21

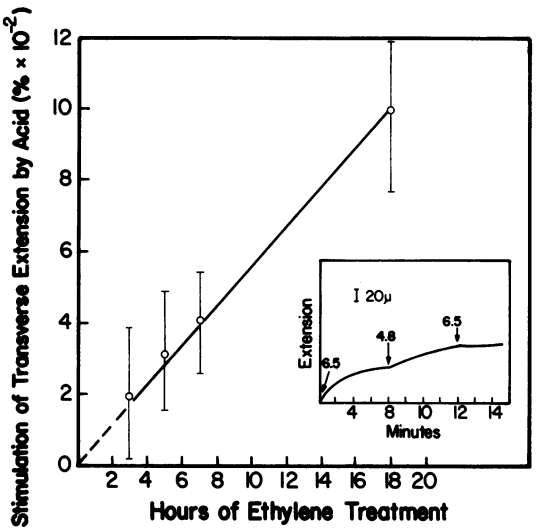

FIG. 5. Effect of ethylene pretreatment on acid-induced transverse extension of frozen-thawed, cored sections. Each point is the average of two experiments, with the actual data points indicated by the bars. There was no detectable acid-induced creep in the controls without ethylene. The insert illustrates an extension response of an 18-h ethylene-treated stem in the presence of pH 6.5 and pH 4.8 K-phosphate.

Consistent with the above reasoning, ethylene dramatically reduces the capacity of pea stems to elongate in response to acid pH. Creep tests on frozen-thawed specimens confirmed that the loss of sensitivity to acid reflected a property of the isolated walls. Interestingly, the inhibitory effect of 5 h ethylene treatment was only detectable at acid pH. Presumably, acid treament unmasks those bonds which are involved in the control of cell shape, specifically, bonds at the interface between the matrix and the cellulose microfibrils. This may account for the failure to detect an early effect of ethylene using the Instron technique (16, 18). On the other hand, the plasmometry studies may measure wall properties occurring at acid pH due to residual proton extrusion during the measurements (22).

Ethylene-induced lateral expansion is inhibited by strong neutral buffers and by orthovanadate. Transverse expansion thus appears to require proton extrusion, since neutral buffers should prevent the auxin-induced drop in cell wall pH, whereas vanadate has been shown to inhibit proton extrusion in peas without affecting either respiration or protein synthesis (14). Ethylene also dramatically increases the transverse acid-induced extensibility of the walls under some conditions. That is, cored sections exhibited acid-enhanced lateral extension, while noncored material did not. It is possible that ethylene may primarily exert its influence on the outer cortical cells. Indeed, Lang *et al.* (13) reported that ethylene appeared to specifically alter the microtubule and microfibril orientations of the cells with polylamellate walls in the outer cortical parenchyma of peas.

Further, the site of auxin responsiveness in peas is located in the outer cell layers (31), and *in vivo* wall acidification may be strongest in this region of the tissue. Exogenous acid buffers acidify both inner and outer tissues, whereas only the outer tissues appear to be modified by ethylene (13). Thus, the tendency of the inner cells to elongate may negate the expansion growth of the outer cells.

The failure of exogenous acid to promote lateral expansion in intact, ethylene-treated stem sections could also indicate that other factors besides proton extrusion and microfibril reorientation are involved in ethylene-induced lateral expansion. For example, there is a discrepancy between the kinetics of sections as reported here and intact seedlings. Intact seedlings and segments with apices attached exhibit a lag time of only 15 min before swelling begins in response to ethylene (2, 12, 17). Warner and Leopold (30) were able to detect an inhibition of elongation in decapitated seedlings in as little as 6 min. Such kinetics can hardly be caused by reoriented cellulose microfibrils, even though it is not clear whether the kinetics of lateral expansion exactly parallel the inhibition of elongation. Burg (2) has reported that ethylene can prevent gravitropic curvature of excised pea internode segments within 15 min, probably due to an inhibition of auxin transport. Ethylene did not alter the rate of cell expansion during this time. Among the reported effects of ethylene on cell wall metabolism are increases in cell wall hydroxyproline (23–26), although the significance of this has been questioned (17), enhancement of pectin levels (15), increases in wall-bound peroxidase (23, 24), and an inhibition of xyloglucan turnover (29). Of these, only the inhibition of xyloglucan turnover occurs rapidly enough to be involved in the rapid response to ethylene. Inasmuch as xyloglucan turnover can be induced by exogenous acid (10), the reduction in xyloglucan turnover could be related to the loss of sensitivity to acid pH.

LITERATURE CITED

1. APELBAUM A, SP BURG 1971 Altered cell microfibrillar orientation in ethylene-treated *Pisum sativum* stems. Plant Physiol 48: 648–652
2. BURG SP 1973 Ethylene in plant growth. Proc Natl Acad Sci USA 70: 591–597
3. BURG SP, A APELBAUM, W EISINGER, BG KANG 1971 Physiology and mode of action of ethylene. Hort Science 6: 359–364
4. CLELAND RE, DL RAYLE 1978 Auxin, H^+-excretion and cell elongation. Bot Mag Tokyo Special Issue 1: 125–139
5. CRAKER LE, C COOKSON, DJ OSBORNE 1978 Control of proton extrusion and cell elongation by ethylene and auxin in the water-plant *Ranunculus sceleratus*. Plant Sci Lett 12: 379–385
6. DE MICHELIS MI, P LADO 1974 Effect of ethylene on auxin- and fusicoccin-induced growth of pea internode segments and on the secretion of H^+ in the incubation medium. Rend Acad Naz Lincei 56: 808–813
7. EISINGER W, L CRONER, L TAIZ 1983 Ethylene-induced lateral expansion in etiolated pea stems. Kinetics, cell wall synthesis, and osmotic potential. Plant Physiol 73: 407–412
8. EISINGER W, SP BURG 1972 Ethylene-induced pea internode swelling. Its relation to ribonucleic acid metabolism, wall protein synthesis, and cell wall structure. Plant Physiol 50: 510–517
9. HENRY EW 1978 An ultrastructural study of ethylene-treated stem segments of Alaska pea (*Pisum sativum*). Cytologia 43: 423–432
10. JACOBS M, PM RAY 1975 Promotion of xyloglucan metabolism by acid pH. Plant Physiol 56: 373–376
11. JACOBS M, L TAIZ 1980 Vanadate inhibition of auxin-enhanced H^+ secretion and elongation in pea epicotyls and oat coleoptiles. Proc Natl Acad Sci USA 77: 7242–7246
12. KANG BG, SP BURG 1973 Influence of ethylene on nucleic acid synthesis in etiolated *Pisum sativum*. Plant Cell Physiol 14: 981–988
13. LANG HM, WR EISINGER, PB GREEN 1982 Effect of ethylene on the orientation of microtubules and cellulose microfibrils of pea epicotyl cells with polylamellate walls. Protoplasma 110: 5–14
14. METRAUX J-P, L TAIZ 1977 Cell wall extension in *Nitella* as influenced by acids and ions. Proc Natl Acad Sci USA 74: 1565–1569
15. MONDAL MH, JF NANCE 1975 Effects of ethylene, kinetin, and calcium on growth and wall composition of pea epicotyls. Plant Physiol 55: 450–454
16. MORRE DJ, WR EISINGER 1968 Cell wall extensibility: its control by auxin and relationship to cell elongation. *In* F Wightman, G Setterfield, eds, Biochemistry and Physiology of Plant Growth Substances. The Runge Press, Ottawa, pp 624–646
17. NEE M, L CHIU, W EISINGER 1978 Induction of swelling in pea internode tissue by ethylene. Plant Physiol 62: 902–906
18. OSBORNE DJ 1977 Auxin and ethylene and the control of cell growth. Identification of three classes of target cells. *In* PE Pilet, ed, Plant Growth Regulation, Proceedings of the 9th International Conference on Plant Growth Substances. Springer-Verlag, Berlin, pp 161–171
19. RAYLE DL, PM HAUGHTON, R CLELAND 1970 An *in vitro* system that simulates plant cell extension growth. Proc Natl Acad Sci USA 67: 1814–1817
20. RICHMOND PA 1977 Control of plant cell morphogenesis by the cell wall: analysis in *Nitella*. Ph.D. thesis. University of Pennsylvania, Philadelphia.
21. RICHMOND PA, J-P METRAUX, L TAIZ 1980 Cell expansion patterns and directionality of wall mechanical properties in *Nitella*. Plant Physiol 65: 211–217
22. RIDGE I 1973 The control of cell shape and rate of cell expansion by ethylene: effects on microfibril orientation and cell wall extensibility in etiolated peas. Acta Bot Néerl 22: 144–158
23. RIDGE I, DJ OSBORNE 1970 Hydroxyproline and peroxidases in cell walls of

Pisum sativum: regulation by ethylene. J Exp Bot 21: 843–856
24. RIDGE I, DJ OSBORNE 1971 Role of peroxidase when hydroxyproline-rich protein in plant cell walls is increased by ethylene. Nature New Biol 229: 205–208
25. SADAVA D, MJ CHRISPEELS 1973 Hydroxyproline-rich cell wall protein: role in the cessation of elongation in excised pea epicotyls. Dev Biol 30: 49–55
26. SADAVA D, F WALKER, MJ CHRISPEELS 1973 Hydroxyproline-rich cell wall protein (extensin): biosynthesis and accumulation in growing pea epicotyls. Dev Biol 30: 42–48
27. STEEN DA, AV CHADWICK 1981 Ethylene effects in pea stem tissue. Evidence of microtubule mediation. Plant Physiol 67: 460–466
28. TAIZ L, J-P METRAUX 1979 The kinetics of bidirectional growth of stem sections from etiolated pea seedlings in response to acid, auxin and fusicoccin. Planta 146: 171–178
29. TERRY ME, B RUBINSTEIN, RL JONES 1981 Soluble cell wall polysaccharides released from pea stems by centrifugation. II. Effect of ethylene. Plant Physiol 68: 538–542
30. WARNER LN, AC LEOPOLD 1971 Timing of growth regulator responses in peas. Biochem Biophys Res Commun 44: 989–994
31. YAMAGATA Y, Y MASUDA 1976 Auxin-induced extension of the isolated epidermis of light-grown pea epicotyls. Plant Cell Physiol 17: 1235–1242
32. YAMAMOTO R, K MAKI, Y YAMAGATA, Y MASUDA 1974 Auxin and hydrogen ion actions in light-grown pea epicotyl segments. I. Tissue specificity of auxin and hydrogen ion actions. Plant Cell Physiol 15: 823–831