

THE CONTROL OF CELL SHAPE AND RATE OF CELL EXPANSION BY ETHYLENE: EFFECTS ON MICROFIBRIL ORIENTATION AND CELL WALL EXTENSIBILITY IN ETIOLATED PEAS

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SUMMARY

Rapidly growing cells of etiolated *Pisum sativum* show two early responses to ethylene: a reduced rate of expansion and an increase in lateral growth. The latter has been explained in terms of an altered orientation of cellulose microfibrils in the cell walls: a change from transverse to longitudinal deposition is shown, using polarization microscopy, within 6 hours of exposure to ethylene.

To explain the ethylene-induced reduction of growth rate, a plasmometric technique was used to study changes in the potential for plastic and elastic extension of cell walls and changes in cell turgor. Both wall extensibility and turgor were found to decline rapidly after exposing intact plants to 1 ppm ethylene and the significance of these changes in relation to ethylene control of cell growth is discussed.

1. INTRODUCTION

Ethylene has two distinct effects on cell expansion in etiolated pea seedlings, a system where the gas is known to be a natural regulator of growth (GOESCHL & PRATT 1968). Firstly, applied ethylene decreases the overall rate of cell expansion so that, although the final cell volumes attained may equal those of air-grown plants, the time taken is 3–5 times longer in ethylene (RIDGE & OSBORNE 1969). Secondly, ethylene increases the ratio of lateral to longitudinal expansion in cortical tissue so that short, 'swollen' cells are produced. The result is a pronounced slowing down and reorientation of cell expansion, and this paper attempts to explain these effects of ethylene chiefly in terms of changes in the structure and properties of cell walls.

It has long been held that the orientation of cellulose microfibrils in plant cell walls is a major factor governing the direction of cell expansion (FREY-WYSSLING 1959; GREEN 1963). For typical cylindrical cells the stresses in the cell walls are twice as great in the lateral as in the longitudinal direction (VAN ITERSON 1937), and yet cell expansion is mainly longitudinal. The explanation lies in the strongly anisotropic nature of the cell walls, which in turn depends on their microfibrillar structure: cellulose microfibrils are deposited at right angles to the cell axis, which so increases resistance to transverse stress that expansion occurs largely in an axial direction. It follows that changes in microfibril orientation might be expected to correlate with an altered direction of cell expansion and, in a few instances, this has been demonstrated. Isodiametric cell

expansion following treatment with colchicine, for example, is related to a random deposition of microfibrils (WARDROP 1956; GREEN 1963); whilst VEEN (1970b) has shown that the predominantly lateral cell expansion in pea stem segments incubated with IAA and 8% sucrose results from a deposition of longitudinally oriented microfibrils. Since ethylene is a natural inducer of lateral cell expansion in peas, it is of considerable interest to determine whether changes in microfibril orientation are involved and, if so, whether random deposition or a precise reorientation occurs. This problem has been approached using the technique of polarization microscopy, and the observations reported here show that ethylene does affect cell wall structure in peas and, in fact, induces longitudinal rather than transverse deposition of microfibrils.

When considering the second aspect of ethylene action, the reduction in rate of cell expansion, I have followed CLELAND (1967) and GREEN (1968) and assumed that the two major factors controlling growth rate are (1) extensibility of the cell walls, WE_x , and (2) cell turgor, P , which may be influenced by changes in either osmotic potential or water potential. Auxin increases growth rate by an effect on cell wall properties which appears as an increase in WE_x (HEYN 1931; CLELAND 1958), but which, if either, of these two parameters, WE_x and P , is affected by ethylene is quite unknown.

Experiments were carried out using the plasmolysis technique of URSPRUNG & BLUM (1924) modified after BURSTRÖM (1964) to determine whether WE_x or P is modified after exposing plants to ethylene. The results show that ethylene not only causes a drastic reduction in the potential for plastic and elastic extension of the cell walls but also appears to decrease turgor pressure.

2. MATERIALS AND METHODS

Seedlings of *Pisum sativum* L. cv. Alaska were grown in sterilized vermiculite at 25°C and 90% relative humidity in darkness. Plants were used after 6 days, all manipulations being carried out in dim green light. For ethylene treatment, seedlings were placed in 6–10 litre glass vessels which were aerated and had ethylene renewed daily, and which contained beakers of saturated KOH solution to prevent accumulation of CO_2 . When plants were to be used for polarization microscopy, the epicotyl was marked with Indian ink just below the hook and 5 and 10 mm below this: the marked segments were excised, measured and fixed at various times after treatment.

2.1. Polarization microscopy

Plant material was embedded in paraplast and sectioned longitudinally so that double cell walls (i.e. the walls of two adjacent cells) could be studied in surface view. All sections were stained with a dichroitic stain, zinc chlor-iodine, and two kinds of observations were made:

- (a) utilizing the dichroism of stained cellulose. Here only the lower prism (polarizer) is used, and the wall shows maximum light absorption when the light vibrates parallel to the microfibril axes; optimum light transmission

occurs when the vibration plane is at right angles to the axes. VEEN (1970a) has further shown that, for pea shoots, the longitudinal axis of pit openings in the walls invariably coincides with the predominant microfibril direction: pit axes, therefore, provided a useful indicator of microfibril orientation.

(b) between crossed nicols. Here the polarizer and analyser are fixed with vibration planes at right angles and the specimen rotated to determine the positions of extinction and maximum brightness. Thus a wall viewed so that microfibrils lie predominantly at 45° to either vibration plane appears bright, but darkens when the microfibrils lie parallel to either plane. (For further discussion of the technique see PRESTON 1952; VEEN 1971).

2.2. Plasmolysis experiments

Intact pea seedlings were treated with ethylene at concentrations of 1–100 ppm for periods of 3–20 hours, with appropriate controls kept in air. Segments 10 mm long were excised from just below the apical hook and batches of 15–20 floated on aqueous solutions of mannitol, 0–0.4 or 0.45 M, for 2 hours in darkness. After recording segment dimensions by photographing or by drawing an enlarged projected image, all segments were plasmolysed in 0.4 or 0.45 M mannitol for a further 2 hours and dimensions again recorded. Measurements of segment length were to the nearest 0.5 mm and standard errors averaged 0.5–0.8%

2.2.1. Determination of elastic and plastic extension and elastic extensibility

Elastic extensibility, E , was defined as the decrease in segment length after immediate plasmolysis, $L_o - L_{pl}$, where L_o is the initial length and L_{pl} the initial plasmolysed length. The elastic or reversible extension of the tissue, RE , after a 2 hour incubation in water, was defined as $L_t - L_{rev}$, where L_t is the final segment length and L_{rev} the length after plasmolysis. Plastic or irreversible extension, IE , was then defined as $L_{rev} - L_{pl}$. Although RE and IE were measured for whole segments, a direct relation to cell wall extension is assumed unless otherwise stated.

As pointed out by CLELAND (1959), this definition of IE is equivalent to the irreversible expansion of walls already present (plastic stretching or PS) only if no new synthesis of wall material occurs. PS is usually measured under conditions which inhibit wall synthesis (at low temperature or in an atmosphere of nitrogen). CLELAND (1959) found that for a 2 hour expansion period IE and PS were identical for *Avena* coleoptile segments, and in the experiments reported here removal of air from intercellular spaces, by lowering pressure with a vacuum pump until segments sank, did not affect the irreversible length attained. I have assumed, therefore, that IE and PS are equivalent in these experiments too.

2.2.2. Determination of water potential, osmotic potential, and turgor pressure

The method was described by BURSTRÖM (1964) and BURSTRÖM, UHRSTRÖM & WURSCHE (1967). Segment length was plotted against mannitol concentration

before and after plasmolysis and a curve for reversible segment length obtained by subtracting irreversible increases in length from final length attained. Knowing the initial segment length, a measure of water potential, ψ , could be obtained from this curve in terms of molal mannitol equivalents. Osmotic potential, π , was determined by graphical extrapolation to the point of incipient plasmolysis, so that from the standard equation:

$$\psi = \pi + P$$

where ψ and π are negative potentials, P could be determined. Segment length rather than volume was plotted since variations in segment diameter proved to be large, between 3 and 7% usually, and, as a further approximation, the matrix potential was neglected.

3. RESULTS

3.1. Polarization microscopy

For control peas grown in air my observations on the microfibrillar structure of cell walls in young apical cells were in complete agreement with those of VEEN (1970a). Cortical cells from the hook region, the apical 5 mm zone, or the sub-apical 5 mm zone, show a predominantly transverse orientation of cellulose microfibrils. Thus the dichroically stained walls are darkest when viewed (using the polarizer only) with the plane of vibration at right angles to the cell axis and the pits are clearly transverse (*fig. 1a*). When viewed with the vibration plane parallel to the cell axis (*fig. 1b*), transverse microfibrils become transparent but the tilting of pit axes and the slight absorption of light indicates the presence of oblique microfibrils. Using the electron microscope, VEEN (1970a) has shown that a thin layer of oblique microfibrils, lying at approximately 45° to the cell axis, is situated at the outer surface of the cell wall, and he suggests that this layer arises by rotation of the originally transverse microfibrils as a result of cell extension. When viewed between crossed nicols the cell walls of apical cells show a bright transverse layer of microfibrils when the cell axis is in the 45° position (*fig. 2a*). With the axis in the 90° position (*fig. 2b*) the tilting of pit angles and the brighter areas of the wall, especially close to the pit borders, again indicate oblique layers of microfibrils, which must lie parallel in the two adjacent cell walls viewed here.

The effects of ethylene treatment on microfibril orientation are illustrated in *figs. 3-5*. After exposure for one day to 100 ppm ethylene, the apical 5 mm zone shows no increase in length (compared to a 4-5 fold increase in air-grown controls), but has increased in width by 35-40%. As shown in *fig. 3a*, the cell walls of this apical tissue still show a strong transverse layer of microfibrils: pit axes are mainly transverse and the wall is maximally dark with the light vibrating at right angles to the cell axis. However, in *fig. 3b*, where the light is vibrating parallel to the cell axis, the wall appears different from that of the air-grown control (cf. *fig. 1b*): vertical, or near vertical, striations can be seen crossing the pit openings and there is a central area which is maximally dark for this position of the polarizer. The former must represent aggregates of

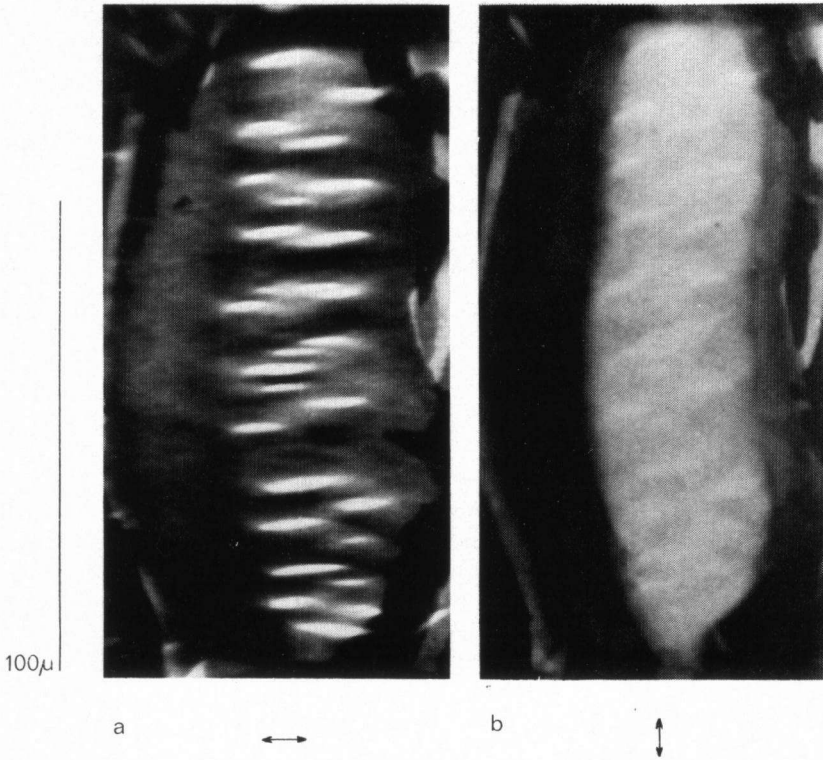


Fig. 1. Cell wall of a mid-cortical cell from the apical 5 mm zone; pea seedlings grown for 7 days in air. Wall showing dichroism after staining with zinc chlor-iodine and viewed with two positions of the polarizer; (a) light vibrating at right angles and (b) parallel to the cell axis. \longleftrightarrow vibration plane of the polarized light.

longitudinal microfibrils and the latter a more diffuse area of longitudinal deposition which, in *fig. 3c*, is visible as a black area when the cell is viewed in the 45° position between crossed Nicols. It must be remembered that these longitudinal microfibrils have appeared during a period when the cells underwent no increase in length; they cannot, therefore, be explained in terms of a reorientation of existing microfibrils as a result of cell extension.

A progressive thickening of the longitudinal layer of microfibrils occurs as ethylene treatment is prolonged (*figs. 4* and *5*). The pit openings appear more rounded or assume a vertical orientation (*figs. 4b* and *5b*), and in *fig. 4c*, where the cell is viewed in the 45° position between crossed Nicols, the wall appears dark with bright spots in a “+”-shaped arrangement around the pits. This appearance can be explained if there are transverse and longitudinal microfibril layers of approximately equal thickness, with areas around the pits where one or other of these layers predominates (*fig. 6*).

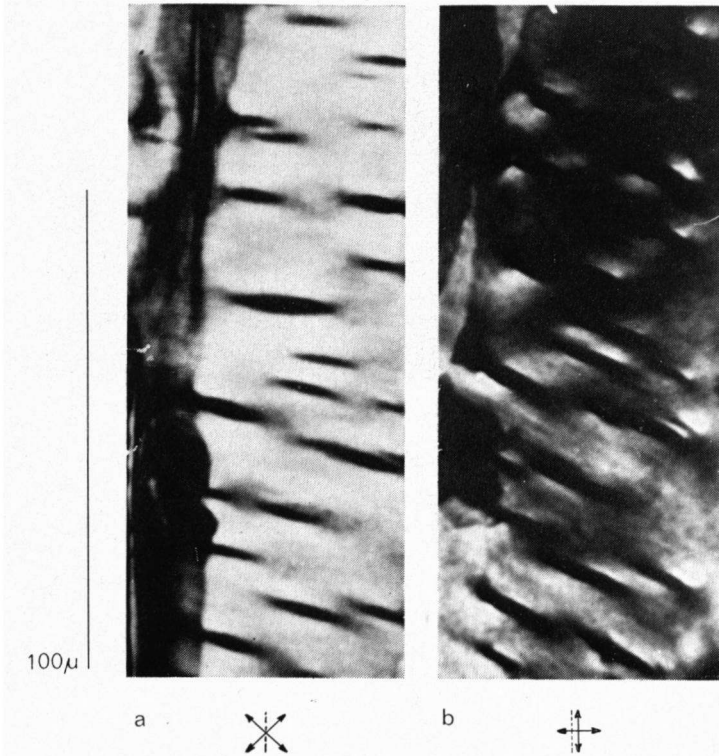


Fig. 2. Apical cell wall from air-grown pea seedlings; wall viewed between crossed Nicols with the cell axis (----) in (a) the 45° and (b) the 90° position.

It seems clear that during growth in ethylene longitudinal rather than transversely oriented microfibrils are deposited on the cell walls. Can longitudinal deposition be construed, therefore, as a causal factor in ethylene-induced lateral cell expansion? Longitudinal microfibrils can be detected in cortical cells some 3–5 mm below the hook within 6 hours of exposure to ethylene (fig. 7). Since at the same time lateral growth in these cells can be detected (10% after 3 hours and 20% after 7 hours), it seems reasonable to infer that the two processes are indeed closely linked.

3.2. Effects of ethylene on elastic and plastic extension

When intact pea seedlings were exposed to 1 ppm ethylene for 3 hours, the elastic and plastic extension of segments during a subsequent 2 hour incubation was substantially reduced relative to air grown control plants (table 1). The reduction in RE averaged 34% and in IE 46%, whilst longer exposure to ethylene resulted in still greater reduction (51% and 90% respectively after 17

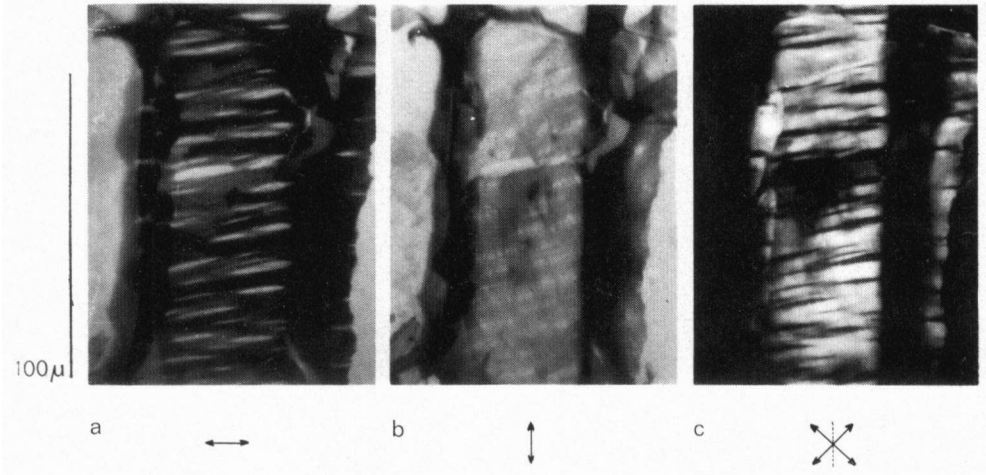


Fig. 3. Apical cell wall from pea seedling exposed for 1 day to 100 ppm ethylene after staining with zinc chlor-iodine. (a) and (b) showing dichroism at two positions of the polarizer. (c) wall viewed between crossed Nicols with the cell axis in the 45° position.

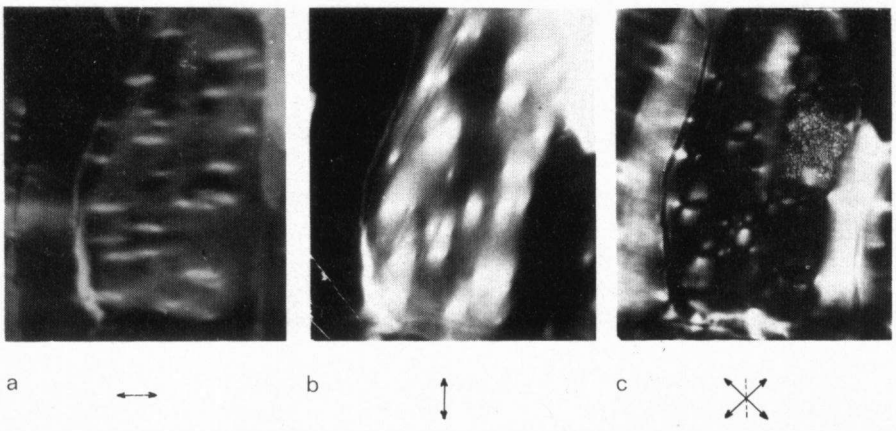


Fig. 4. Apical cell wall from pea seedling exposed for 2 days to 100 ppm ethylene. Staining and viewing positions as for *fig. 3*. Note vertical axes of pits in (b) and bright spots arranged $+$ -wise in (c).

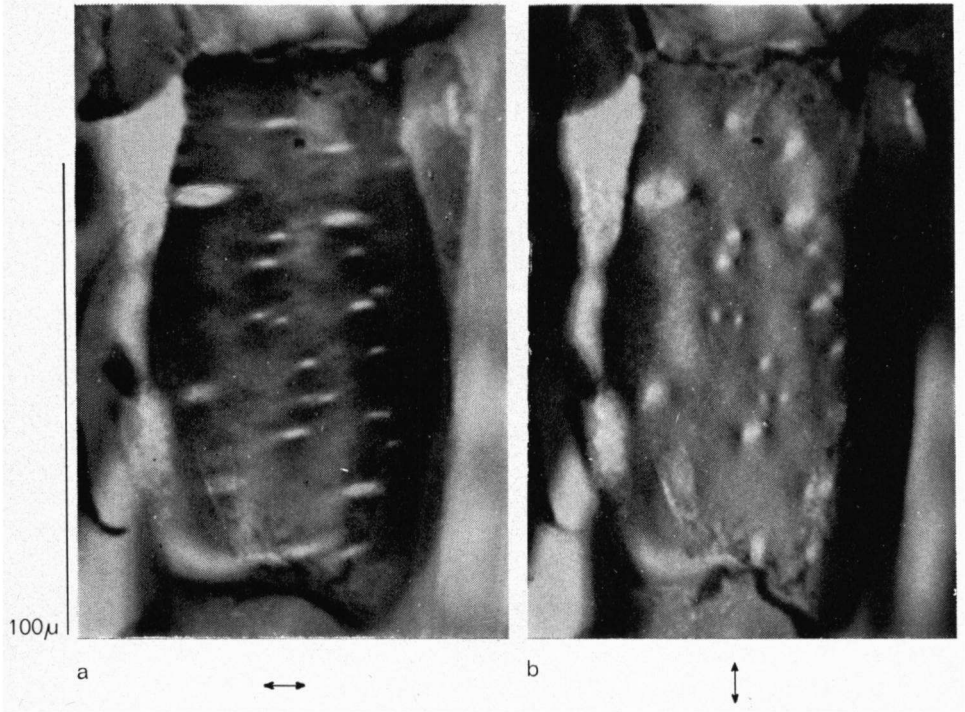


Fig. 5. Apical cell wall from pea seedling exposed for 4 days to 100 ppm ethylene. Wall stained and viewed as for *fig. 3a* and *3b*. Note approximately equal light absorbance for both positions of the polarizer.

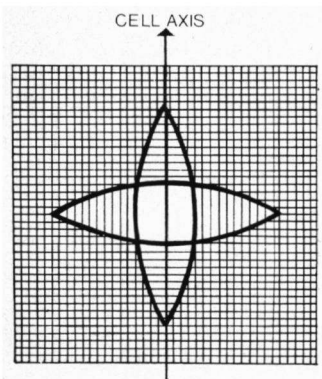


Fig. 6. Schematic representation of microfibril arrangement around pit openings to explain the + -shaped bright spots seen in *fig. 4c*: areas where only 1 microfibril layer is present appear bright between crossed Nicols with the cell axis in the 45° position.

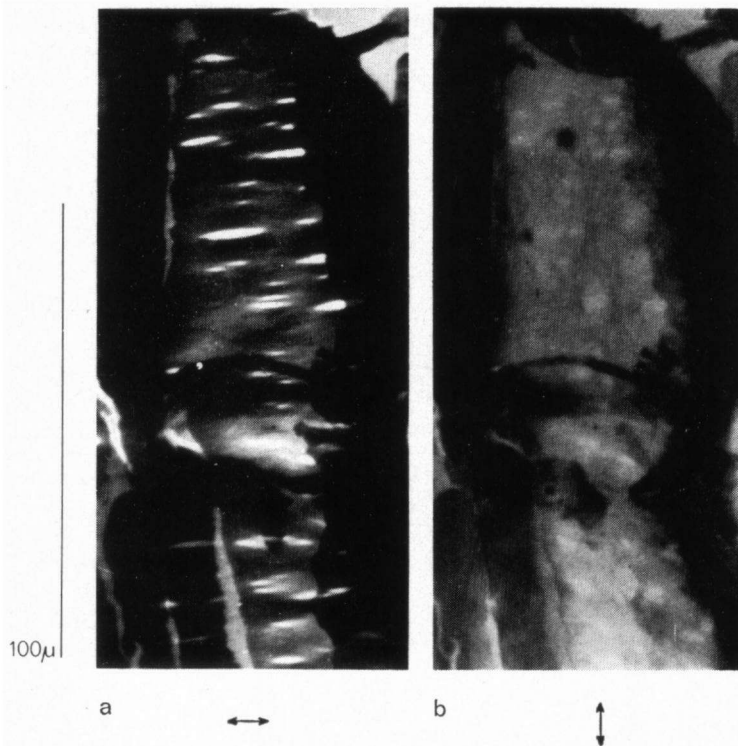


Fig. 7. Apical cell wall from the mid-cortex 3 mm below the hook. Pea seedlings 7 days old exposed for 6 hours to 100 ppm ethylene. Wall showing dichroism, stained and viewed as for fig. 3a and 3b.

hours). Concentrations of 10 or 100 ppm ethylene were no more effective than 1 ppm over a 3 hour exposure period but induced significantly greater reductions over 17 hours (*table 1*). Tissue swelling is appreciable after 17 hours in ethylene and, since tissue extensibility is a function of tissue diameter, this factor would reduce tissue extension quite apart from changes in the cell walls. However, swelling in 1 ppm and 10 ppm ethylene is identical, so that increases in tissue diameter cannot explain the greater effectiveness of higher ethylene levels: some additional effect relating to changes in the cell walls must be involved.

Table 1 also shows the reduction in elastic extensibility, E , (reduction in length on immediate plasmolysis) after ethylene pretreatment: the effects are proportional to both length of pretreatment and ethylene concentration. Ethylene appears to cause a stiffening or setting of the cell walls, which reduces elastic extensibility; it would be interesting to examine the effects of low temper-

Table 1. Effect of ethylene on wall extension and elastic extensibility. Pea seedlings pretreated with ethylene or kept in air, and elastic (reversible) and plastic (irreversible) extension measured after incubating 10 mm apical segments for 2 hours in water. Elastic extensibility determined as the reduction in length of 10 mm segments upon immediate plasmolysis. Figures in brackets show the % reduction due to ethylene.

Ethylene ppm	Hours	Plastic Extension	Elastic Extension	Elastic Extensibility
0		0.508 ±0.059	0.922 ±0.065	0.542 ±0.037
1	3	0.244 ±0.055 (-52.0%)	0.630 ±0.040 (-31.7%)	0.316 ±0.059 (-41.7%)
0		0.558 ±0.066	0.920 ±0.074	0.556 ±0.061
1	3	0.072 ±0.062 (-87.7%)	0.394 ±0.073 (-57.2%)	0.250 ±0.057 (-65.0%)
0		0.702 ±0.100	0.896 ±0.123	0.342 ±0.067
10	17	0.370 ±0.084 (-47.3%)	0.500 ±0.110 (-44.6%)	0.182 ±0.068 (-46.8%)
0		0.576 ±0.113	0.876 ±0.118	0.462 ±0.110
10	17	0.000 ±0.090 (-100%)	0.234 ±0.093 (-73.3%)	0.000 ±0.096 (-100%)

ature or anaerobiosis on this process in order to determine whether metabolic reactions are involved.

When elastic or plastic extension of air-grown control tissue is plotted against the osmotic concentration of the external solution, OP_e , both are found to increase linearly with OP_e above the point of incipient plasmolysis (*fig. 8*). Ethylene pretreatment decreases RE and IE for all values of OP_e , but for RE the slope of the line decreases sharply for mannitol concentrations of 0.2 M or less. Ethylene is, therefore, more effective in reducing elastic extension above a certain turgor value which ranged between 0.1 and 0.2 M mannitol in different experiments. The situation bears some resemblance to that found in *Avena* coleoptiles incubated with auxin (CLELAND 1959); auxin increased elastic (and also plastic) extension only above a critical turgor value.

3.3. Effects of ethylene on osmotic potential, water potential and turgor

Typical results for a 3 hour pretreatment with 1 ppm ethylene are shown in *fig. 9* and three effects of ethylene should be noted; a slight decrease in osmotic potential, π , from (-) 0.364 to (-) 0.355 molal mannitol; an increase in water potential, ψ , from (-) 0.220 to (-) 0.266 molal mannitol; and a consequent reduction in turgor pressure, P, from 0.144 to 0.087 molal mannitol. Average percent changes for these 3 parameters in 3 or 5 replicate experiments are given in *table 2*, where it is also shown that the apparent lowering of turgor in ethylene is not a transitory effect since no recovery is detectable after a 17 hour pretreatment. However, bearing in mind that the plasmolysis method gives

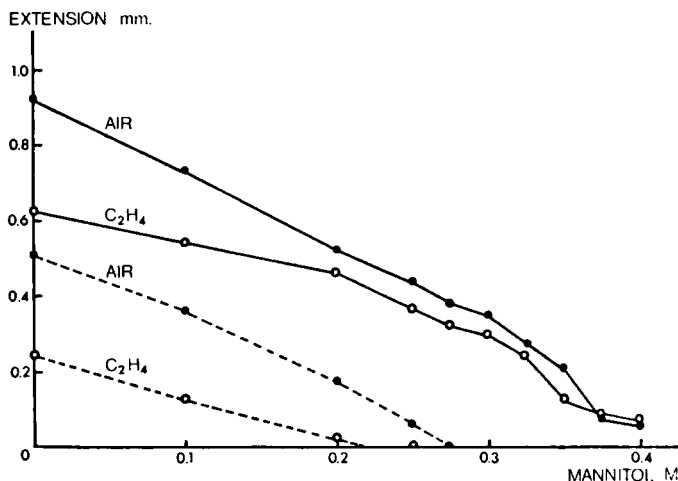


Fig. 8. Effect of ethylene on elastic and plastic extension at different OP_e values. — elastic (reversible) extension. ---- plastic (irreversible) extension. Ethylene pretreatment at 1 ppm for 3 hours. 10 mm apical segments excised and incubated in solutions of mannitol 0–0.4 M for 2 hours.

only an upper limit of turgor during growth, and that turgor may be substantially below the equilibrium value during rapid growth (RAY & RUESINK 1963), then the apparent reduction in turgor found in the slowly-growing tissue of peas exposed to ethylene may be an overestimate. It would clearly be of great value if other methods could be used to substantiate these findings.

Table 2. Effect of ethylene on osmotic potential, water potential and turgor pressure. Intact seedlings pretreated with 1 ppm ethylene or kept in air. Plasmolysis experiments carried out with 10 mm apical segments. Each experiment repeated 5 times and results show the average % change in ethylene \pm standard deviation.

Hours in Ethylene (1 ppm)	% Change		
	Osmotic Potential	Water Potential	Turgor Pressure
3	-2.55	+16.80	-33.2
3	-2.55 \pm 1.97	\pm 16.80 \pm 5.73	-33.2 \pm 4.25
17	-1.87 \pm 1.77	\pm 15.58 \pm 2.35	-43.95 \pm 4.80

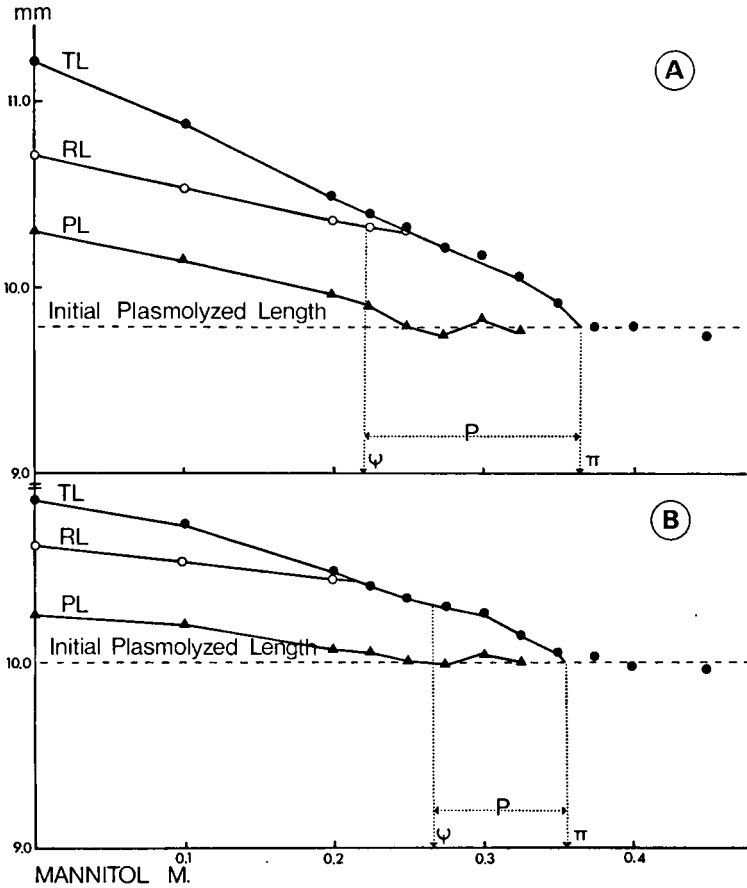


Fig. 9. Effect of ethylene on osmotic potential (π), water potential, ψ , and turgor pressure, P. Pea seedlings kept in air (A), or pretreated for 3 hours in 1 ppm ethylene (B). 10 mm apical segments excised and incubated in mannitol solutions, 0–0.45 M.

Total length attained = TL.

Length after subsequent plasmolysis = PL.

Initial plasmolyzed length (IPL) = length after immediate plasmolysis.

RL = reversible segment length, TL – (PL – IPL).

4. DISCUSSION

Altered patterns of pea cell growth in ethylene have been related to three factors: deposition of longitudinal microfibrils, reduced extensibility of the cell walls, and reduced turgor. The switch from transverse to longitudinal microfibril deposition corresponds closely in time with the onset of ethylene-induced lateral cell expansion, and strongly suggests a causal connection between the two. BURG & BURG (1968) made a similar suggestion but held that deposition

of discrete bands of longitudinal thickening on the outside of the cell wall were the main cause of lateral growth in ethylene. I have found diffuse longitudinal deposition over the entire wall surface, as VEEN (1970b) found for pea stem segments incubated with IAA and sucrose. Moreover Veen showed, using the electron microscope, that this kind of deposition was on the inner surface of the cell wall, and in a recent paper by APELBAUM & BURG (1971), which appeared after my work was completed, EM observations also showed longitudinal microfibrils on the inner surface of the cell wall after 24 hours in ethylene. Apelbaum & Burg suggest that microfibril re-orientation arises as a *result* of strains set up by lateral expansion whereas I concur with VEEN (1970b) in the view that lateral cell expansion itself results from the re-orientation of microfibrils.

If longitudinal microfibril deposition is the main cause of lateral growth in ethylene, it follows that cell wall synthesis is a necessary condition for such lateral growth. There is some evidence to support this view. When apical segments from air-grown etiolated peas are incubated in the presence of ethylene then, although growth in length is always inhibited, lateral swelling occurs only when sucrose is present in the medium (RIDGE unpublished), and WINTER (1966) has shown that cellulose synthesis in such segments is limited by the availability of sugars. Similarly, swelling of intact peas in ethylene is strongly inhibited by removal of the cotyledons (MICHENER 1938), again suggesting that a supply of sugars for cell wall synthesis could limit lateral cell expansion.

When we consider ethylene inhibition of the rate of cell expansion, R , then the situation is far from clear. Both the fall in WE_x and the fall in P may be contributing factors, but which is the more important is, at present, impossible to say. Moreover, wall extensibility and turgor cannot be regarded as totally independent variables governing R . In single cells of *Nitella*, for example, a relatively small decrease in turgor (20% or so) reduces growth rate and WE_x temporarily to zero followed by a gradual rise in WE_x (GREEN & KING 1966). During long-term exposure to ethylene yet another factor must be considered: the increase in thickness of cell walls revealed by electron microscope studies (OSBORNE, RIDGE & SARGENT 1970). This thickening is detectable within 24 hours, and since WE_x is inversely proportional to wall thickness (LOCKHART 1965) it follows that WE_x must decrease. However, it seems likely that increased wall thickness is a result, rather than a primary cause, of the early decrease in growth rate, there being no evidence that ethylene alters the rate of wall synthesis during this period.

How then can the early rapid decline in the potential for plastic and elastic extension in ethylene be explained? If one assumes that increases in wall thickness, deposition of longitudinal microfibrils and increases in tissue diameter are initially of minor importance, there seem to be two main possibilities. First, as mentioned above, the apparent reduction in turgor may cause a reduction in wall extensibility; but considering other higher plant systems (CLELAND 1959) the fall in WE_x seems too large to be accounted for solely in this way. Secondly, ethylene may induce active stiffening of cell walls. In many systems

ethylene and auxin are antagonistic in their effects (RIDGE & OSBORNE 1969; OSBORNE, RIDGE & SARGENT 1970); thus it is possible that ethylene directly inhibits the plasticising action of auxin on pea cell walls, or, alternatively, induces some rapid change in wall structure (cross linking?) such that sensitivity to auxin is reduced.

Perhaps the surprising feature of ethylene-treated pea shoots, especially when exposed to high concentrations of the gas, is that cell growth occurs at all: despite an apparent reduction in turgor and highly inextensible cell walls, cell expansion does take place albeit very slowly and over a much longer period than for controls in air. In summary, the results show that within 3 hours of exposure to ethylene there is a general slowing down of cell expansion, which is accompanied by a reduction in cell wall extensibility (both plastic and elastic) and a fall in turgor, apparently resulting from an increase in water potential and a slight decrease in osmotic potential. After 3–6 hours in ethylene, cortical cells begin to expand in a lateral direction and this is accompanied by continuous deposition of longitudinal microfibrils on the cell walls. It need scarcely be stressed that changes in cell wall properties and structure appear to play a vital role in the control of cell growth by ethylene.

ACKNOWLEDGEMENTS

This work was carried out in the laboratory of Prof. Dr. M. H. van Raalte, University of Groningen, for whose hospitality, help and advice I am deeply grateful. I wish to thank also Dr. B. M. Veen and Dr. D. R. Kreger for many useful discussions, and finally, the Governing Body of Lady Margaret Hall, Oxford, for the award of a Susette Taylor Travelling Research Fellowship.

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