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*Short Communication*

# Altered Cell Microfibrillar Orientation in Ethylene-treated *Pisum sativum* Stems

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The roots and stems of plants typically swell when they are exposed to ethylene (1–3, 5, 8), or supraoptimal auxin (2, 3, 5, 8, 20), and root hairs are induced to form (1, 5, 6, 8). When supraoptimal IAA causes pea subapical stem sections to swell, the optical birefrigence pattern of the cell wall changes, and newly deposited wall microfibrils tend to be oriented in a longitudinal rather than radial direction (20, 21). Ethylene also alters the birefrigence of pea stem subapical cell walls when it causes the tissue to swell (3, 4, 9), producing a characteristic banded pattern. This same pattern occurs in pea stem tissue which has swelled after treatment with supraoptimal IAA (4, 9). The similar effects of ethylene and supraoptimal IAA on pea tissue have been attributed to the fact that supraoptimal IAA induces ethylene formation (2, 3).

According to the multinet hypothesis (13, 19), cells elongate predominantly in a longitudinal direction because of the restraining influence of newly deposited, radially oriented microfibrils. If a cell is to expand radially under the influence of ethylene or supraoptimal IAA, newly deposited microfibrils no longer should be oriented in a radial direction. The present communication describes the effect of ethylene on microfibrillar organization in various regions of the cell wall, and shows it to be similar to that resulting from auxin treatment (20, 21).

Pea seedlings were grown in vermiculite for 7 days in complete darkness and then treated continuously in a desiccator with 50 $\mu$l/liter ethylene for 24 to 96 hr, except for one complete aeration each day. Within 24 hr the seedlings displayed a typical triple response to ethylene and thereafter swelling intensified progressively throughout the 96-hr experimental period. Subapical stem pieces were fixed with 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, for 12 hr, rinsed with buffer, postfixed with 1% osmium tetroxide in phosphate buffer for 1 hr, dehydrated with an ethanol series, and embedded in Epon-Araldite epoxy resin. After sectioning, the tissue was stained with uranyl acetate and lead citrate. For light microscope studies, freehand sections were made from swollen subapical tissue without fixation, and the sections were stained with 0.05% toluidine blue O (16).

The effects of ethylene, IAA, kinetin, BIA,[2] BA, and colchicine on the growth of excised 1-cm subhook sections from 7-day-old control plants were studied singly and in combination by incubating lots of 10 sections in 125-ml Erlenmeyer flasks containing 10 ml of 2% sucrose, 5 mM potassium phosphate buffer, pH 6.8, and 5 mM cobalt chloride solution plus the added hormone or hormones. All manipulations were carried out under dim green light, the flasks were sealed with vaccine caps, ethylene injected with a syringe when required, and tissue incubated in the dark with gentle shaking for 18 hr at 23 C. Ethylene production was then determined by gas chromatographing 5 ml of air from each flask (2), and the tissue was weighed and measured. The concentrations of colchicine, BIA, kinetin, and BA used in these studies were selected to provide maximum swelling with little or no growth inhibition; higher concentrations inhibited growth markedly and did not induce significantly more ethylene production.

Control tissue from intact seedlings had the wall structure typical of elongating cells (Fig. 1a), with radially oriented microfibrils at the inner wall surface, more randomly distributed microfibrils in the center of the wall, and longitudinally oriented microfibrils in the outer region of the wall. The cell depicted in Figure 1a is an epidermal cell, but similar wall structure was noted in cortical and pith cells. After 24 hr of treatment with ethylene a very different wall structure appeared uniformly in epidermal, pith, and cortical cells of the swollen subapical zone. This structure is illustrated in Figure 1b. The microfibrils at the inner surface of the wall are oriented in a predominantly longitudinal direction, whereas those in the center of the wall are radially oriented. The newly deposited longitudinal microfibrils at the inner surface should restrict longitudinal expansion and allow the cell to grow in a radial direction. Those microfibrils originally laid down in a radial direction before ethylene was applied are now located nearer to the center of the wall; they are maintained in a radial configuration by the forces created during radial cell expansion. The microfibrils in the outer portion of the wall had already been pulled into a predominantly longitudinal orientation before ethylene was applied. However, the gas greatly slows cellular elongation, and therefore any further tendency for these fibrils to be pulled into a longitudinal pattern. Instead, because of the forces created during radial expansion, these microfibrils tend now to be pulled into a radial orientation. The wall microfibrillar pattern observed in control tissue was not changed during 48 hr of additional growth of the seedlings; the pattern observed in ethylene-treated tissue was similar at 24 and 48 hr. The experiment was repeated 3 times, and in each case the subapical zones from five control and five ethylene-treated seedlings were fixed and prepared for electron microscopy. Several randomly selected sections were examined from each subapex, and without exception the ethylene-treated and control cells had the distinctly different wall structures depicted in Figures 1a and 1b.

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[2] Abbreviations: BIA: benzimidazole; BA: benzyladenine.

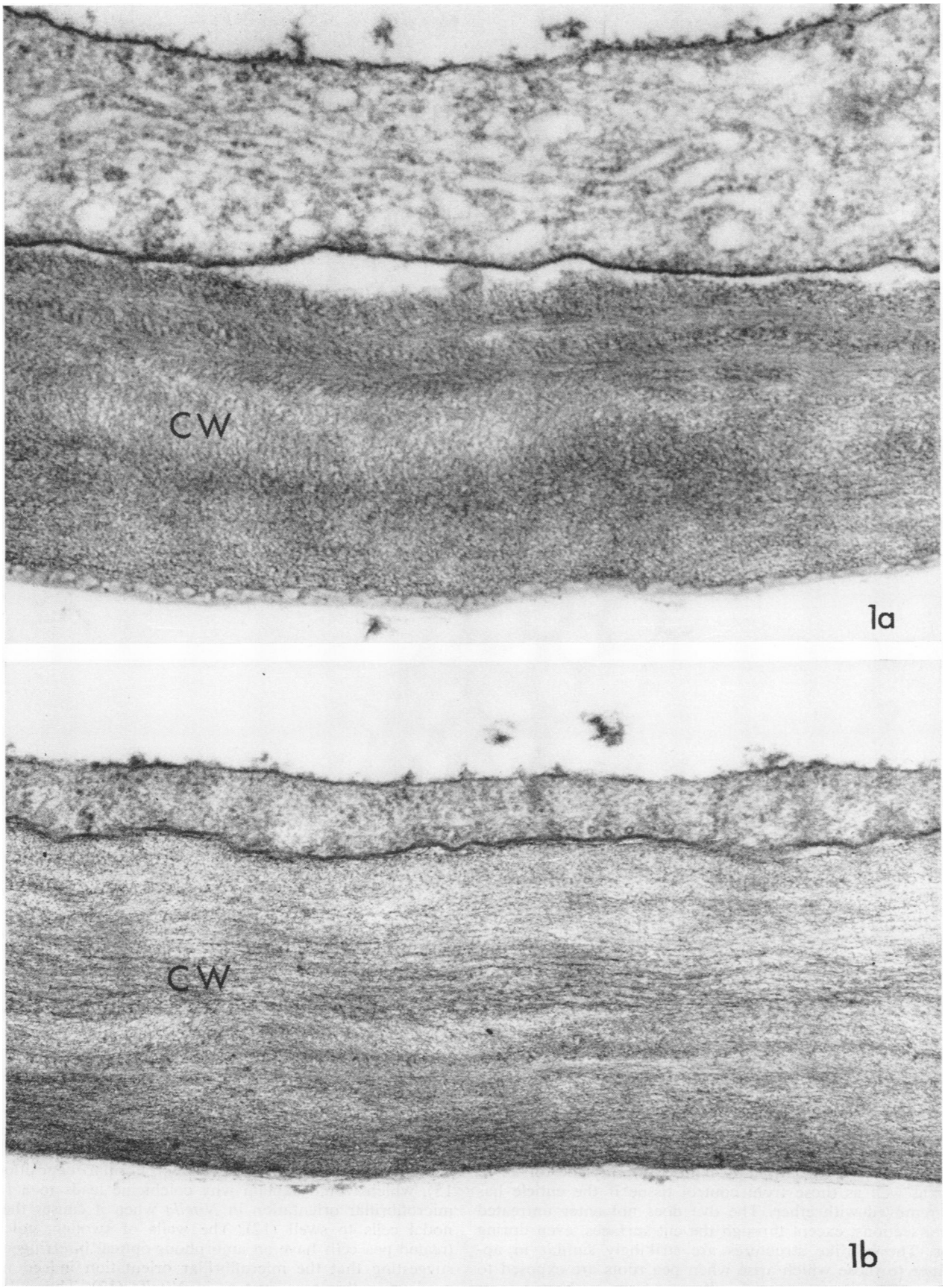


FIG. 1. Cross sections through the subapical zone of pea stems showing epidermal cells of control plants (a) and plants treated with ethylene for 24 hr (b). Note the different microfibrillar patterns in the cell walls (CW). × 78,000.

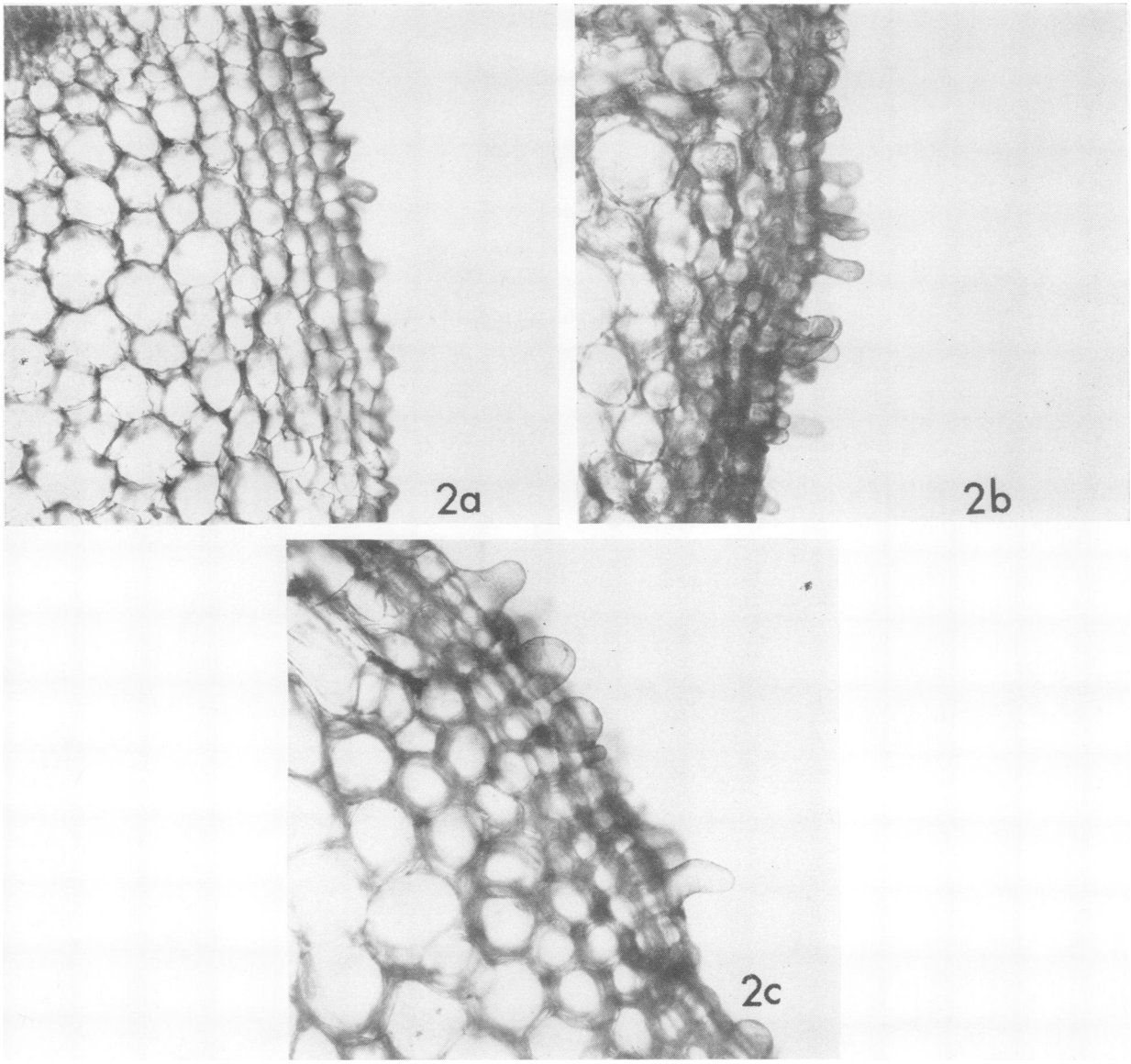


FIG. 2. Cross section through the subapical zone of pea seedlings treated with ethylene for 48 (a), 72 (b), and 96 (c) hr. Note the progressive increase in size of the epidermal and cortical cells, and development of hairlike structures. × 105.

All the tissues of the stem subapex continue to expand radially for at least 96 hr in the presence of ethylene, whereas in the absence of the gas cells stop growing in 24 to 48 hr (4). After 48 hr the epidermal cells, because of the changed microfibrillar orientation, bulge out into hairlike structures which continue to develop for at least an additional 48 hr (Fig. 2a–c). These hairs break through the cuticle of the stem, causing the tissue to develop an extreme permeability toward water-soluble substances such as various dyes. Methylene blue, for example, penetrates rapidly through the epidermal layer and within 5 to 10 min completely permeates both sections taken from plants exposed to ethylene for 24 to 48 hr as well as those from control tissue if the cuticle has been removed with ether. The dye does not enter untreated control sections, except through the cut surfaces, even during 24 hr. The hairlike structures are strikingly similar in appearance to those which arise when pea roots are exposed to ethylene (5). Only epidermal cells can form such structures because all other tissues of the stem and root are restrained in their radial expansion by subtending cells.

Numerous agents cause pea stem sections to swell; for example, relatively high concentrations of BIA (3, 11, 18), kinetin and other cytokinins (10), colchicine, and vinblastine sulfate (4). When BIA causes swelling, an optical birefringence pattern arises (18) which is indistinguishable from that observed after ethylene, supraoptimal IAA, kinetin, or BA treatment (3, 4, 9). Like IAA and ethylene, BIA alters the orientation of the cell wall microfibrils (18). Colchicine and vinblastine sulfate act by a different mechanism, however. They prevent polymerization of microtubules in animal cells and colchicine also is known to do so in plant cells (15, 17). The microtubules may orient microfibrillar deposition (14, 15), which would explain why colchicine leads to a random microfibrillar orientation in *Nitella* when it causes the internodal cells to swell (12). The walls of swollen, colchicine-treated pea cells have an amorphous optical birefringence (9), suggesting that the microfibrillar orientation indeed may be random in this case, just as in *Nitella* (12). Thus all agents which cause swelling have in common the property of changing microfibrillar orientation. In addition they all retard pro-

line incorporation into a pronase-extractable fraction of the cell wall of etiolated pea subapical tissue (4, 9), and fail to cause swelling or effect proline incorporation if the tissue has been treated with red light (9). They differ in that auxins, ethylene, BIA, and BA apparently redirect microfibrillar orientation while colchicine leads to a random deposition.

It has been proposed that the swelling caused by kinetin is due to induced ethylene production (10), for 10 to 200 $\mu$M kinetin doubles the rate of ethylene production in pea tissue (1, 10). However, the dose response curve for ethylene-induced swelling of pea subapical tissue is a log-function of ethylene concentration so that it is necessary to increase the ethylene content of the tissue about 50-fold in shifting from a threshold to complete response (2). Consequently, although kinetin and BA slightly increase the rate of ethylene production (Table I), this cannot be the cause of the intense swelling response which ensues in each case. This point is well illustrated by the behavior of tissue exposed to 1 $\mu$M IAA, for such tissue produces more ethylene than that grown in the presence of 0.2 $\mu$M IAA plus added cytokinin and yet it does not swell, whereas the kinin-treated tissues swell as intensely as if exposed to 1 ml/liter ethylene (Table I). In order to induce sufficient ethylene production to cause a substantial, nearly complete swelling response it is necessary to add 10 $\mu$M IAA and raise the rate of ethylene evolution to a value more than 50 times higher than that caused by 1 $\mu$M IAA or any of the other treatments. In the presence of 10 $\mu$M IAA, added ethylene causes very little additional swelling; when 100 $\mu$M IAA are used and the rate of ethylene production increased still further, added ethylene is totally without effect on the growth of the tissue, indicating that the swelling response caused by auxin-induced ethylene finally is complete at this rate of ethylene production (Table I). Yet in the presence of the same amount of auxin-induced ethylene or 1 ml/liter added ethylene the other agents still are able to produce a synergistic or additive effect. For example, 10 $\mu$M kinetin cause very intense additional swelling when added in the presence 10 $\mu$M IAA, without any substantial stimulation of ethylene production, and in the presence of 0.2 $\mu$M IAA and 1 ml/liter ethylene, added BIA produces more swelling than ethylene or either compound alone (Table I). Moreover, while concentrations of ethylene able to cause swelling prevent a spontaneous curvature from developing in excised pea sections during the first 2 to 3 hr of incubation (2) and supraoptimal IAA has the same effect, neither BIA, BA, colchicine, or kinetin induce sufficient ethylene production to effect this response to the gas. These data demonstrate that kinins, BIA, and colchicine must cause radial cellular expansion by some mechanism other than ethylene action.

The changes in wall microstructure induced by ethylene are accompanied by biochemical changes in cell wall composition. Thus ethylene reduces $^{14}$C-proline incorporation into extensin (4, 9); this may prevent cross linking and rigidification of the wall (7) which would explain why wall expansion and incorporation of $^{14}$C-glucose into the wall continue. Control tissue, on the other hand, stops growing within 1 to 2 days and at that time the walls become rich in hydroxyproline (7), and still incorporate large amounts of $^{14}$C-proline but only small amounts of $^{14}$C-glucose. When cells are removed from ethylene the process is reversed; $^{14}$C-proline incorporation increases, $^{14}$C-glucose incorporation decreases, and growth quickly ceases. These biochemical changes will be the subject of a future communication.

Supraoptimal IAA and ethylene both cause newly deposited microfibrils to be deposited at the inner wall surface in a predominantly longitudinal direction (20, 21) (Fig. 1b). This finding adds further credence to the suggestion (2, 3) that IAA-induced swelling results from enhanced ethylene production rather than a direct action of IAA on growth. Radial expansion caused by kinetin, BIA, BA, and colchicine also involves changes in wall structure, but these are not due to induced ethylene production.

Table I. *Effect of IAA, BIA, BA, Ethylene, and Colchicine on Growth and Ethylene Production of Pea Subhook Sections*

Sections were incubated for 18 hr. Growth measurements are average values of 4 to 16 replicates; ethylene production values are averages for 4 to 12 replicates.

| IAA Concentration | Treatment | Increase | | $C_2H_4$ Production for 10 Sections |
|---|---|---|---|---|
| | | Length | Weight | |
| $\mu$M | | % | % | $\mu$l |
| 0 | None | 60 | 75 | 1.4 |
| | Kinetin, 10 $\mu$M | 48 | 75 | 2.1 |
| | $C_2H_4$, 1 ml/liter | 36 | 73 | — |
| 0.2 | None | 110 | 140 | 2.2 |
| | Kinetin, 100 $\mu$M | 46 | 116 | 3.8 |
| | BA, 100 $\mu$M | 45 | 110 | 3.6 |
| | Colchicine, 1 mM | 56 | 144 | 1.8 |
| | BIA, 2 mM | 67 | 140 | 1.9 |
| | $C_2H_4$, 1 ml/liter | 59 | 124 | — |
| | BIA, 2 mM, and $C_2H_4$, 1 ml/liter | 44 | 122 | — |
| | | | | — |
| 1 | None | 120 | 160 | 5.3 |
| | $C_2H_4$, 1 ml/liter | 52 | 144 | — |
| 10 | None | 60 | 160 | 53 |
| | Kinetin, 10 $\mu$M | 30 | 152 | 58 |
| | $C_2H_4$, 1 ml/liter | 52 | 155 | — |
| 100 | None | 50 | 150 | 110 |
| | $C_2H_4$, 1 ml/liter | 52 | 147 | — |

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