Modification by Ethylene of the Cell Growth Pattern in Different Tissues of Etiolated Lupine Hypocotyls

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ABSTRACT
The influence of ethylene on growth in etiolated lupine (*Lupinus albus* L.) hypocotyls was studied in ethephon-treated plants. Ethephon reduced the length and increased the diameter of hypocotyls. At the end of the hypocotyl growth period (14 days), the fresh weight was reduced by 53%, and the dry weight was reduced by 16%. Thus, ethylene reduced water uptake in the tissues to a greater extent than the incorporation of new materials. Light microscopic measurements showed that the thickness of tissues was stimulated by ethylene, the vascular cylinder and cortex exhibiting greater increases (55 and 45%, respectively) than pith (28%) or epidermis (12%). Ethephon modified the cell growth pattern, stimulating lateral cell expansion and cell wall thickness, while reducing cell elongation. The response to ethylene varied in the different tissues and was higher in cortex and pith cells than in the epidermis cells. The ethylene-induced cell expansion in the cortex varied according to the localization of cells in the tissue: the central and subepidermal layers showed little change, whereas the innermost layers exhibited the greatest increase. Electron microscopy revealed that ethylene increased both the rough endoplasmic reticulum and dictyosomes, suggesting that ethylene stimulated the secretion of cell wall materials. In untreated seedlings, the pattern of cell growth was similar in cells from the epidermis, cortex, and pith. The final cell size varied along the hypocotyl, the cells becoming shorter and broader the closer to the basal zones of the organ.

Several recent studies have confirmed that growth in heterogeneous multicellular systems, such as coleoptiles or hypocotyls, involves the cooperation of functionally different tissues (7, 8, 18, 19). The phenomenon of tissue tension, first recognized by Sachs (22), has been intensively studied during the last few years, and its role in the growth of coleoptiles and hypocotyls has become more clear. It has been proposed that tissue tension and the IAA-induced extensibility of the outer epidermal wall constitute the limiting processes for organ growth (7). The localization of IAA-responsive cell growth (7) as well as the putative IAA receptor in the epidermis (5, 10) strongly supports the model.

According to Penny and coworkers (18, 19), this model may apply to the lupine hypocotyl. In this organ, a recent study (24) demonstrated that the IAA polarly transported in the stele migrated sideways to the outermost tissues, as was also shown for maize and oat coleoptiles (2). Furthermore, morphometric studies showed that variations in cell growth rate as well as in cell wall thickness correlated with the variations of IAA content during lupine hypocotyl growth (13, 14, 23). These results suggest the participation of IAA in the control of cell growth in lupine hypocotyls.

In addition to IAA, the influence of ethylene in the growth of stems is widely documented (for reviews, see refs. 1, 3, 15-17, and 21). Cell growth response to ethylene depends on the type of cell (16) as well as the cell's sensitivity to ethylene (12, 15, 21). Although some data suggest that cell responsiveness to ethylene could be mediated by the endogenous level of IAA (15, 26), there is little information concerning the role of the different tissues in the ethylene-mediated growth of stems, unlike the case of IAA, which has been extensively studied.

In a previous paper, we reported that lupine hypocotyl growth was inhibited by exogenous ACC2 and ethephon, the response being dependent on the concentration, age of the seedlings, and the localization along the hypocotyl (12). In addition, an extensive study showing variations in ethylene production, ACC content, and ethylene-forming enzyme activity during hypocotyl growth was carried out recently (20). From the above, some role for the ethylene produced by etiolated lupine hypocotyls might be suspected in cell growth.

In the present paper, we describe the influence of ethylene on cell growth in different tissues (epidermis, cortex, vascular tissues, pith) and the cell growth pattern exhibited by these tissues during the normal growth of etiolated lupine hypocotyls. Bearing in mind previous studies, we discuss the role of the IAA-ethylene interaction on the control of this cell growth pattern.

MATERIAL AND METHODS

Growth of Plants

Lupine seeds (*Lupinus albus* L. cv Multolupa) were imibed for 24 h in water or, alternatively, in an aqueous solution of 6.6 mM ethephon (commercial Ethrel, 48% 2-chloroethylphosphonic acid) and germinated in damp vermiculite at 25°C in darkness. Length, fresh weight, and dry weight of the hypocotyls in control and treated plants were measured on a five-plant uniform sample from 3- to 17-d-old seedlings. To study the cell growth pattern in control plants, 3-d-old hypocotyls were marked with ink delimiting four 5-mm-long zones (Fig. 6).

### Footnotes

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Microscopy

For EM, tissue segments (2 mm long) were excised 10 mm below the cotyledons of 6-d-old plants (Fig. 1). For light microscopy, 2-mm segments were cut from specified locations along the hypocotyl (Figs. 1 and 6). The tissue was fixed at 4°C for 2 h in 3% glutaraldehyde, washed several times in sodium cacodylate buffer (0.1 m, pH 7.3), and postfixed for 4 h in 1% OsO₄. Sucrose was added to washing and postfixation solutions to obtain the same osmolality as in the fixation solution. The tissue was washed in buffer and 10% aqueous solutions of ethanol for 1 h, stained for 1 h in 2% aqueous uranyl acetate, dehydrated in a gradient of ethanol, and embedded in epoxy resin as described by Luft (11). Semithin and ultrathin sections were cut using a Reichert-Jung ultramicrotome. Semithin sections were stained with toluidine blue and viewed with a light microscope (Photomicroscope II; Carl Zeiss, Oberkochen, Germany). Ultrathin sections (50–70 nm) were cut from blocks, covered with Forwar, stained in uranyl acetate and lead citrate, and viewed with a Zeiss EM 10 electron microscope (Carl Zeiss).

Measurement of Cell Growth Parameters

Cell size and tissue thickness were measured on semithin sections using the light microscope with an ocular micrometer. Cell length was measured in longitudinal sections, and cell diameter (the cell wall excluded) and tissue thickness were measured in transverse sections. In the study of the influence of ethylene, sections were excised from the middle zone of 6-d-old hypocotyls (Fig. 1). However, to study the cell growth pattern in control plants, three regions from different locations along the hypocotyls of 3-, 6-, and 12-d-old seedlings were used. From each region, a longitudinal section (2 cm) and a transverse section (2-mm radius × 2-mm length) were cut from the upper and lower end. The study was done twice.

The different cell types of the vascular cylinder were identified by EM. For each ultrathin section, five to 10 electron micrographs were obtained. Cell diameter and cell wall thickness were measured directly on the electron micrographs (final magnification between ×4,250 and ×15,250). Data are expressed as mean values of 25 to 30 independent measurements ± SE.

RESULTS AND DISCUSSION

Influence of Ethylene on Hypocotyl Growth

Ethephon treatment caused a reduction in the length and an increase in the diameter of the hypocotyls (Fig. 1). Hypocotyl growth from untreated seeds showed sigmoidal kinetics, the linear period starting after day 3 and the growth ceasing after day 12 (Fig. 2A). In ethephon-treated seeds, normal growth occurred during the first 3-d period, after which the growth rate was very slow (Fig. 2A). Ethephon reduced the final hypocotyl length by 85%, whereas the hypocotyl diameter increased by about 40% (Fig. 1).

The variation in weight to length ratio in control plants (Fig. 2B) showed an increase in fresh weight and a slight decrease in dry weight from 3 to 7 or 10 d, both stabilizing after this period. As has been discussed (13), at the beginning of cell elongation, there is an increase of water content in the cell without a proportional increase in cell size, probably due to an increase in the vacuolar size; during later stages of elongation, water uptake occurs simultaneously with the incorporation of new materials into the elongating cells. On the other hand, ethephon produced a continuous increase in both the fresh weight to length and dry weight to length ratios, as a consequence of the radial expansion of the hypocotyl (Fig. 2B). At the end of the growth period, the fresh weight of the ethylene-treated tissue was reduced by 53% relative to controls and the dry weight was reduced by 16%. Thus, ethylene inhibited water uptake to a greater extent than the incorporation of new materials into the tissues.

Influence of Ethylene on Cell Growth

Measurements performed in the light microscopic preparations showed that the tissue thickness (epidermis, cortex, vascular cylinder, and pith) was greater in ethylene-treated plants than in control plants (Table I). The vascular cylinder and cortex exhibited greater increases (55 and 45%, respectively) in thickness than pith (26%) or epidermis tissues (12%) (Table I).

Cells from comparable locations in control compared with treated hypocotyls showed larger diameter and shorter length in treated seedlings (Fig. 3). According to the Osborne hypothesis (16), this ethylene response corresponds to type I cells, in which ethylene promotes expansion and inhibits elongation. In addition, cell wall thickness and, consequently, cell wall surface and volume increased in treated seedlings.
greater than the highest plants (Fig. 4). Also, (data seedlings. Points, and A, dry weight) larger than hypocotyl length in similar ethylene (Fig. 3). The response to ethylene in cortex and pith was greater than in epidermis cells. EM preparations revealed that ethylene also increased cell expansion and cell wall thickening of the different cell types in the vascular cylinder (Table II).

The effect of ethylene on cell diameter in the cortex varied according to the radial location of cells in the tissue: the larger cells from the central layers and those adjacent to the epidermis showed less change than the intermediate layers, whereas the innermost layers showed the greatest increase in diameter (Fig. 4). Also, radial variation in cell diameter in control plants is shown in Figure 4, with the central layers reaching the highest values. Similar results were observed in the pith cells (data not shown). Because the number of cell layers was similar in treated and control plants, the above results indicate that the ethylene-induced thickness of the different tissues, shown in Table I, was caused by an increase in cell expansion of the constituent cells.

From the present results it is concluded that the cell growth response to ethylene depends on the type of cell as well as the location of the cells in the tissues.

Electron micrographs of cortex and pith cells revealed several ultrastructural differences between control and treated plants (Fig. 5). Both in cortex and pith, ethylene treatment seemed to increase the cytoplasmic density. As previously reported, the most significant change in cell structure was observed in the RER, which increased markedly in ethylene-treated plants. In addition, dictyosomes were more abundant in treated plants. Because both RER and dictyosomes are involved in the formation of the cell wall, the above

<table>
<thead>
<tr>
<th>Table I. Influence of Ethephon Treatment on Tissue Thickness</th>
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<tr>
<td>The thickness of tissues was measured on light microscopic preparations of transverse sections through the middle zone of 6-d-old hypocotyls (Fig. 1). Data correspond to the means of 20 measurements ± SE. Variation in relation to the control is indicated.</td>
</tr>
<tr>
<td>Tissue</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Epidermis</td>
</tr>
<tr>
<td>Cortex</td>
</tr>
<tr>
<td>Vascular cylinder</td>
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<tr>
<td>Pith</td>
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</table>
results suggest that ethylene stimulated the secretion of cell wall materials. In fact, Figure 5 clearly shows that the number of membrane vesicles that are near or fusing with the plasmalemma was higher in treated plants. These data are in agreement with the high dry weight to length ratio in ethephon-treated plants as compared with control plants (Fig. 2B). Moreover, data in Figure 3 indicate an increase in the cell wall volume of pith and cortex cells after ethylene treatment.

**Pattern of Cell Growth in Control Plants**

In untreated seedlings, the pattern of growth in cells located at different positions along the hypocotyl showed similar features in the three tissues studied (epidermis, cortex, and pith) (Fig. 6). In each zone of the hypocotyl (apical, middle, and basal), the cell length increased and the cell diameter showed little change during hypocotyl ageing. On the other hand, the final size at the end of the growth period varied along the hypocotyl; the cells becoming shorter and broader from the apical to the basal zones. In spite of the fact that cell diameter increased basipetally, no significant variation in hypocotyl diameter was found along the organ during the growth period. A morphometric study revealed that cell wall thickness in the apical cells was twice that in the basal cells at the end of the growth period (14); i.e. the thinner apical cells had thicker cell walls. Although longitudinal variation of cell diameter in the vascular cylinder was not measured, the above data may help explain the consistency of hypocotyl diameter along the organ.

As shown in Figure 6, cell elongation in the cortex and pith was synchronized, both reaching similar final lengths. On the other hand, epidermal cells exhibited a shorter final length than other cells in the same zone. This means that the number of cells per column was greater in the epidermis than in the other tissues. In longitudinal sections from the middle zone of 6-d-old hypocotyls, some occasional mitosis was observed in epidermal cells but not in cortex or pith cells. Although the stimulus for cell division is unknown, an increase in the number of epidermal cells seems necessary for the maintenance of hypocotyl elongation.

Because present results demonstrate that ethylene reduces cell elongation and stimulates lateral cell expansion as well as cell wall thickening in lupine hypocotyls, the question is whether endogenous ethylene might be involved in the hormonal regulation that produces this pattern of cell growth in untreated controls. Recent data showed that ethylene production and ACC content varied along the lupine hypocotyl as well as during hypocotyl ageing, the highest values appearing in the apical region and in the youngest seedlings (20). However, sensitivity to ethylene-induced growth inhibition in lupine hypocotyl depended on the cell growth status, the youngest actively growing apical cells being less sensitive than the older slowly growing basal cells (12). This could explain the variation of cell size shown in Figure 6.

In addition to ethylene, the role of IAA on cell growth of lupine hypocotyls must be considered. As was reported previously (14), apical cells maintained higher IAA concentrations than basal cells during the elongation period. This should explain the greater final length of apical cells. Furthermore, the longitudinal gradient of cell wall thickness was positively correlated with the endogenous IAA content, the highest values of both being located in the apical growing cells (14). Recently, a radial gradient in cell wall thickness was found in sunflower hypocotyls, in which the epidermis and subepidermis had a thicker cell wall than the inner tissues (6). In this organ, the amount of IAA was about four times greater in the outer (epidermis and subepidermis) than in the inner tissues.

### Table II. Influence of Ethephon Treatment on the Growth of Different Cells from the Vascular Cylinder

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cell Type</th>
<th>Control</th>
<th>Treated</th>
<th>Increase Due to Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D (μm)</td>
<td>T (nm)</td>
<td>D (μm)</td>
<td>T (nm)</td>
</tr>
<tr>
<td>Phloem</td>
<td>Companion</td>
<td>5.0 ± 0.2</td>
<td>231 ± 30</td>
<td>8.0 ± 0.5</td>
</tr>
<tr>
<td>Phloem</td>
<td>Parenchyma</td>
<td>9.3 ± 0.6</td>
<td>224 ± 31</td>
<td>11.2 ± 0.7</td>
</tr>
<tr>
<td>Phloem</td>
<td>Sieve elements</td>
<td>8.6 ± 0.6</td>
<td>223 ± 17</td>
<td>12.0 ± 0.9</td>
</tr>
<tr>
<td>Xylem</td>
<td>Young vessels</td>
<td>4.0 ± 0.3</td>
<td>377 ± 24</td>
<td>6.5 ± 0.5</td>
</tr>
</tbody>
</table>
These results suggest an involvement of IAA in cell wall thickening during cell elongation. Because, according to Kutschera (6), the synthesis of cell wall material and cell elongation are independent processes, an indirect influence of IAA in cell wall thickening through IAA-induced ethylene might be suspected. Experiments showing increased ethylene production rates during IAA-induced growth in lupine hypocotyls support this view (20).

Taken together, these results suggest that the IAA-ethylene interaction is a critical factor in the control of cell growth in etiolated lupine hypocotyls. From the available data, at least three different components can be envisaged in this interaction: (a) the direct influence of both hormones, promotion of cell elongation by IAA, inhibition of cell elongation, and stimulation of lateral cell expansion by ethylene; (b) the indirect influence of IAA through ethylene synthesis induced by the high, but otherwise physiological, IAA concentrations present in growing tissues; (c) the indirect influence of IAA on ethylene growth response sensitivity. As proposed by Osborne and coworkers (15, 26), the sensitivity and specificity of ethylene in cell growth could be related to the IAA content of the cells. The longitudinal distribution of IAA (23) and the variation in sensitivity to ACC- or ethephon-induced growth inhibition along lupine hypocotyls (12) suggest that the smaller the IAA content, the greater the ethylene sensitivity, confirming the view of Osborne (15). With regard to the role of IAA and ethylene on growth, it must also be noted that, unlike the evidence for tissue specificity of IAA action, the data here indicate that ethylene affects growth in a variety of tissues.

ACKNOWLEDGMENT

We are grateful to P. Thomas for checking the English of the manuscript.
Figure 6. Variation of cell size during hypocotyl growth in control seedlings. Cells from epidermis, cortex, and pith in three regions (zone A, apical; zone M, middle; zone B, basal) of 3-, 6-, and 12-d-old hypocotyls were measured. Columns, mean values of 20–25 measurements; vertical bars, SE of cell length. SE of cell diameter was irrelevant. Pith and cortex cells from the second layer near the vascular cylinder were measured. The location of zones along the hypocotyl is indicated in the scheme, which shows the hypocotyl size at each age.

LITERATURE CITED