Effects of Ethylene on the Kinetics of Curvature and Auxin Redistribution in Gravistimulated Roots of Zea mays

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ABSTRACT

We tested the involvement of ethylene in maize (Zea mays L.) root gravitropism by measuring the kinetics of curvature and lateral auxin movement in roots treated with ethylene, inhibitors of ethylene synthesis, or inhibitors of ethylene action. In the presence of ethylene the latent period of gravitropic curvature appeared to be increased somewhat. However, ethylene-treated roots continued to curve after control roots had reached their final angle of curvature. Consequently, maximum curvature in the presence of ethylene was much greater in ethylene-treated roots than in controls. Inhibitors of ethylene biosynthesis or action had effects on the kinetics of curvature opposite to that of ethylene, i.e., the latent period appeared to be shortened somewhat while total curvature was reduced relative to that of controls. Label from applied [3H]-indole-3-acetic acid was preferentially transported toward the lower side of stimulated roots. In parallel with effects on curvature, ethylene treatment delayed the development of gravity-induced asymmetric auxin movement across the root but extended its duration once initiated. The auxin transport inhibitor, 1-N-naphthylphthalamic acid reduced both gravitropic curvature and the effect of ethylene on curvature. Since neither ethylene nor inhibitors of ethylene biosynthesis or action prevented curvature, we conclude that ethylene does not mediate the primary differential growth response causing curvature. Because ethylene affects curvature and auxin transport in parallel, we suggest that ethylene modifies curvature by affecting gravity-induced lateral transport of auxin, perhaps by interfering with adaptation of the auxin transport system to the gravistimulus.

Wheeler and Salisbury (19) reported that inhibitors of ethylene biosynthesis or action retard shoot gravitropism, and they suggested that ethylene mediates gravitropic curvature in these shoots. However, questions have been raised regarding both the statistical significance of the inhibitions observed and the specificity of the high concentrations of inhibitors used in this study (14). Harrison and Pickard (6) studied the role of ethylene in the negative gravitropic response of tomato hypocotyls. They found that gravitropism can occur without substantial changes in ethylene production and also that curvature is unaffected by raising or lowering ethylene in the hypocotyls. They concluded that ethylene does not mediate the primary negative gravitropic response of tomato shoots.

Hensel and Iversen (8) examined the role of ethylene in the gravitropic response of roots of Lepidium sativum. They found that pretreatment of the roots with 0.2 μL L⁻¹ ethylene for 6 h eliminated gravitropic curvature. Since growth was inhibited nearly 100% by this treatment, it is not surprising that gravitropism was suppressed. However, in this same report, they showed that shorter pretreatments with ethylene promoted curvature even though growth was partially inhibited. We have observed a similar phenomenon in maize roots (4). These results indicate an effect of ethylene on gravitropism that is independent of the effect of this gas on growth.

The goals of the present study were to characterize the effects of ethylene on the kinetics of root gravitropism and to examine the physiological basis of these effects, with special reference to potential effects of ethylene on (a) the sensitivity of roots to auxin and (b) activity of the auxin transport system.

MATERIALS AND METHODS

Plant Material

Caryopses of maize (Zea mays L., cv Golden Cross Bantam) were soaked in distilled water for 10 h prior to planting. The grains were placed between wet paper towels on vertical opaque plastic trays and germinated at room temperature (22–25°C) under fluorescent laboratory lighting (intensity 175 μmol·m⁻²·s⁻¹). After 2 d, seedlings with straight primary roots (approximately 2 cm long) were selected and attached to the inside of the bottom half of a plastic Petri dish using a small amount of floral tape. The half-dish was lined with moist filter paper and the seedling was oriented with the root parallel to the inside surface of the dish bottom.
Measurement of Gravitropic Curvature

The half-Petri dishes containing the seedlings were mounted vertically inside a desiccator jar lined with wet paper towels and the seedlings were allowed to equilibrate for 1 h prior to experimentation. The lid of the desiccator jar was fitted with a rubber stopper containing a short length of glass tubing fitted with a serum vial stopper through which samples of ethylene could be injected. At zero time the desiccator jar was opened, the Petri dishes were rotated 90° to gravistimulate the roots, the lid was replaced and the appropriate amount of ethylene was injected into the jar. Controls were handled the same way but with no injection of ethylene. The seedlings were photographed at 20 min intervals after the beginning of stimulation, and angle of curvature was measured from enlargements of the photographs using a protractor. To check the effects of mixing on the kinetics of the effects of ethylene on gravitropism, a magnetic stirrer was used to circulate the air in the chamber during some of the experiments. The kinetics of the ethylene response were not appreciably different with and without stirring.

Pretreatment of Roots

In some experiments, the roots were pretreated with inhibitors of ethylene biosynthesis or action or with inhibitors of auxin transport. The seedlings were mounted vertically with the roots immersed in oxygenated aqueous solutions of the inhibitors for 90 min. The seedlings were then mounted in the Petri dishes and used for gravitropism experiments as described above.

Curvature Induction by Centrifugation

To stimulate roots at forces greater than 1 g, seedlings were mounted vertically in centrifuge tubes that were fixed in a vertical position within a centrifuge (Hitachi model 05P-21). The seedlings were centrifuged for 10 min at speeds providing the desired centrifugal force. The seedlings were then mounted vertically in a humid chamber and the time course of curvature toward the previous centrifugal side was measured as described above.

Transport of Label Applied as 3H-IAA

We measured the effects of ethylene on the movement of label from 3H-IAA in both gravistimulated and centrifuged roots. The transport experiments were done by applying an agar donor containing 3H-IAA as described by Young et al. (20). Agar (1.5%) sheets (1.0 cm × 1.0 cm × 1.5 mm) were incubated overnight in distilled water (pH adjusted to 6.4 with NaOH). They were then cut into 1.5 mm cubes. The blocks were allowed to equilibrate with a small volume of 3H-IAA (1.084 mC/mL, Amersham) such that equilibrated blocks contained 15 × 10^3 cpm 3H-IAA/block (approximately 0.4 μM).

To measure auxin transport across the root, donor blocks were applied to one side of vertically oriented roots within the elongation zone about 3 mm behind the tip, and the seedlings were stimulated by rotating the Petri dishes 90°. Half of the seedlings were oriented so that the donor block was on the top of the root and half with the donor on the bottom. Following the transport period, the donor blocks were placed into separate scintillation vials for determination of radioactivity (Beckman model LS6800 scintillation spectrometer). To determine the amount of label moving to the other side of the root, a 0.5 cm long semicylinder of root tissue was excised from the side of the root immediately opposite the donor. The tissue segment was placed in a scintillation vial and radioactivity was measured after allowing the vials to stand at room temperature overnight. For experiments using roots that had been prestimulated by centrifugation, the donors were applied to vertically oriented roots following centrifugation, and transport was measured both in the direction of prior centrifugation and in the opposite direction. These experiments were done both in the absence and presence of applied ethylene during the transport period.

RESULTS

Effect of Ethylene on Root Growth

Table I shows the concentration dependence of ethylene effects on root growth. We observed increasing inhibition of root growth as the concentration of ethylene was increased from 0 to 10 μL L⁻¹. Further increase in ethylene (up to 150 μL L⁻¹) caused no additional inhibition of root growth.

Effect of Ethylene on the Time Course of Gravitropic Curvature

Figure 1 shows the effect of three different concentrations of ethylene on the time course of gravitropic curvature in primary roots of maize. Ethylene at 1 μL L⁻¹ appeared to delay the response somewhat but had little effect on the overall pattern of curvature thereafter. Higher concentrations of ethylene (10 μL L⁻¹, 100 μL L⁻¹) also appeared to delay curvature. However, at these concentrations, overall curvature was enhanced. The enhancement of curvature resulted from a prolonging of the period of rapid curvature. As a result the ethylene-treated roots continued to curve after the controls had reached a final angle of about 60°. In many cases the ethylene-treated roots curved substantially beyond 90°.

Table I. Concentration Dependence of Ethylene Inhibition of Root Elongation

<table>
<thead>
<tr>
<th>Ethylene Concentration</th>
<th>Inhibition</th>
</tr>
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<tbody>
<tr>
<td>μL L⁻¹</td>
<td>%</td>
</tr>
<tr>
<td>0</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>10</td>
<td>46</td>
</tr>
<tr>
<td>100</td>
<td>51</td>
</tr>
<tr>
<td>150</td>
<td>52</td>
</tr>
</tbody>
</table>
Figure 1. Effect of ethylene on the time course of gravitropic curvature in primary roots of maize. Roots were placed horizontally at zero time in the absence of applied ethylene (control) or in the presence of the indicated concentrations of applied ethylene. Inset shows the time course of curvature during the first 3 hr on an expanded scale. As an indication of variability, standard deviations were ± 7, 9, 9.6, 11.7, 9.3, and 8.6° for hours 1 to 6 of the control. Comparable SD values for curvature in the presence of 100 μL L⁻¹ ethylene were ± 3, 7.2, 10.3, 24.5, 25.5, and 24°.

Effect of Ethylene Biosynthesis Inhibitors on Gravitropic Curvature

The latent period of the gravitropic response was shortened somewhat in roots pretreated with inhibitors of ethylene biosynthesis (AVG,² AOA, cobalt) (Fig. 2). After 1 hr of stimulation, pretreated roots had curved more than controls. However, the rate of curvature in pretreated roots declined after the first hour and total curvature after 6 hr was less than that of controls. Pretreatment of roots with silver nitrate, an inhibitor of ethylene action (16), delayed the response and reduced the final curvature somewhat (Fig. 3). Silver nitrate also prevented the induction of exaggerated curvature by ethylene (Fig. 3).

Effect of 1-N-naphthylphthalamic Acid on Gravitropic Curvature in the Presence and Absence of Ethylene

Pretreatment of roots with the auxin transport inhibitor NPA (17), strongly inhibited their gravitropic response. NPA also prevented the long-term promotive effect of ethylene on root gravitropism (Fig. 4).

² Abbreviations: AVG, aminoethoxyvinylglycine; AOA, aminoxyacetic acid; NPA, 1-N-naphthylphthalamic acid.
Effect of Ethylene on the Response of Maize Roots to Applied Gradients of Auxin

To determine whether or not ethylene modifies the sensitivity of roots to auxin, we examined the effects of applied auxin gradients on curvature induction in roots in the presence and absence of applied ethylene. Agar blocks containing IAA in concentrations ranging from 0.1 μM to 1 mM were applied to one side of the elongation zone (about 3 mm from the root tip) of vertically oriented roots in the presence and absence of 100 μL L⁻¹ ethylene. Although the roots curved toward the side to which auxin was applied, the magnitude of the curvature was the same in the presence and absence of ethylene (data not shown).

Dose-Response Relationship for Curvature Development following Lateral Centrifugation of Roots

When vertically oriented seedlings were centrifuged for 10 min with the centrifugal force applied laterally across the root, the roots developed curvature when kept in a vertical position following the centrifugation. Curvature was toward the previous centrifugal direction. Maximum curvature developed following centrifugation at about 20g (inset, Fig. 5). Figure 5 shows the time course of curvature in roots centrifuged at 20g for 10 min and then held vertically in the presence or absence of 100 μL L⁻¹ ethylene. In the absence of ethylene, curvature reached a maximum of about 25° after 20 min. Thereafter, the roots began to curve back toward the vertical, reaching the vertical at about 60 min. The pattern of curvature in the presence of ethylene was similar except that the magnitude of the curvature was greatly reduced.

Effect of Ethylene on Gravity-induced Asymmetric Movement of Label from ³H-IAA across the Elongation Zone

Earlier work has shown that label from applied ³H-IAA moves preferentially toward the lower side of gravistimulated maize roots (11). We found that ethylene alters the kinetics of this movement (Table II). In ethylene-treated roots, gravity-induced movement of label was delayed relative to that of controls. However, asymmetric movement of label across ethylene-treated roots was prolonged relative to that of controls. We also examined the effects of ethylene on movement of label across roots stimulated by centrifugation (Table III). In these experiments roots were prestimulated either by orienting horizontally (1g) or by centrifuging at 20g. Movement of label across the root was then measured during the first 30 min following stimulation, with the roots mounted vertically in the presence or absence of ethylene. There was no preferential movement of label toward the previous lower side of roots stimulated at 1g. If anything, there was slight preferential

Figure 4. Effect of NPA on the time course of gravitropic curvature of primary roots of maize in the presence or absence of applied ethylene. Roots pretreated with 1 μM NPA where indicated. Ethylene applied at 100 μL L⁻¹. As an indication of variability, standard deviations were ± 3.6, 9.6, 10.2, 17.1, 26.5, and 33.3° for hours 1 to 6 in the presence of ethylene. Comparable so values for roots pretreated with NPA and gravistimulated in the presence of ethylene were ± 3, 7, 12, 18.1, 20.3, and 23°.

Figure 5. Effect of ethylene on curvature following lateral centrifugation of primary roots of maize at 20 g. Vertically oriented roots were centrifuged for 10 min at 20 g and then maintained in a vertical position during curvature development. Curvature was toward the previous centrifugal side. Ethylene applied at 100 μL L⁻¹. Maximum so was ± 8°. Inset shows the centrifugation dose-response relationship for subsequent curvature development.
Table II. Asymmetric Movement of Label from \(^3\)H-IAA Across the Elongation Zone of Gravistimulated Maize Roots in the Presence and Absence of Ethylene

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Prestimulation</th>
<th>T → B</th>
<th>B → T</th>
<th>Ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min</td>
<td>cpm</td>
<td>cpm</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>45</td>
<td>330 ± 27</td>
<td>248 ± 15</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>155</td>
<td>174 ± 19</td>
<td>151 ± 16</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>128 ± 18</td>
<td>132 ± 11</td>
<td>0.9</td>
</tr>
<tr>
<td>Ethylene</td>
<td>45</td>
<td>257 ± 25</td>
<td>280 ± 20</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>172 ± 5</td>
<td>164 ± 14</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>149 ± 10</td>
<td>110 ± 7</td>
<td>1.3</td>
</tr>
</tbody>
</table>

* Ratio of movement of label from top to bottom (T → B)/bottom to top (B → T).

movement toward the opposite side (also observed by Young et al. [20]). In roots prestimulated at 20g there was obvious preferential movement of label (asymmetry = 1.5) toward the previous centrifugal side during the first 30 min following stimulation. When this experiment was repeated in the presence of 100 \(\mu\)L \(^{-1}\) ethylene, asymmetric movement of label following prestimulation at 20g was greatly reduced.

**DISCUSSION**

We found that gravitropism in maize roots occurred both in the presence of high levels of ethylene and in the presence of inhibitors of ethylene biosynthesis or action. Inhibitors of ethylene biosynthesis or action have been shown in previous work to promote maize root growth (13). Our results support the conclusion of Harrison and Pickard (6) that ethylene does not mediate the primary differential growth response causing gravitropic curvature. However, the dramatic effects of ethylene on the kinetics of curvature indicate that ethylene can influence one or more steps in the response mechanism. Ethylene appeared to increase the latent period of curvature while inhibitors of ethylene action or biosynthesis shortened the apparent latent period. Although ethylene appeared to delay the gravitropic response somewhat, once initiated the response was prolonged. This led to exaggerated curvature with roots often curving well beyond 90°.

We have considered two potential explanations for the observed promotion of gravitropic curvature by ethylene. These explanations are based on the tenets of the Cholodny-Went theory (ref. 18, Chap 10) of root gravitropism. According to this theory, curvature results from gravity-induced accumulation of auxin on the lower side of the elongation zone. The elevated level of auxin on the lower side leads to growth inhibition there, causing the root to curve downward. There is evidence that auxin accumulates along the lower side of gravistimulated roots (Table II and ref. 11). It is also well known that roots show time-dependent adaptation to elevated levels of auxin, i.e. roots are initially strongly inhibited by applied auxin but soon (often within 1 h) adapt to the elevated auxin level and begin to grow rapidly even in the presence of the applied auxin (2, 5, 7). If adaptation to elevated auxin levels plays a role in root gravitropism, ethylene might prolong curvature by interfering with the ability of roots to adapt to high auxin levels, i.e. by maintaining high sensitivity to auxin (12). This would prolong the growth-inhibiting effects of elevated auxin on the lower side of the root and lead to exaggerated curvature. We tested the effects of ethylene on adaptation of maize roots to auxin by measuring curvature induction in vertical roots upon unilateral application of auxin in the presence and absence of ethylene. Since curvature was the same with and without applied ethylene, we conclude that ethylene does not affect adaptation to applied auxin, at least in vertically oriented roots.

A second possible explanation for the ability of ethylene to cause exaggerated curvature relates to potential effects of ethylene on the auxin transport system. In gravistimulated roots there is asymmetric movement of label from applied auxin toward the lower side of the root (Table II and ref. 20). In control roots there is a close correlation between the development of asymmetric auxin movement and the development of curvature (20). Our data indicate that ethylene affects gravity-induced asymmetric auxin movement. This conclusion is supported by the following observations: (a) The promotive effect of ethylene on curvature was reduced in roots treated with the auxin transport inhibitor, NPA (Fig. 4). (b) Ethylene delayed the initiation of asymmetric auxin movement in stimulated roots but sustained the asymmetry once

Table III. Effect of Ethylene on Asymmetric Movement of Label from \(^3\)H-IAA Across the Elongation Zone of Maize Roots Stimulated by Centrifugation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Centripetal to Centrifugal</th>
<th>Centrifugal to Centripetal</th>
<th>Ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm</td>
<td>cpm</td>
<td></td>
</tr>
<tr>
<td>1g</td>
<td>336 ± 43</td>
<td>352 ± 39</td>
<td>0.9</td>
</tr>
<tr>
<td>20g</td>
<td>319 ± 7</td>
<td>200 ± 20</td>
<td>1.5</td>
</tr>
<tr>
<td>20g, ethylene</td>
<td>147 ± 17</td>
<td>115 ± 11</td>
<td>1.3</td>
</tr>
</tbody>
</table>

* Ratio of movement of label from centripetal side to centrifugal side/movement from centrifugal side to centripetal side. For the horizontally stimulated 1g controls, ratio indicates movement of label from top to bottom/movement bottom to top.
inhibited (Table II). Inhibitors of ethylene biosynthesis or action had the opposite effect. (c) The ability of ethylene to inhibit curvature following short term stimulation at 20g (Fig. 5) was paralleled by inhibition of short-term auxin transport and by reduction of auxin asymmetry (Table III).

In a review of leaf epinasty, Kang (10) noted that the downward bending of the petiole is usually attributed to higher levels of auxin on the top of the petiole. He proposed that ethylene causes epinasty by affecting adaptation of the auxin transport system to the gravity stimulus. More specifically, he suggested that ethylene interferes with the adaptation of the gravity sensor so that the sensor becomes overstimulated leading to net movement of auxin in the "wrong" direction, i.e. toward the upper side of the petiole.

Our data can be interpreted within the context of Kang's model as follows. Gravistimulation leads to enhancement of an auxin transport system directing auxin toward the lower side of the root. As a part of the normal control of curvature, this auxin transport system shows time-dependent adaptation to the stimulus so that, after 2 to 3 h, downward transport declines in spite of the fact that the root has not yet reached a vertical orientation. Approach of the root to a final angle of curvature involves changes in the strength of the stimulus (change in angle with respect to gravity as the root approaches the final angle of curvature) and changes in the degree of adaptation of the transport system to the prevailing stimulus. We propose that ethylene somehow interferes with the ability of the auxin transport system to adapt to gravistimulation. Consequently downward auxin movement is prolonged resulting in exaggerated curvature.

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LITERATURE CITED


