Abscission: The Initial Effect of Ethylene Is in the Leaf Blade

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ABSTRACT

The leaf blade of cotton (Gossypium hirsutum L. cv. Stoneville 213) was investigated as the initial site of ethylene action in abscission. Ethylene applied at 14 μl/l to intact 3-week-old plants caused abscission of the third true leaf within 3 days. However, keeping only the leaf blade of this leaf in air during ethylene treatment of the rest of the plant completely prevented its abscission for up to 7 days. This inhibition of abscission was apparently the result of continued auxin production in the blade since (a) the application of an auxin transport inhibitor to the petiole of the air-treated leaf blade restored ethylene sensitivity to the leaf in terms of abscission; (b) repeated applications of naphthaleneacetic acid to the leaf blade of the third true leaf, when the entire plant was exposed to ethylene, had the same preventive effect on abscission of this leaf as keeping its leaf petiole in air; and (c) the inhibitory effect of ethylene on auxin transport in the petiole, which is reduced by auxin treatment, was also reduced by placing the leaf blade in air.

The reverse treatment of exposing only the leaf blade of the third true leaf to 14 μl/l of ethylene, while the rest of the plant was kept in air, also did not cause abscission for up to 5 days. Auxin transport in the petioles of these leaves, however, was inhibited over 80% within 2 days and this effect presumably accounted for their increased sensitivity to ethylene during the subsequent exposures of the whole leaf to the gas.

These results suggest that an initial and essential function of applied ethylene in abscission is to reduce the amount of auxin transported out of the leaf blade. This reduction together with the inhibitory effect of ethylene on auxin transport in the petiole reduces the auxin level at the abscission zone to a point where the cells in this region become responsive to the more direct action of the gas (e.g., enzyme induction and secretion). This sequence of events accounts for the lack of abscission unless ethylene is applied to both the leaf blade and the abscission zone.

Auxin and ethylene are without doubt two of the more important regulatory agents involved in the natural control of abscission (3, 25, 32). The dominant role of auxin in abscission is one of retardation, while that of ethylene is clearly one of promotion. The interplay between auxin and ethylene in their mutually antagonistic roles in abscission has been the subject of numerous studies. From these studies has emerged the concept that the ability of ethylene to initiate cell separation in the abscission zone through enzyme induction (2, 4, 23, 34) and secretion (5) depends largely upon the sensitivity of this tissue to ethylene (1, 3, 16). The principal factor governing this sensitivity to ethylene seems certainly to be auxin. Several types of evidence point to this conclusion. First, auxin applied to freshly cut petiole explants (16, 27), debladed petioles (3, 21), intact plants (9), or plants with their roots removed (21), prevents ethylene induced abscission. Second, lowering the auxin level at the abscission zone by excising and aging the abscission zone tissue (1, 6, 16), removing the leaf blade (3), or applying an inhibitor of auxin transport to the petiole (8, 28, 30) potentiates the action of ethylene.

If the auxin level at the abscission zone must first be lowered before ethylene can induce cell separation, the question arises as to the mechanism(s) and target tissue(s) involved in this auxin lowering process when intact plants are exposed to the gas. While several studies (9, 11, 15, 18, 22, 36) have dealt with the possible mechanisms involved in the reduction of auxin (e.g., transport inhibition, destruction, synthesis), none have specifically dealt with the problem of defining in the intact plant the target tissue where these processes principally occur. The technique of isolating and keeping one of the leaf blades of intact cotton plants in air while treating the rest of the plant with ethylene, or vice versa, was used in the present study to answer this question.

The experiments reported here demonstrate that the leaf blade is the initial target tissue of exogenously applied ethylene, where some essential function of ethylene must first be performed before abscission can occur. Results indicate that this essential function of ethylene in the leaf blade is to reduce the amount of auxin transported out of the blade. A preliminary report of this study has been published (12).

MATERIALS AND METHODS

Plant Culture. Cotton (Gossypium hirsutum L. cv. Stoneville 213) plants were grown in a controlled environmental growth room (2200 ft-c, 14-hr photoperiod, relative humidity 50 ± 5% day and night; temperature 27 C day, 18 C night) in 15.2-cm plastic pots, containing a peat moss-verticulite mixture (Jiffy-Mix, Jiffy Products of America, West Chicago, Ill.) mixed with sand (3:1 v/v), and were watered daily with Hoagland’s nutrient solution.

Ethylene Treatment. The general technique of treating the third true leaf of intact 3-week-old cotton plants with air, and the rest of the plant with ethylene, or vice versa, is illustrated in Figure 1. Small Lucite chambers consisting of two equal halves were fitted together over the third true leaf blade and sealed with a gasket of closed cell neoprene 0.64 cm thick. A slit cut in the gasket allowed the petiole to pass directly through the center of the gasket. Lanolin was used to make this seal air tight and elastic bands were placed around the leaf cham-

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ber to hold the two halves of the leaf chamber firmly together against the gasket material. The plants were then placed into a larger Lucite chamber of 320 liter capacity. Using this arrangement and the appropriate gas flow equipment, the isolated leaf blade could be exposed to either air or ethylene while the rest of the plant received the same or the opposite treatment.

Two large Lucite chambers, each accommodating up to 16 plants were used in this study. The internal environmental conditions in these chambers were 1800 ft-c 14-hr photoperiod; relative humidity 76% day, and 50% night; temperature 28.5 C day, 18 C night. Gas flow through the leaf chambers was maintained at a constant rate of 300 cc/min while flow through the larger chamber was held constant at 18 l/min. The inlet and outlet ports on the small chambers were located at opposite corners. Air or ethylene (14 μl/l) was pumped into a manifold located inside the large chamber which distributed the gases to the various flow meters regulating the flow into each leaf chamber. A slight negative pressure was maintained on the exit manifold to facilitate the removal of the effluent air. New tubing was used in each experiment to avoid ethylene contamination problems. The flow design was such that ethylene from the same source could be used to treat isolated leaf blades and entire plants simultaneously, thereby ensuring that the differences observed were not due to differences in ethylene concentration. The concentration of ethylene in the air was monitored throughout each experiment.

Auxin Transport. The classical donor-receiver agar cylinder technique (10) was used to measure auxin transport capacity in 5-mm petiole sections. The donor agar cylinders contained 2 μM naphthaleneacetic acid-1-14C with a radiochemical purity of 98.2% as determined by paper chromatography (20) and liquid scintillation counting (19). Petiole sections were cut from the distal portion of the petiole 1 cm below the leaf blade. Sections were allowed to transport auxin for 4 hr in the dark at 30 C. The sections were then cut in half, placed directly into a dioxane scintillator fluid (26), extracted overnight on a shaker at 4 C, and counted. Donor and receiver agar cylinders were assayed in a similar manner.

Naphthaleneacetic Acid Leaf Treatments. Leaf blades of the third true leaf of 3-week-old cotton plants were immersed twice each day in 0, 1, 10, 100, 1000 μM naphthaleneacetic acid solutions containing 0.01% Tween 20. Plants were continuously exposed to 14 μl/l of ethylene for 10 days except for brief periods when the plants were removed from ethylene for treatments.

RESULTS

Ethylene was totally incapable of inducing abscission of the third true leaf, even after 7 days, when the leaf blade was maintained in air (Fig. 2, lower curve). These results were in marked contrast to the rapid abscission which occurred when the whole leaf was exposed to ethylene (Fig. 2, upper curve). Under these conditions 20% of the third true leaves were exposed to ethylene.
had abscised by the end of the 1st day with 100% abscission having occurred by the 3rd day. By the end of the 5th day all of the other leaves on these plants had abscised (data not shown). As reported for cotton (29), ethylene caused the younger leaves to abscise first. The older, fully expanded, first true leaf was the last leaf to abscise. Keeping the third true leaf blade in air had no significant effect on the abscission rate of the other leaves on the plant, because by the end of the 5th day all of these leaves had also abscised. Exposing the third true leaf blade to ethylene without or with the leaf chamber had no effect on the rate of abscission (data not shown).

Five such experiments of this nature were conducted for a maximum length of 12 days. After being purged with air 7 to 12 days in the leaf chambers, the leaf blade began to senesce. Shortly after visible senescence was observed, the leaf abscised. Occasionally after 7 days an abscission zone would form at a point on the petiole just outside the leaf blade chamber. The formation of an abscission zone in a region where it does not normally occur is of interest in light of previous studies where a similar phenomenon has been observed (14, 37, 38).

The auxin transport capacity in the petiole of the third true leaf under various treatment conditions is presented in Table 1. As reported (9), a 2-day ethylene exposure of the entire leaf reduced auxin transport capacity in the petiole by 79% when compared to the control (entire plant in air). Keeping the leaf blade in air reduced the effectiveness of ethylene in terms of transport inhibition but it did not completely prevent this effect of the gas. Auxin transport was reduced 79% when the entire leaf was treated with ethylene and 42% when the leaf blade was maintained in air. Under the latter condition auxin transport capacity continued to decline and reached 60% inhibition by the 6th day. Auxin transport capacity could not be determined at the end of 6 days in the petioles of the third true leaves of plants where the entire leaf was in ethylene, since these leaves had all abscised by the end of the 3rd day.

To determine if the abscission preventive effect of maintaining the leaf blade in air was related to auxin flow from the leaf blade to the abscission zone, the auxin transport inhibitor DPX18402 (8) was applied as a lanolin ring to the petiole (Fig. 3). The left-hand portion of Figure 3 again shows the preventive effect of keeping the leaf blade only in air. These petioles were treated with plain lanolin and served as the controls. The right-hand portion of Figure 3 shows the effect of applying a lanolin ring containing 100 μg/g of DPX1840 (about 50 mg) around the petiole 1.5 cm below the leaf blade at the time the plants were placed into ethylene.

DPX1840 applied in this manner completely nullified the abscission preventive effect of keeping the leaf blade in air. After DPX1840 treatment the air-treated leaf blades abscised just as rapidly as those in ethylene. These rather dramatic results strongly suggested that auxin was involved in the preventive effect of keeping the leaf blade in air.

The results presented in Figure 3 also confirm the reported (8, 28) synergistic effect of DPX1840 with ethylene in abscission. When the entire plant was treated with ethylene without DPX1840 application, 33% of the leaves abscised by the end of the 1st day. Adding DPX1840 to the petiole under the same conditions increased the abscission percentage obtained on the 1st day to 68%. DPX1840 applied to the third true leaf of cotton without added ethylene did not cause abscission (data not shown).

The data presented above, especially the DPX1840 data, strongly suggested that the abscission preventive effect of keeping the leaf blade in air was due to the prevention of an essential abscission requiring auxin-ethylene interaction in the leaf blade. Since blocking transport in the petiole with DPX1840 overcame the effect of keeping the leaf blade in air, it appeared that the essential ethylene function was to reduce the flow of auxin out of the leaf blade. If this hypothesis was correct, then the application of a synthetic auxin to the leaf blade in the presence of ethylene should also prevent abscission.

To test this idea naphthaleneacetic acid was applied at various concentrations as a dip treatment to only the third true leaf blade (Fig. 4). As previously described (9, and Figs. 2 and 3) ethylene induced rapid abscission of the third true leaf blade reaching 100% abscission in the experiment by the end of the 2nd day. Increasing the concentration of naphthaleneacetic acid from 1 μM to 1 mM resulted in a progressively greater reduction of ethylene-induced abscission. At 1 and 10 μM all of the third true leaves eventually abscised, but at 0.1 mM less than one-half of these leaves abscised in 10 days and at 1 mM none of them abscised. At the higher naphthaleneacetic acid concentrations, abscission of the first and second true leaves was also reduced but not as dramatically as that of the treated third true leaf.

Since the action of ethylene on the leaf blade is an absolute requirement if rapid abscission is to occur, it is of interest to

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ask whether or not this effect alone can trigger abscission. To answer this question the leaf blade of the third true leaf was treated with 14 μl/l of ethylene while the rest of the plant was exposed to air. As indicated by the data presented in Figure 5, exposure of only the leaf blade to ethylene does not induce abscission during a 5-day exposure period. Only when the leaf blade, petiole, and abscission zone receive ethylene does rapid abscission occur.

Treatting only the leaf blade with ethylene resulted in a significant decline in the auxin transport capacity even though the petiole itself was never exposed to the gas (Table II). In fact, the degree of inhibition after 2 days was essentially the same as that observed in similar leaves where the whole plant was treated with ethylene. Transport capacity was reduced 80% when the whole leaf received ethylene and 82% when only the leaf blade was treated with ethylene. The degree of transport inhibition did not change significantly after the 2nd day.

**DISCUSSION**

Considerable evidence (1, 3, 16, 32) suggests that a cellular aging process must occur in the cells of the separation region before they become fully responsive to the abscission stimulating effects of ethylene. Changes in cell sensitivity have largely been attributed to changes in the auxin concentration of the cells in this region (7, 16). Several workers (9, 11, 13) have suggested that ethylene participates in lowering the auxin concentration in the cell separation region and, hence, in changing the sensitivity of these cells, through its reported effects on auxin transport (9, 11), destruction (22, 31), and synthesis (36). Of these three, the effect of ethylene on auxin transport has been most closely correlated with the abscission process.

In the present study the leaf blade was isolated from ethylene to ascertain whether or not this tissue is a potential site of ethylene action in abscission. If no change in the rate or extent of abscission is observed under such conditions, then ethylene obviously performs no essential function in this tissue. However, as the data presented in Figure 2 indicate, this is not the case. Isolating the leaf blade from ethylene prevented abscission, clearly demonstrating that treatment of only the abscission zone and petiole with ethylene is not sufficient to induce rapid abscission.

These results suggest that as long as the flow of auxin from the leaf blade continues unaltered ethylene cannot induce abscission. If this interpretation is correct, then blocking the flow of auxin from the leaf to the abscission zone with an auxin transport inhibitor should result in rapid abscission. As the data presented in Figure 3 demonstrate, the potent inhibitor of auxin transport DPX1840 (8) does in fact restore ethylene sensitivity to the leaf. DPX1840 treatment completely negated the preventive effect of keeping the leaf blade in air. Additional support for this idea is provided by the data presented in Figure 4 demonstrating that treatment of only the third true leaf blade with naphthalenecarboxylic acid under conditions where the entire plant was exposed to ethylene also prevented abscission. Naphthalenecarboxylic acid clearly blocks the effect(s) of ethylene in the leaf blade just as it does in the petiole and abscission zone (9, 27).

It is well established (9, 11) that ethylene at the concentration used in this study severely reduces auxin transport in the petiole of cotton within 24 hr. Therefore, the question arises as to why ethylene did not fulfill the same role as DPX1840 since the petiole was exposed to the gas for 7 days. The data

![Fig. 4. Effect of ethylene (14 μl/l) on abscission of the third true leaf of cotton under conditions where the leaf blade is treated with increasing concentrations of naphthalenecarboxylic acid (NAA) and the entire plant is exposed to ethylene. Each datum point is the average per cent abscission of the treated third true leaf of 10 plants.](image)

**Table II. Comparison of Auxin Transport Capacity in Petiole of Third True Leaf of Cotton under Various Treatment Conditions Involving Air and Ethylene**

<table>
<thead>
<tr>
<th>Treatment Condition</th>
<th>2 Days</th>
<th>4 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entire plant in air</td>
<td>56 %</td>
<td>52 %</td>
</tr>
<tr>
<td>Entire plant in C,H, 14 μl/l</td>
<td>11 %</td>
<td>10 %</td>
</tr>
<tr>
<td>Leaf blade in C,H, rest of leaf and plant in air</td>
<td>10 %</td>
<td>8 %</td>
</tr>
</tbody>
</table>

1 Per cent transport capacity

\[
\frac{\text{cpm in basal segment + receiver}}{\text{cpm in section + receiver}} \times 100.
\]

*Also see reference 9 and Fig. 2.*
presented in Table I, suggest a possible answer to this paradox. Auxin transport capacity was reduced 79% after 48 hr when the entire leaf was treated with ethylene but only 42% when the leaf blade remained in air. By keeping the leaf blade in air the petiole was presumably provided with auxin, which, as previously reported (9, 33), reduces the ability of ethylene to inhibit auxin transport. In contrast to ethylene, DPX1840 inhibited auxin transport over 90% within 4 hr under all treatment conditions (data not shown). This marked difference between the extent and rapidity of transport inhibition by ethylene and DPX1840 would appear to explain the inability of ethylene to cause abscission the same as DPX1840 under these conditions.

Note-worthy with regard to the relative amount of auxin which might be expected to reach the abscission zone under conditions where the leaf blade was held in air is a comparison of this amount with that expected to reach the abscission zone when the whole leaf is exposed to ethylene. Spectrofluorometric determinations (35) of IAA levels in the leaf blade following a 48-hr exposure of the whole leaf to 14 \mu\text{g}/l of ethylene indicate that IAA levels are reduced by 65% (unpublished data). Following the same period of time auxin transport is inhibited 79% (Table I). If 100 units of IAA were transported through the petiole per unit of time, prior to ethylene exposure, then following a 48-hr ethylene treatment the amount of IAA would be reduced to only 7 units (65% reduction in the amount of auxin in the leaf plus a 79% reduction in auxin transport). On the other hand, when the leaf blade is maintained in air the amount of IAA would only be reduced to 58 units since no change in IAA levels of the leaf blade occurs (unpublished data) and transport is reduced only by 42% under these conditions. While this type of assessment is admittedly over-simplified, it does serve to illustrate that a 7-fold difference in auxin content at the abscission zone under these two treatment conditions could easily occur.

Ethylene apparently alters auxin synthesis, conjugation, or destruction in the leaf blade, since the total extractable auxin content, as indicated above, is reduced by 65% following a 48-hr exposure to ethylene (unpublished data). Effects on synthesis are suggested by the results of two studies (18, 36) indicating that ethylene inhibits the conversion of tryptophan to IAA in Coleus while effects on destruction are suggested by the enhanced IAA oxidase activities observed in cotton leaves following ethylene treatment (22). Added to these potential auxin lowering actions of ethylene is the observed fact that ethylene reduces auxin transport in the veinous tissues of the leaf blade in essentially the same manner as it does in the petiole (unpublished data). Thus, reduced auxin transport and synthesis, plus enhanced destruction, may contribute to the overall decline in auxin efflux from the leaf blade.

Exposure of the petiole and abscission zone to ethylene, but not the leaf blade, does not result in abscission (Fig. 2). Similarly, exposure of only the leaf blade to ethylene also does not cause abscission (Fig. 5), even though auxin transport is severely inhibited (Table II). This is not too surprising since the localized application of potent auxin transport inhibitors to the petiole also does not trigger abscission in the absence of applied ethylene. Presumably, there is a lack of abscission under these conditions because there is insufficient ethylene at the abscission zone to trigger enzyme induction (2, 23, 34) and secretion (5). This view is supported by the observation that the sensitivity of leaves to ethylene is increased by pretreating only the leaf blade with ethylene. When leaf blades of the third true leaf were pretreated for 48 hr with 14 \mu\text{g}/l of ethylene and then the entire plants were placed in 3 \mu\text{g}/l of ethylene, the leaves abscised, on the average, 1 day earlier than those which were not pretreated with ethylene.

The results obtained in this study indicate that rapid delocalization of cotton by ethylene involves an essential auxin lowering function in the leaf blade. This effect coupled with an ethylene-mediated auxin transport inhibition in the petiole quickly drops the concentration of the auxin at the abscission zone to a point where the cells in this region become responsive to the gas in terms of enzyme induction (2, 23, 34) and secretion (5), leading to leaf drop. All of these ethylene-mediated processes are readily reversible since the removal of ethylene at any time up to the time of actual cell separation halts the abscission process (9, 17).

In contrast to the role of ethylene in the petiole and abscission zone, the role of the ethylene in the leaf blade during natural abscission is not presently clear. Whether or not the increase in ethylene production during senescence (11, 24) contributes significantly to the natural decline in auxin content is not known. The answer to this question must await further clarification as to the exact nature of those processes in the leaf involved in this decline plus an assessment of the sensitivity of these processes to ethylene.

**LITERATURE CITED**