

[¹⁴C]Ethylene Metabolism during Leaf Abscission in Cotton¹

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ABSTRACT

Changes in ¹⁴C₂H₄ metabolism in the abscission zone were monitored during cotton (cv. Deltapine 16) leaf abscission. Rates of ¹⁴C₂H₄ oxidation to ¹⁴CO₂ and tissue incorporation in abscission zone segments cut from the second true leaf of nonabscising leaves of intact plants were similar (about 200 disintegrations per minute per 0.1 gram dry weight per 5.5 hours) and relatively constant over a 5-day period. Deblading to induce abscission caused a dramatic rise in ¹⁴C₂H₄ oxidation, but tissue incorporation was not markedly affected. This rise occurred well before abscission, reaching a peak of 1,375 disintegrations per minute per 0.1 gram dry weight per 5.5 hours 2 days after deblading when abscission was 40%. The rate then gradually declined, but on day 5 when abscission reached completion, it was still nearly three times higher than in segments from nonabscising leaves. Application of 0.1 millimolar abscisic acid in lanolin to the debladed petiole ends increased the per cent abscission slightly and initially stimulated ¹⁴C₂H₄ oxidation. In contrast, naphthaleneacetic acid applied in a similar manner delayed and markedly inhibited both abscission and ¹⁴C₂H₄ oxidation.

Petiole segments cut 1 centimeter from the abscission zone of intact second true leaves also incorporated and oxidized ¹⁴C₂H₄ to ¹⁴CO₂ but at rates two and six times higher, respectively, than that of comparable adjacent abscission zone segments. However, in marked contrast to the abscission zone segments, no changes in oxidation were observed when the leaves were debladed to induce abscission.

These results demonstrate that: (a) prior to abscission, the ethylene oxidation, but not the tissue incorporation pathway, rapidly increases in the abscission zone; (b) this increase does not occur in adjacent petiole tissue; and (c) changes in the rate of oxidation and per cent abscission brought about by hormone treatments parallel one another. The possible significance of these changes in ethylene metabolism is discussed with respect to the hypothesis that ethylene action and metabolism are directly related.

Contrary to the conclusions reached earlier about ethylene metabolism (1, 2, 16, 18), recent studies of the metabolic fate of ethylene have demonstrated that plant tissues do metabolize significant amounts of ethylene. Generally, the amount of ethylene metabolized is relatively small (4, 6, 8-10, 13), but a recent report (15) indicates that this may not always be the case.

In all of the tissues examined in this laboratory (4, 6, 8, 9), two distinguishable pathways for ethylene metabolism have been identified. One pathway leads to the incorporation of ethylene into water-soluble tissue metabolites while the other leads to the oxidation of ethylene to CO₂. Since both pathways generally function simultaneously and respond similarly to various treatments, they

appear to be interrelated, yet they can be shown to be distinct. For example, antiethylene levels of CO₂ greatly inhibit ethylene oxidation to CO₂ without affecting tissue incorporation whereas Ag⁺ has the opposite effect (10). Also during specific growth or developmental changes, one pathway may increase rapidly and surpass that of the other by severalfold before subsiding (8, 9). Characterization of this two-component ethylene metabolic system in various plant tissues (4, 6, 8-10, 13) and a careful analysis of how ethylene metabolism responds to changes in ethylene tissue sensitivity (10) have led to the suggestion that this metabolic system is not simply a detoxification mechanism but instead is the initial event in the ethylene action sequence.

One of the important natural physiological effects of ethylene that has not yet been fully investigated with respect to ethylene metabolism is the process of leaf abscission. Although an earlier study of ethylene metabolism in cotton and bean abscission explants was conducted in this laboratory (5), the specific radioactivity of the labeled ethylene available at that time was too low (22 mCi/mmol) to permit a satisfactory investigation of this type. Since the specific radioactivity now available is over five times higher (119 mCi/mmol), a reevaluation of ethylene metabolism during abscission was undertaken. Results indicate that when cotton plants are debladed to induce abscission, ethylene oxidation increases in the abscission zone tissue prior to abscission but not in the adjacent petiole tissue.

MATERIALS AND METHODS

Plant Culture. Cotton plants (*Gossypium hirsutum* L. cv. Deltapine 16) were grown in a controlled environmental growth room (3,800 ft-c; 18-h photoperiod; RH, 75 ± 5% night and day; temperature 27 C day, 21 C night) in plastic trays (28 × 33 × 13 cm) containing a peat moss-Vermiculite sand mixture (3:1, v/v). After emergence, the plants were watered each morning with Hoagland nutrient solution and each evening with distilled H₂O.

Abscission Induction and Hormone Treatment. All experiments involving hormone treatments included 28 trays containing about 50 plants each. When plants were 17 days old, the second true leaf blade along with part of the petiole was removed in 21 of the trays by cutting the petiole 5 cm above the stem-petiole juncture. Immediately after cutting, plain lanolin (about 25 mg) was applied with a syringe to the end of each 5-cm petiole stump in seven of the trays. An equal number of trays with debladed plants received lanolin containing either 0.1 mM NAA² or ABA. These lanolin treatments were repeated again the next 2 days, but before retreatment, 5 mm of petiole tissue was cut off the distal end of each petiole stump. The plants in seven of the planting trays were left intact throughout the course of the experiment until abscission zone segments were needed from these plants.

Each day of the experiment the per cent abscission was determined by applying a 4.5-g weight to the distal end of the petiole stump of 15 plants just below the lanolin treatment. None of the

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² Abbreviation: NAA: naphthaleneacetic acid.

leaves of the intact control plants abscised throughout the course of the experiment.

Abscission Zone and Petiole Segments. On the day of deblading (day 0), abscission zone segments were cut from only intact plants. On subsequent days similar segments were also cut from the lanolin treatments. This operation consisted of first removing the upper part of the plant by cutting the stem directly above where the petiole and stem join together. This was most easily accomplished by sliding a razor blade along the upper side of the petiole until it severed the stem. This cut was made directly below the lateral bud so that it was also removed. The abscission zone segments were then obtained by cutting 1.5 to 2 mm on both sides of the abscission zone region. This region of cell separation is visible in cotton and appears as a narrow, darker green band at the base of the petiole. During the entire harvesting operation, all plants and freshly cut segments were maintained between wet paper toweling to avoid desiccation. In some experiments, petiole segments were also cut from the second true leaf. These segments were of similar length (3–4 mm) and were cut 10 mm from the base of the petiole.

When petioles had already abscised, a 1- to 1.5-mm piece was cut from the stem side of the abscission scar and a similar piece was cut from the base of the abscised petiole stump. These two pieces constituted one abscission zone segment. The abscised petioles were collected frequently and always maintained in a high humidity environment until cutting. Although 40 and 60% abscission of the debladed and ABA-treated petioles, respectively, had abscised by day 2, abscission zone segments were cut from only nonabscised petioles on this day. Thereafter, segments from abscised petioles were also used.

$^{14}\text{C}_2\text{H}_4$ Treatment. For $^{14}\text{C}_2\text{H}_4$ treatment 20 freshly cut abscission zone or petiole segments were placed into 2.5-ml vials and each vial placed inside a separate 62-ml modified Erlenmeyer glass-stoppered flask (Fig. 1 of ref. 6). Prior to placing the abscission zone or petiole segments into the incubation flasks, sufficient distilled H_2O was added to the flask to cover the bottom (4 ml), and 1 ml of 1.5 N NaOH was placed in the small, side well container to trap $^{14}\text{CO}_2$. The flasks were sealed, and enough purified $^{14}\text{C}_2\text{H}_4$ (7) injected to yield a final concentration of 20 $\mu\text{l}/\text{l}$ which was subsequently verified by GC analysis. The flasks were incubated on a shaker for 5.5 h in the dark at 22 C.

Following $^{14}\text{C}_2\text{H}_4$ treatment the flasks were opened, and the NaOH removed and placed directly into a liquid scintillation vial. After drying under a vacuum to remove dissolved $^{14}\text{C}_2\text{H}_4$, 4 ml of water and 15 ml of Handifluor (Mallinckrodt) were added to the vials, and they were counted for radioactivity with an over-all counting efficiency of 74% as determined by the external standard method using n - ^{14}C hexadecane. The abscission zone or petiole segments were removed from the incubation flasks, lyophilized to remove dissolved ethylene, and subsequently weighed. Dry weights for abscission zone tissue segments were 33 to 40 mg per replicate or flask (20 segments per flask) while petiole segments ranged from 16 to 23 mg. The dried tissue samples were digested for 30 min in glass vials with 2 ml of 30% HClO_4 heated to 90 C in a water bath. One ml of 30% H_2O_2 was then added to clear the solution and after adding 4 ml of water and 15 ml of Handifluor, the tissue was liquid scintillation counted for radioactivity at an over-all efficiency of 50%. Preliminary experiments were conducted to verify that no ^{14}C tissue counts were lost during the tissue digestion and clearing procedure.

Two blank flasks were also included during each $^{14}\text{C}_2\text{H}_4$ exposure. These blank flasks contained 4 ml of distilled H_2O and 1 ml of 1.5 N NaOH but no tissue segments. The amount of radioactivity found in the NaOH of these flasks was subtracted from that found in the NaOH of the tissue flasks. Blanks consistently contained only 80 to 100 dpm per flask in the NaOH after drying. This was under conditions where the total initial $^{14}\text{C}_2\text{H}_4$ per flask was approximately 14.5×10^6 dpm. This background amount was

one-fifth to one-sixth the amount observed in the NaOH of flasks containing tissue segments during peak periods of metabolism.

The 1st day of the experiment (day 0), when the leaves were first debladed, abscission zone segments were cut from only the intact or nondebladed plants for $^{14}\text{C}_2\text{H}_4$ treatment. Two flasks containing 20 segments each and two blanks were included in this initial exposure. On the 1st through the 5th day, abscission zone segments were cut from intact plants and plants with debladed petioles treated with either plain lanolin or lanolin containing NAA or ABA. During this period, there were eight tissue flasks and two blanks per day. Comparisons of $^{14}\text{C}_2\text{H}_4$ metabolism in abscission zone *versus* petiole tissue were conducted in a similar fashion except these experiments did not involve hormone treatments. All experiments were conducted a minimum of two times with similar results.

RESULTS

Deblading induces leaf abscission in cotton (14). Under these experimental conditions with young plants, abscission of the debladed, plain lanolin-treated petioles began 1 to 2 days after removing the leaf blade and steadily increased reaching 80% by day 3 and 100% by day 5 (Fig. 1C, debladed). ABA, a known promoter of abscission (2, 3, 11), increased the per cent abscission when applied at 0.1 mM in lanolin to the cut ends of the petioles. As in this and all other treatments, no abscission occurred 1 day after deblading (day 1); but on days 2 through 4, ABA increased abscission by 10 to 20%. In contrast, NAA, a well known antago-

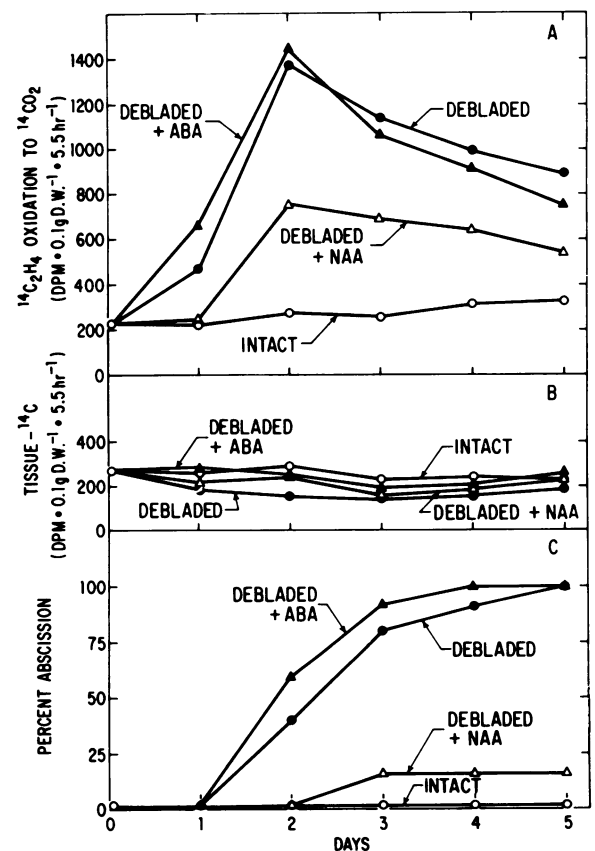


FIG. 1. A: time course of oxidation of purified $^{14}\text{C}_2\text{H}_4$ to $^{14}\text{CO}_2$ in 3- to 4-mm abscission zone segments cut from the second true leaf of intact cotton plants or from similar leaves whose leaf blades were removed and the petiole ends immediately treated with either plain lanolin (debladed) or lanolin-containing NAA or ABA to alter the rate of petiole abscission. B: radioactivity from $^{14}\text{C}_2\text{H}_4$ incorporated into these same abscission zone segments. C: time course of abscission in similarly treated second true leaves.

nist of abscission (2, 14), greatly suppressed abscission when applied in the same manner. Even by day 2 when 40% of the debladed control petioles had already abscised, no abscission was apparent in the NAA-treated petioles. On the 3rd day, 16% of the petioles did abscise, but no further abscission occurred during the entire experiment.

Abscission zone segments cut from the second true leaves of cotton plants not induced to abscise (intact controls) were found to oxidize $^{14}\text{C}_2\text{H}_4$ to $^{14}\text{CO}_2$ and to incorporate $^{14}\text{C}_2\text{H}_4$ at similar and constant rates of approximately 200 dpm/0.1 g dry weight \cdot 5.5 h throughout the course of the 5-day experiment. Whereas deblading, followed by plain lanolin treatment (debladed controls), had no marked effect on $^{14}\text{C}_2\text{H}_4$ tissue incorporation (Fig. 1B), it greatly stimulated $^{14}\text{C}_2\text{H}_4$ oxidation to $^{14}\text{CO}_2$ in the same abscission zone segments (Fig. 1A). The rate of oxidation more than doubled 1 day after deblading when abscission was not yet apparent (Fig. 1C). By day 2 it reached a peak of 1375 dpm/0.1 g dry weight \cdot 5.5 h which was five times that of similar segments cut from the intact controls. This peak rate was observed when 40% abscission had occurred (Fig. 1C), and as abscission progressed over the next 3 days, oxidation slowly declined. However, it was still 2.7 times higher than the intact controls when abscission reached completion on day 5.

Both ABA and NAA had no marked effect on $^{14}\text{C}_2\text{H}_4$ tissue incorporation during the entire experiment (Fig. 1B), even though ABA promoted abscission by 10 to 20%, and NAA greatly reduced it. However, this lack of effect on incorporation was not true for $^{14}\text{C}_2\text{H}_4$ oxidation. ABA stimulated oxidation by 39% on day 1 and to a lesser degree on day 2 during peak activity. Thereafter, the rate declined slightly faster than that of the debladed controls. NAA also affected $^{14}\text{C}_2\text{H}_4$ oxidation but in the opposite manner. NAA inhibited peak activity by nearly 50% (Fig. 1A) on day 2 and delayed by 1 day the initial rise in oxidation caused by deblading.

In another experiment not involving hormone treatments, $^{14}\text{C}_2\text{H}_4$ metabolism in adjacent petiole tissue was investigated to determine if the dramatic increase in ethylene oxidation also

occurred in this tissue. As in previous experiments (Fig. 1, A and B), the same trends in ethylene metabolism were observed in abscission zone segments cut from intact and debladed plants (Fig. 2, A and B). The rates of $^{14}\text{C}_2\text{H}_4$ tissue incorporation and oxidation for the intact control abscission zone segments were very similar to each other and to that of tissue incorporation in the debladed plants (Fig. 2, A and B). As before, this was not true for oxidation which reached a peak rate four times that of the intact controls on day 2 before slowly declining. $^{14}\text{C}_2\text{H}_4$ oxidation and tissue incorporation in the petiole segments responded quite differently. For example, the rates of $^{14}\text{C}_2\text{H}_4$ tissue incorporation and oxidation in petiole segments from the intact plants were two and six times higher, respectively, than that in the intact control abscission zone tissue. Deblading did not induce any significant change in ethylene metabolism, clearly indicating that removal of the leaf blade causes a localized increase in ethylene oxidation only in the abscission zone tissue.

DISCUSSION

Although the exact role of ethylene metabolism in plants has not been fully elucidated, circumstantial evidence suggests that this metabolism may represent the initial biochemical event triggering ethylene action (4, 6, 8–10). The current paucity of information concerning the biochemical mechanism of ethylene action (1, 2, 16, 18) makes it impossible at the present time to demonstrate directly the existence of such a relationship. However, other less direct approaches are possible such as establishing the presence of this metabolic system in ethylene-responsive tissues and demonstrating that in these tissues there is a close interrelationship between ethylene action and metabolism. This was the approach taken in the present cotton abscission study. If ethylene metabolism were a prerequisite for ethylene action in abscission, this metabolism must not only be present but it would also be expected to increase prior to abscission. Since the abscission zone is the most likely place for such changes to occur, this tissue was investigated.

Under conditions where abscission was induced by deblading and altered by hormone treatments, ethylene metabolism was found to occur in the abscission zone region and changes in the rate of oxidation and per cent abscission were found to parallel one another. Although the incorporation of ethylene into the tissue was hardly affected by deblading, oxidation doubled the day before any abscission had occurred, and it remained several-fold higher throughout the abscission period. Treatment of the petiole ends with ABA increased abscission slightly and had a similar effect on ethylene oxidation. NAA treatments, on the other hand, had a delaying and inhibiting effect on abscission and oxidation. These results with NAA and ABA represent the first report indicating that plant hormones are capable of altering ethylene metabolism. However, how direct these effects may be is not clear since they were not applied directly to the abscission zone.

The observed changes in ethylene oxidation in the abscission zone following deblading did not occur in the adjacent petiole tissue. This difference highlights the unique physiological responsiveness of the abscission zone tissues to ethylene which has contributed to the concept of "target cells" (17). It may be that the enhanced responsiveness of explants to ethylene after aging (12) occurs because ethylene oxidation must first be induced before ethylene can exert its full effect on the cell separation process. This idea is consistent with the concept that auxin, produced in the leaf blade, must be continuously supplied to the abscission zone tissue via active basipetal transport to reduce ethylene action effectively (1, 2). As seen in Figure 1A, NAA suppresses the appearance and intensity of the ethylene oxidation pathway in the abscission zone region. Therefore deblading, without exogenous auxin application, would lead first to a reduction in auxin supply

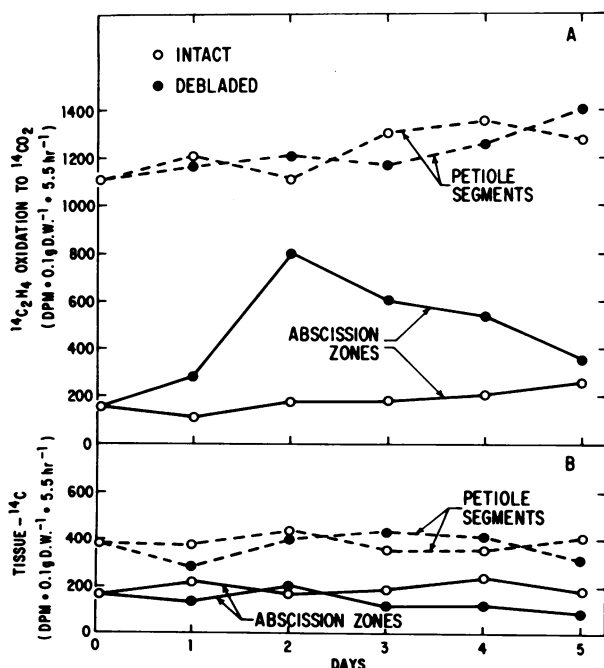


FIG. 2. A: time course of oxidation of purified $^{14}\text{C}_2\text{H}_4$ to $^{14}\text{CO}_2$ in 3- to 4-mm petiole or abscission zone segments cut from intact or debladed second true leaves of cotton. B: radioactivity from $^{14}\text{C}_2\text{H}_4$ incorporated into these same segments. Petiole segments were cut 1 cm from the abscission zone.

at the abscission zone followed by enhanced ethylene oxidation. This increase might account for the enhanced ethylene responsiveness which is clearly apparent 1 day after deblading (12). The inability of ethylene to induce abscission under conditions of high auxin would then be explainable in terms of a suppression of the ethylene oxidation pathway.

It is presently unclear why ethylene metabolism in the petioles should be severalfold higher than in the abscission zones prior to abscission. Perhaps this level of metabolism reflects ethylene actions in the petiole associated with normal growth processes. Since ethylene oxidation in the abscission zone rises to about the level always present in the petioles (1,000–1,200 units in the petiole as against 800 to 1,400 in the abscission zone during peak activity) perhaps the induction of abscission overcomes some type of suppressive effect on ethylene metabolism allowing it to rise to a more normal, physiologically active state.

The significance of the changes in ethylene oxidation without a concomitant change in tissue incorporation is unclear but not unusual (8, 9). Since ethylene oxidation and tissue incorporation are independent pathways (10), it is not too surprising that they should respond differently. In peas (10), it does appear that both pathways must operate since inhibiting either one independently greatly reduces ethylene responsiveness. Perhaps the ratio at which one pathway operates relative to the other may have some influence on the ultimate ethylene effect. The fact that two pathways exist for ethylene metabolism that are potentially subject to differential regulation increases the complexity of the system. However, if this system is directly coupled to ethylene action as suggested, the presence of two independently regulated pathways may account for the wide divergence of ethylene responses that

can occur in different tissues depending on their physiological state.

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