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Abscission

THE ROLE OF ETHYLENE MODIFICATION OF AUXIN TRANSPORT[1]

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ABSTRACT

The role of ethylene-mediated reduction of auxin transport in natural and ethylene-induced leaf abscission was studied in the cotton (*Gossypium hirsutum* L., cv. Stoneville 213) cotyledonary leaf system. The threshold level of ethylene required to cause abscission of intact leaves was between 0.08 and 1 μl/l with abscission generally occurring 12 to 24 hours following ethylene fumigation. The threshold level of ethylene required to reduce the auxin transport capacity in the cotyledonary petiole paralleled that required for stimulation of abscission. In plants where cotyledons are allowed to senesce naturally there is a decline in auxin transport capacity of petioles and increase in ethylene synthesis of cotyledons. The visible senescence process which precedes abscission requires up to 11 days, and increases in ethylene production rates and internal levels were detected well before abscission. Ethylene production rates for entire cotyledons rose to 2.5 mμl g^{-1} hr^{-1} and internal levels of 0.7 μl/l were observed. These levels appear to be high enough to cause the observed decline in auxin transport capacity. These findings, along with those of others, indicate that ethylene has several roles in abscission control (*e.g.*, transport modification, enzyme induction, enzyme secretion). The data indicate that ethylene modification of auxin transport participates in both natural abscission and abscission hastened by exogenous ethylene.

The ability of ethylene to inhibit auxin transport is well documented (9–11, 16, 24, 39, 40, 46). Equally well documented (see "Reviews" 17, 18, 29, 31, 48) is the ability of auxin to retard or prevent abscission. The amount of auxin reaching the abscission zone and the distribution of auxin both proximal and distal to the abscission zone appear to play an important role in this preventive action of auxin (8, 17, 29, 48). In view of the ability of ethylene to reduce the basipetal auxin transport capacity and to function as a potent accelerator of abscission (see "Reviews" 13, 47, 50), the gas may function indirectly to regulate abscission through its effect on basipetal auxin transport. Morgan and Gausman (40) first suggested such a role for ethylene in abscission, and this idea has been supported by others (14, 16).

Auxin applied to mature cotton plants (25) or cotton explants (38) prior to ethylene fumigation or treatment with ethylene-releasing agents such as Ethrel prevents the promotive effect of ethylene on leaf abscission. A similar preventive action of auxin on abscission can also be demonstrated when auxin is applied distally to abscission explants (7, 19). These results indicate that the stimulatory effect of ethylene on abscission is realized only after the endogenous auxin level has declined and some cellular aging has occurred (1, 7, 19). Burg (14) has reviewed the evidence for a role of ethylene in this aging process and has concluded that ethylene may participate in this phenomenon through its effects on auxin destruction (26, 39), transport (9–11, 16, 24, 39, 40, 46) and synthesis (52) No evidence has yet been provided, however, which establishes a causal relationship between abscission and any of these auxin-ethylene interactions.

This paper presents a series of experiments designed to test the hypothesis that either endogenous or exogenously applied ethylene influences or controls abscission, in part, through its effect on basipetal auxin transport.

MATERIALS AND METHODS

Plant Culture. Cotton plants (*Gossypium hirsutum* L., var. Stoneville 213) were grown in a greenhouse or in a controlled environment room (2000 ft-c; 15-hr photoperiod; relative humidity 56 ± 5% day, 60 ± 5% night; temperature 29.4 ± 1 C day, 23.9 ± 1 C night). Plants were watered with a modified Hoagland's solution (41) and were grown in 15.2-cm plastic pots containing sand or a peat-perlite mixture (20) or in wooden flats containing vermiculite.

Ethylene Concentration *versus* Percentage of Leaf Abscission. Cotton plants were fumigated with ethylene in 12-liter bell jars under controlled environmental growth room conditions. Ethylene concentrations were determined by gas chromatography (38) following a 30 min equilibration period. Bell jars were removed at 12-hr intervals; the plants were aerated for 15 min, and abscission was measured by applying a 3-g force to the distal end of each petiole.

The ethylene fumigation concentrations during the initial 12-hr enclosure period of the first experiment were 90, 15, 1 and 0.08 μl/l ethylene. These concentrations were duplicated with a precision of ± 5% in all subsequent fumigation periods and experiments. The bell jars enclosing the control plants never contained more than 0.015 μl/l ethylene at the end of any 12-hr period.

Ethylene Concentration *versus* Auxin Transport Inhibition. Intact 25-day-old cotton plants grown in a controlled environment room were fumigated with ethylene in the dark for 24 hr at 27 ± 1 C in Plexiglas chambers of approximately 160 liter capacity. Plants placed in an identical chamber but receiving only room air served as the controls. CO_2 was removed with

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10% KOH on filter-paper wicks. Ethylene, CO_2 and O_2 concentrations were determined by gas chromatography at the end of the fumigation period. CO_2, an apparent competitive inhibitor of ethylene action in abscission (4–6, 57), was never found to be higher than 0.20% following ethylene pretreatment, and O_2 never fell below 20%.

Immediately following ethylene pretreatment the cotyledons which were still attached to the plants were harvested. No more than one cotyledon was taken from any one plant. Cotyledonary petiole sections 10 mm in length were then excised starting 10 mm below the juncture of the petiole and the leaf blade and were placed in Plexiglas holders with the proximal end resting on receiver agar cylinders. Donor agar cylinders containing 2 μM NAA-1-^{14}C (50.6 mc/mmole, 15.6 μl volume) were then applied to the distal end of each section (9). The radiochemical purity of the NAA-1-^{14}C as determined by paper chromatography (23) followed by liquid scintillation counting of the paper chromatogram (21) was 97.4%.

Donor agar cylinders were in contact with the sections for 4 hr. During transport the sections were enclosed in the dark at 27 C in glass chambers and were purged with air at 200 ml per min. The air flowing out of the chambers was passed through gas dispersion tubes placed in 8 ml of 10% KOH to trap ^{14}C released as $^{14}CO_2$ (9). The radioactivity collected in the traps was determined by the method described by Schwertner and Morgan (49). As previously found using NAA-1-^{14}C (9, 11, 39), the amount of $^{14}CO_2$ recovered never differed significantly from that collected from the blank chamber containing only donor agar cylinders.

Three separate experiments of this nature were conducted. Experiments 1 and 2 contained two replications per treatment with four petiole sections per replication. In these two experiments each donor and receiver agar cylinder was placed directly into 15 ml of a dioxane scintillator fluid (33) following transport. The petiole sections were cut in half following transport, and the 0- to 5-mm apical and 5- to 10-mm basal segments from each replication were grouped into separate lots and were ground for 1 min on a VirTis tissue grinder in 4 ml of 40% ethanol. Duplicate 1-ml aliquots of the tissue extract were counted on a liquid scintillation counter. Experiment 3 contained 6 petiole sections per treatment. Each donor, receiver and 5-mm apical and basal segment was placed directly into scintillator fluid (33) following transport and subsequently was counted.

All samples were placed overnight on a shaker in the dark before counting. Samples were counted at 41% efficiency for ^{14}C, and all data were corrected for quenching. External standard and channel ratio data showed that there was no difference in quenching by the ethylene-treated and control samples.

Cotyledonary Age *versus* Transport Capacity, Ethylene Production, and Internal Ethylene Concentration. Plants grown in a greenhouse and plants grown in a controlled environment room were used in this study. Two separate harvests of cotyledons were made from each group of plants. When the greenhouse grown plants were 51 days old, approximately one-half of the cotyledons were harvested and grouped on the basis of color into dark green, yellow-green, and yellow cotyledons. The remaining cotyledons were harvested when the plants were 54 days old. Two successive harvests of cotyledons from plants grown in a controlled environment room were also made at 36 and 37 days after planting. From each of the color classes, 25 cotyledons were selected. The yellow cotyledons which were harvested were still attached to the plants and were turgid at the time of harvesting. They readily fell from the plants, however, when gently touched (*i.e.*, the separation layer was almost complete).

The rate of ethylene production by the cotyledons was determined by sealing 15 of the 25 cotyledons of each color class in small chromatography jars of approximately 360 ml capacity. The ends of the petioles were placed into small vials containing distilled water to avoid desiccation of the leaves. The containers with cotyledons were left open in the dark at 30 C for 4 hr to minimize wounding effects (28, 35) and then sealed for 6 hr. Duplicate 5-cc air samples were assayed for ethylene by gas chromatography at the end of the enclosure period.

Petiole sections 15 mm in length were excised from the remaining 10 cotyledons of each color class starting 5 mm below the juncture of the leaf blade and petiole. These were used to determine the auxin transport capacity of the petioles of the three classes of cotyledons. Donor and receiver agar cylinders were applied to the sections as previously described for stem sections (9). Donor agar cylinders containing 1 μM IAA-1-^{14}C (15.54 μl volume) were applied to the distal end of the petiole sections and incubated in the dark at 30 C for 2 hr. The radiochemical purity of the IAA-1-^{14}C was 94.0%, determined as for NAA-1-^{14}C above. Following transport, the donor agar cylinders were removed, and the sections were segmented into three successive 5-mm segments, frozen, and subsequently homogenized in 5 ml of 40% ethanol for 2 min. Duplicate 1-ml aliquots were infrared dried and planchet counted.

In subsequent experiments, groups of 20 cotyledons were harvested for the various color classes, and internal ethylene concentrations were then determined by the vacuum extraction technique (12).

RESULTS

Ethylene Concentration *versus* Percentage of Leaf Abscission. The percentage of leaf abscission at various time intervals as influenced by various ethylene concentrations is shown in Figure 1. Significant abscission was not observed until the end of the second 12-hr enclosure period. The magnitude of abscission was directly related to the ethylene concentration. The percentage of leaf abscission at 1, 15, and 90 μl/l ethylene was 13, 41, and 78% respectively. As the length of the total exposure period was increased from 24 to 48 hr, there was a gradual increase in the percentage of leaf abscission, except at 0.08 μl/l ethylene where no abscission occurred.

The youngest, just expanding terminal leaves and the cotyledons were the first leaves to abscise in all three experiments.

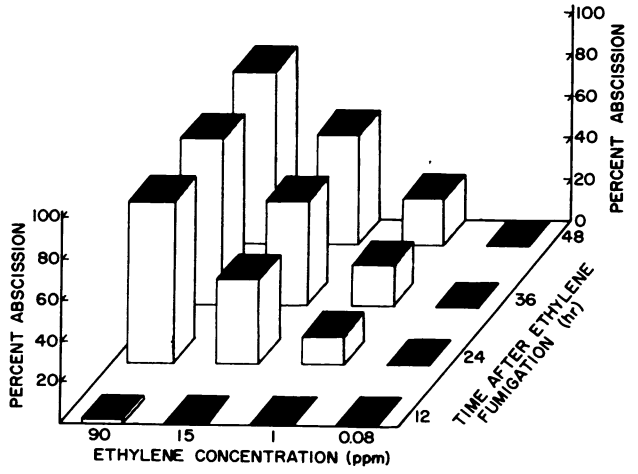

FIG. 1. Percentage of abscission of leaves from vegetative cotton plants at various time intervals following ethylene fumigation. Each value represents the average of three experiments (26-, 29-, and 25-day-old cotton plants, respectively) with each experiment containing six plants per treatment.

At the 90 and 15 μl/l ethylene concentrations, all of the youngest leaves had abscised at the end of 48 hr and 86 and 72% of the cotyledons had abscised. The last leaf to abscise in all three experiments was the first fully expanded true leaf. At 90 μl/l only 39% of the first primary leaves had abscised at the end of the experiment while at least 78% of all other leaves had already abscised.

Ethylene Concentration *versus* Auxin Transport Inhibition. The percentage of reduction in basipetal auxin transport capacity in cotyledonary petiole sections following 24-hr pretreatment periods with various ethylene concentrations is shown in Figure 2. The threshold level of ethylene required to reduce auxin transport was approximately 0.1 μl/l ethylene. Inhibition of transport increased linearly with increasing ethylene concentration up to approximately 10 μl/l ethylene. At 1 μl/l ethylene transport was reduced approximately 40%, whereas at 10 μl/l it was reduced approximately 75%. Between 50 and 100 μl/l ethylene auxin transport was maximally inhibited (85%), and higher concentrations of ethylene had no further effect. Since significant abscission occurred with ethylene levels above 1 μl/l in 24 hr (Fig. 1), yet transport was measured only in those cotyledonary petioles still attached to the plant at the end of the ethylene treatment, the data in Figure 2 may underestimate the effect of ethylene on auxin transport during the period in question.

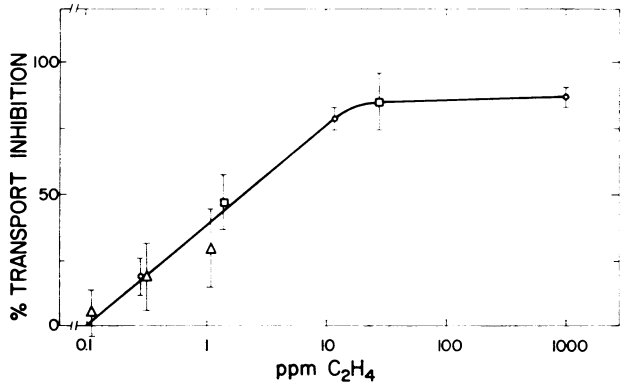

FIG. 2. Percentage of reduction in basipetal auxin transport capacity in cotyledonary petiole sections following a 24-hr pretreatment period with various concentrations of ethylene. Total uptake and recovery in control and ethylene-pretreated plants did not differ significantly. The percentage of reduction in transport was calculated on the basis of the percentage of total uptake which was transported out of the absorbing (0–5 mm) segment. Bars through data points represent standard deviations.

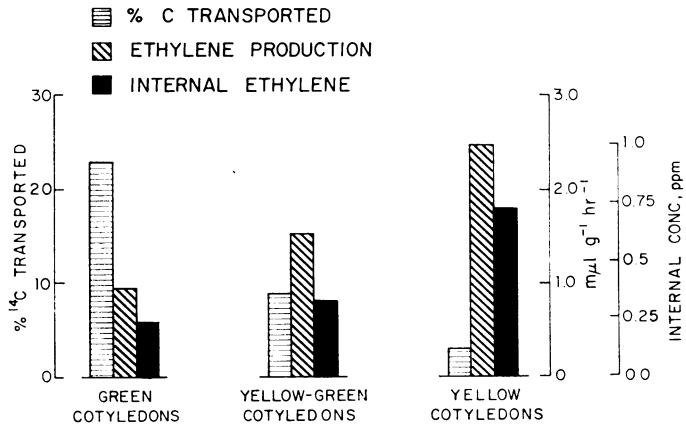

FIG. 3. Basipetal auxin transport capacity, ethylene production rate, and internal ethylene concentration of three physiological ages of cotton cotyledons. Auxin transport and ethylene production rate data are the average of two separate determinations from both greenhouse and growth room grown plants. Internal ethylene concentration data are the average of two experiments using growth room grown plants.

A double reciprocal plot of the data presented in Figure 2 was made with the same general assumptions made by others applying such kinetic treatments to physiological processes (15, 22, 37). The V_{max} obtained from this plot was 87% and the Michaelis-Menten constant was 2.22 μl/l ethylene in the gas phase or 9.5 nM.

Cotyledonary Age *versus* Transport Capacity, Ethylene Production, and Internal Ethylene Concentration. The basipetal auxin transport capacity, ethylene production rate, and internal ethylene concentration of the three color classes of cotyledons are presented in Figure 3. Cotton cotyledonary abscission in the intact plant under favorable conditions is preceded by a gradual color transition from dark green to bright yellow, a process requiring approximately 11 days for plants grown in a growth room. The color classes of cotyledons used in this study therefore represent cotyledons of three different physiological ages.

Basipetal auxin transport capacity in the cotyledons was found to decrease with increasing age. Concomitant with the decline in auxin transport capacity with age was an increase in the rate of ethylene production. The internal ethylene concentrations reflected the increased ethylene production rates with age or senescence.

DISCUSSION AND CONCLUSIONS

The hypothesis under consideration in the present study is that ethylene influences or controls abscission through its effect on auxin transport. It has been established, at least for *Coleus* (30, 53, 55), bean (36), and cotton (51), that as the petiole becomes progressively older there is a gradual decline in the basipetal auxin transport capacity. The auxin transport velocity does not decline. Ethylene produces an effect similar to aging on auxin transport capacity (10, 16). Jacobs (31) has pointed out how similar the family of basipetal auxin transport curves from progressively older *Coleus* petioles is to those obtained from plants given progressively longer exposures to ethylene (compare Fig. 12 of [30] to Fig. 3 of [16]). Furthermore, it appears more than a coincidence that the pulvinus tissue of bean explants should be the principal site of ethylene production (28) and ethylene responsiveness (19) and also possess a reduced capacity to transport auxin (32). It also appears relevant that both ethylene production rates and internal levels are from two to six times greater in the cotton leaf petiole, the auxin transport pathway between blade and abscission zone, than in the blade (35).

If the disruption of auxin transport by ethylene plays a regulatory role in abscission, this disruption must occur prior to abscission. In general, ethylene fumigation resulted in leaf abscission only after the plants were exposed to ethylene for 12 hr (Fig. 1). Only 2 to 3 hr is required for ethylene to significantly disrupt basipetal auxin transport in intact plants (9, 16, 39) or excised stem sections (10), and the reduction increases with the length of exposure to ethylene. Thus, the 12-hr lag period before abscission occurs appears adequate for ethylene to have significantly disrupted auxin transport prior to abscission.

Ethylene concentration of 1 μl/l and above stimulated abscission, whereas 0.08 μl/l resulted in no abscission even after 48 hr (Fig. 1). A similar threshold level of ethylene necessary to induce abscission has recently been reported by Wiese and

DeVay (56). If an ethylene-mediated disruption of auxin transport is important in abscission, then there should be a similar threshold level for the effect of ethylene on transport, and this was observed (Fig. 2).

The results presented in Figures 1 and 2 were obtained with exogenously applied ethylene and are thus pertinent to the action of ethylene as a defoliant. A different question is whether ethylene-mediated auxin transport modification is involved in natural abscission. To answer this question we attempted to determine the relationship between the natural decline in basipetal auxin transport in the petiole with age and the level of ethylene in the petiole. As previously observed (51), the basipetal auxin transport capacity was found to decline gradually during senescence (Fig. 3). More important, however, is the finding that this phenomenon is paralleled by a gradual increase in ethylene production and internal ethylene content (Fig. 3). These results are consistent with the data of Jackson and Osborne (28), which show a progressive increase in ethylene production by tissue surrounding the abscission zone prior to abscission and a decline in ethylene production only after separation was complete.

There are several indications that the vacuum extraction technique (12) gives a close approximation of actual internal ethylene levels. The method will accurately (±5–10%) determine added ethylene in amounts ranging from levels equal to those in nonfumigated control plants up to 11 μl/l (12). McAfee (35) found a consistent correlation between vacuum extracted internal ethylene levels and production rates in cotton leaves and petioles.

It is also possible to assess the physiological significance of the ethylene production rates observed (Fig. 3). Burg (14) has suggested that an ethylene production rate of 3 to 5 mμl $g^{-1}hr^{-1}$ approximates an internal ethylene concentration of from 1 μl/l to a few μl/l. Application of this ratio to cotton cotyledons suggests that the maximum production rate of 2.5 mμl $g^{-1}hr^{-1}$ reported here (Fig. 3) should reflect an internal ethylene concentration of 0.5 to 0.8 μl/l. It is important to note that McAfee (35) found both production rates and internal ethylene levels to be two to six times higher in petioles than leaf blades. Thus, this value may well be conservative.

Important considerations bearing on the significance of the absolute internal ethylene concentration in the tissue are (*a*) the length of time the tissue is exposed to this level of ethylene and (*b*) the sensitivity of the tissue to this level of ethylene. It must be remembered that cotton cotyledonary abscission under natural conditions requires several days. During this period of time the ethylene produced by the petioles is increasing (Fig. 3, also 28) and, assuming this level is above the threshold required to reduce transport, auxin transport is steadily declining. As auxin transport declines, there is evidence (45; unpublished data) that the sensitivity of the transport system to ethylene increases. Therefore, a low level of ethylene production initially may reduce auxin transport sufficiently to increase the sensitivity of the tissue to the gas and thus greatly amplify the effect of a rather small total increase in ethylene production during abscission (Fig. 3).

Shortly following excision, the transport capacity in bean petiole sections (44, 45) and explants (44) rapidly declines, but this decline can be prevented if auxin is supplied to the excised tissue immediately following excision (45). Furthermore, such treatment also prevents the inhibitory action of ethylene in transport. We have observed a similar effect with cotton stem sections. If excised sections are incubated in auxin solutions of 1 μM IAA or greater during ethylene fumigation, the effect of ethylene on transport is partially blocked (unpublished data). In contrast, auxin supplied several hours following excision actually promotes the decline in transport capacity (see Table V of 45). As Osborne and Mullins (45) noted, these effects are remarkably similar to the preventive effects of auxin on abscission when supplied to bean explants immediately after excision (stage I) in contrast to the stimulating effect of auxin supplied several hours following excision (stage II). Burg (14) has suggested that the ethylene produced by freshly cut bean explants (about 3 mμl $g^{-1}hr^{-1}$) reflects an optimally stimulatory level of ethylene which is approximately 1 μl/l. Since this level of ethylene should also be effective in reducing auxin transport (Fig. 2), a consideration of this effect in explaining the preventive action of auxin on abscission seems warranted.

The experiments conducted in this study indicate that senescence, an increase in ethylene production, and a decrease in auxin transport are all closely related and are an integral part of the complex series of events which occur during abscission. These observations suggest that endogenously produced ethylene may function in part to regulate leaf abscission through its effect on auxin transport. While the results supporting such a theory are equivocal, the strong influence exhibited by both auxin (see "Reviews" 17, 18, 29, 31, 48) and ethylene (see "Reviews" 13, 47, 50) on abscission, the close correlation between increased ethylene production and a decline in auxin transport with senescence (Fig. 3), and the close association of senescence with abscission (19, 28, 29, 34, 43) indicate that such a relationship may exist.

The results obtained in this study fit well into the framework of the aging-ethylene hypothesis of abscission of Abeles (2), which has been supported by the data of Webster (54) and Morré (42). The primary role of auxin as a juvenility factor in this hypothesis is to retard aging, thereby preventing an ethylene mediated induction of cell-wall degrading enzymes. These studies, however, have been primarily involved with the role of ethylene in the hydrolytic enzyme induction process. The results presented here allow one to view the induction process, studied primarily in explants in which the native auxin supply has been removed and wounding has produced a significant stimulation of ethylene synthesis (14), within the over-all sequence of events in the intact plant.

On the basis of the data presented here, one avenue for this induction process is through an ethylene-mediated inhibition of auxin transport. As the leaf senesces, ethylene production increases, reducing the auxin supply (juvenility factor) to the cells of the separation layer via an effect of ethylene on auxin transport and perhaps also destruction (26, 39) and synthesis (52). These effects of ethylene do not exclude the possibility that other factors may also be important in the regulation of the auxin supply at the abscission zone. As a result of this reduction of auxin supply, the aging process in these cells is set into motion, and the cells become increasingly sensitive to ethylene. Ultimately the separation-layer cells pass the threshold from insensitivity to sensitivity to ethylene, and the initiation of RNA and protein synthesis (5, 6, 54) occurs, leading to the formation of cell wall degrading enzymes (3, 27, 42) and perhaps their release or secretion from the cytoplasm to the cell wall (Abeles, personal communication, published in Plant Physiol. 47: 7–9, 1971 after the present paper was accepted for publication).

This series of events would give ethylene several roles in abscission. The increased amounts of ethylene which are produced as the leaf ages would reduce auxin transport, thereby increasing the sensitivity of the cells in the abscission layer to ethylene, and would also induce the formation of the enzymes required for abscission and regulate their secretion into the cell wall.

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