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Ethylene-induced Leaf Abscission Is Promoted by Gibberellic Acid[1]

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ABSTRACT

Gibberellic acid (GA_3) promoted leaf abscission from cotton (*Gossypium hirsutum* L.) plants exposed to ethylene. With mature plants, only the rate of abscission was increased, but when vegetative plants were exposed to ethylene for 4 days or less, the amount of abscission was increased markedly. Promotion of abscission occurred at near saturating ethylene levels (10 μl/liter), over a wide range of GA_3 concentrations, and with both GA_3 and GA_7.

GA_3 promoted abscission when Ethephon was substituted for ethylene and at locations not receiving direct application of GA_3. The magnitude of the abscission promotion by GA_3 was greater than that resulting from auxin transport inhibitors or abscisic acid. The characteristic inhibition of abscission by auxin occurred. The responses suggest that endogenous gibberellins may be involved in rapid abscission of apical leaves from vegetative cotton plants exposed to ethylene. Application of GA_3 may offer an additional option in agricultural manipulation of abscission and dehiscence.

Recent research on cotton has characterized the relationship of leaf age or position of insertion on the stem to abscission induced by ethylene (21). When vegetative plants of several ages are exposed to defoliating levels of ethylene, the young, partially expanded and unexpanded leaves abscise faster or to a greater degree than basal (mature but not senescent) leaves on the same plant. This response could be due to either differences in the mechanism of abscission between expanding and mature leaves or to differences in the hormone complement of the respective leaves. The balance of hormones is easily modified by applications of those substances considered active: thus, the latter hypothesis was tested first. If some aspect of the hormone balance in young leaves causes them to abscise more rapidly than those more basal on the same plant, then more of the critical substance should promote fall of basal leaves or delay fall of apical leaves. The results of such experiments are presented here.

MATERIALS AND METHODS

Seeds of Stoneville 213 cotton (*Gossypium hirsutum* L.) were planted in peat-perlite medium, watered with a modified Hoagland's solution, and grown in a growth room maintained at a 15-hr day of 2400 ft-c of cool white fluorescent and incandescent light, 27 C day, 22 C night, relative humidity uncontrolled.

Plants 25 ± 1 days old unless otherwise specified were inventoried for leaf number and development, treated with various plant growth substances, and placed in gas-tight growth chambers with recirculating air flow. GA_3 was applied as an aqueous spray to wet the leaves or with a brush to selected parts of the plant. Growth conditions in the chambers were the same as above; in addition, relative humidity was maintained at 50% in the day and at 60% at night. The chambers were constructed with glass on the top and two sides, and the light sources were above the chambers. Ethylene was metered into one chamber to the desired level, and a second chamber was used as the control. Ethylene levels were verified by gas chromatography (19), and depletion of CO_2 was prevented by maintaining the constant entry of 25% fresh air.

Leaf abscission in response to a 5-g weight applied to the end of each petiole was observed daily and recorded by leaf position on the plants. All data are averages of four plants per pot (one exception, Fig. 2) and five pots per treatment except where otherwise noted. After termination of an ethylene or control treatment, plants were returned to the growth room in which they were germinated, and their subsequent behavior was observed.

RESULTS

The typical pattern of ethylene-induced abscission described earlier (21) was duplicated in this study (data not given). Young leaves, in particular those ready to unfold and expand, abscised first, followed by younger and older leaves (see controls, Figs. 4 and 5). Leaf 1 (most basal) was usually least sensitive to ethylene. With time, leaves became more sensitive to ethylene beginning at the base and progressing upward. Progressively older plants exhibited more abscission at a given ethylene level (Figs. 1 and 4 in ref. 21, and the unpublished data mentioned above).

Application of GA_3 and naphthaleneacetic acid to plants gave the most pronounced results. GA_3 promoted leaf abscission, particularly with the three youngest ages (Fig. 1). The typical inhibition of abscission by auxin (12, 19) occurred with the 25-day-old plants, and it was clearly demonstrated in confirming experiments for both IAA and naphthaleneacetic acid at 10^{-3} M.

ABA and kinetin (6-furfuryladenine) at 10^{-4} M produced moderate changes in ethylene-induced abscission (results not

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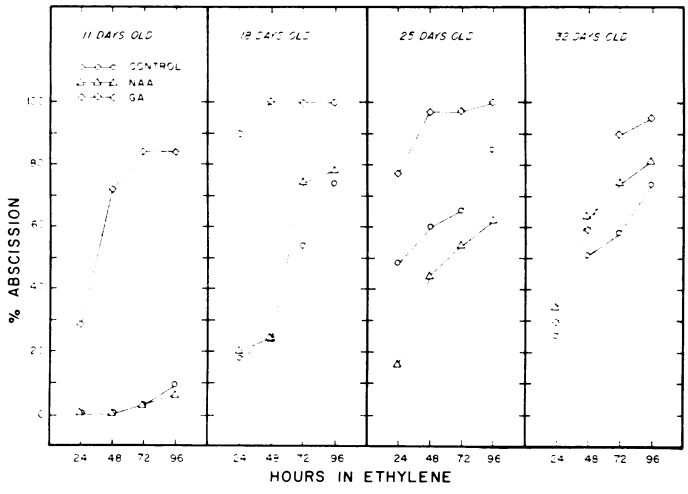

FIG. 1. Effect of 10^{-4} M GA_3 and naphthaleneacetic acid (NAA) on leaf abscission induced by 10 μl ethylene/l air. Total per cent abscission of cotyledons and leaves on plants of the ages indicated during 4 days exposure to ethylene. Data are averages of two pots per treatment. There was no abscission from control, GA; and naphthaleneacetic acid-treated plants in a chamber without ethylene.

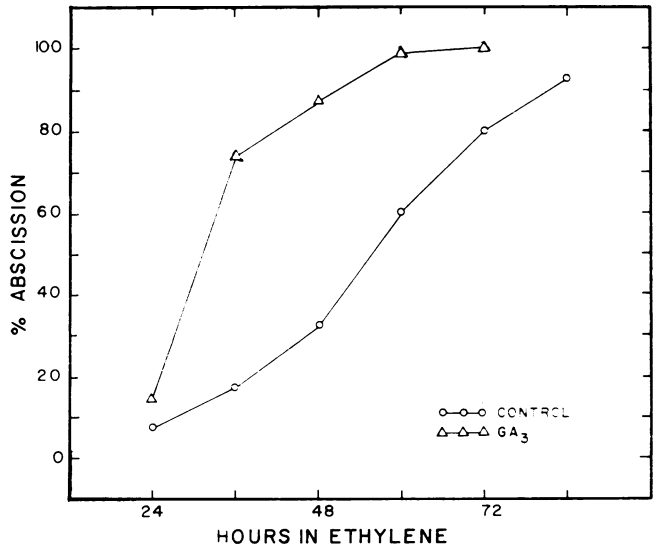

FIG. 2. Effect of 10^{-4} M GA_3 on leaf abscission induced by 10 μl ethylene/l air. Total per cent abscission after 3 days on individual mature cotton plants.

shown). ABA promoted abscission, particularly of basal leaves, and the effect decreased with increasing age of plants. The effect of kinetin was less pronounced, but it tended to retard abscission of the oldest leaves. None of the compounds tested caused any abscission in control plants (in the chamber without ethylene). The relative effects of auxin, GA_3, ABA, and kinetin (10^{-3} M) were verified with 32-day-old plants in 2 μl ethylene/l air. After 96 hr IAA reduced abscission more than half, GA_3 more than doubled it; the effects of ABA and kinetin were much less pronounced.

Since the promotive effect of GA_3 was greatest, it was studied further. GA_3 increased the initial rate of abscission of leaves from mature, fruited cotton plants exposed to ethylene (Fig. 2), but the amount of abscission from the control and treated plants became essentially equal as the experiment progressed. These observations were verified in additional replicated tests. GA_3 and GA_7 were equally effective in promoting ethylene-induced abscission, and both were more effective than the auxin transport inhibitor Alanap (Fig. 3). Auxin transport inhibitors such as Alanap (N-1-naphthylphthalamate), DPX-1840 (3,3a-dihydro-2-(*p*-methoxyphenyl)-8H-pyrazolo-(5,1-*a*)isoindol-8-one), TIBA (2,3,5-triiodobenzoic acid), and morphactins (2-chloro-9-hydroxyfluorene-9-carboxylic acid) promote ethylene-mediated leaf abscission (20, 22).

The promotion of ethylene-induced abscission by GA_3 was determined over the concentration range from 10^{-2} to 10^{-6} M (Fig. 4). The extreme concentrations were only slightly more or less effective, respectively, than 10^{-3}, 10^{-4}, and 10^{-5} M GA_3.

When Ethephon (Ethrel) was substituted for ethylene, promotion of abscission by GA_3 was less pronounced (Table I). For abscission of leaves of vegetative cotton plants, Ethephon has a fairly sharp threshold concentration range; thus, 10^{-1} M often caused complete defoliation and 10^{-3} M gave little. When concentrations between 2×10^{-2} and 5×10^{-2} M were tested, the enhancement effect of GA_3 was expressed. GA_3 and DPX-1840 were tested together and alone (data not given). GA_3 most effectively promoted abscission, and together with DPX-1840 the response was neither synergistic nor additive.

The enhancement of ethylene-induced abscission by auxin transport inhibitors can be achieved with the material applied only to the petioles (20); so, the relationship of location of application and effect of GA_3 was determined. Generally, application of GA_3 to either stems, petioles, or leaf blades enhanced leaf abscission over controls, but the degree of response depended upon plant age. Application to the blade was slightly more effective on 24-day-old plants (Fig. 5), whereas petiole application was more effective on 31-day-old plants (data not presented). The localized application of GA_3 promoted abscission of untreated leaves above (leaves 7 and/or 8, Fig. 5 and leaves 6 to 8, Fig. 6). Thus, the GA_3 effect was not restricted to the site of application.

DISCUSSION

In retrospect, the determination that GA_3 promotes ethylene-mediated leaf abscission should not have been too sur-

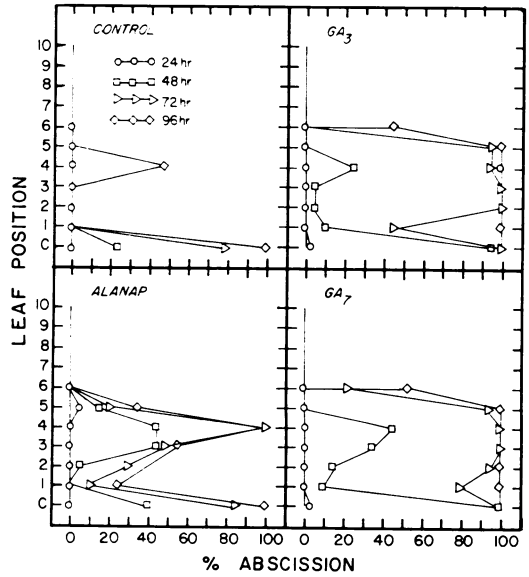

FIG. 3. Effect of 10^{-4} M GA_3, GA_7 or Alanap on leaf abscission induced by 1.75 μl ethylene/l air. Total per cent abscission of cotyledons (C) and leaves at different positions for 4 days.

prising. Carns *et al.* (8) found that GA_3 accelerated abscission of cotton explant petioles, and that observation has been repeated and extended in a number of more recent studies on cotton (2–4, 9, 13, 18, 23), *Coleus* (5, 24), and other plants (10, 11, 14). All of these studies employed debladed petioles, and none of them examined the interaction of GA and ethylene. The opposite effects of GA and ethylene in several responses have been well recognized, but their similar effects on senescence related processes have not been overlooked (17, 25). The present findings may extend the understanding of the natural regulation of abscission and allow improvement of agricultural defoliation practices.

The anatomy of abscission has been carefully studied, and some differences in effects of GA, ethylene, and other substances have been recognized. In cotton, natural abscission involves both cell division and breakdown of cell walls (1, 4, 15, 16). Cell division is also a component of natural abscission in beans (6, 27). GA promotes cell division in abscission zones (2, 4, 18), but the effect of ethylene on cell division is less clear. High levels of ethylene (1666 μl/l air) inhibited cell division which occurred extensively in "normal" (control) bean leaf explants (6). No difference in cell division in ethylene-treated and control bean explants was reported in other studies (27, 28), but variations in the levels of endogenous GA may result in some differences in response to applied ethylene. Inhibition of both cell division and DNA synthesis appears to be rather general actions of ethylene (7). The char-

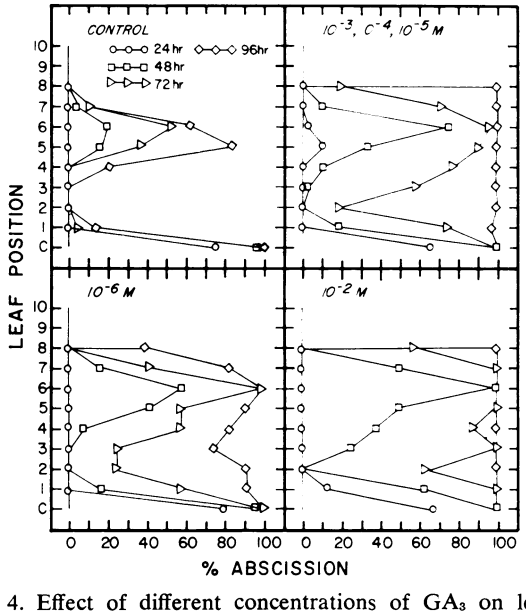

FIG. 4. Effect of different concentrations of GA_3 on leaf abscission induced by 1.56 μl ethylene/l air. Total per cent abscission of cotyledons (C) and leaves at different positions for 4 days. Data are averages of three pots per treatment; data for the 10^{-3}, 10^{-4}, 10^{-5} M treatments are presented as averages due to similarity of results.

Table I. *Effect of GA_3 on Ethrel-induced Abscission from 25-day-old Cotton Plants*

Experiment	Ethrel Concn (M)						
	0	1×10^{-2}	2×10^{-2}	3×10^{-2}	4×10^{-2}	5×10^{-2}	1×10^{-1}
	% abscission						
Experiment I (48 hr)							
+GA[1]	0	70					81
−GA	0	32					68
Experiment II (36 hr)							
+GA[2]	0		93	95	90	95	
−GA	0		53	56	65	68	

[1] GA_3 spray 1×10^{-2} M.
[2] GA_3 spray 1×10^{-3} M.

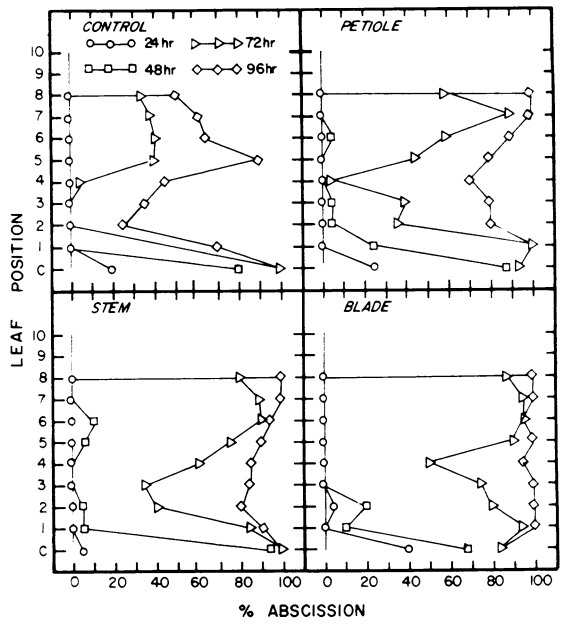

FIG. 5. Effect of site of application of GA_3 on leaf abscission induced by 4.28 μl/l ethylene. Total per cent abscission at different positions for 4 days. GA_3 at 10^{-1} M in 40% aqueous ethanol applied with a brush. Blades 8 and above and petioles 7 and above were not treated.

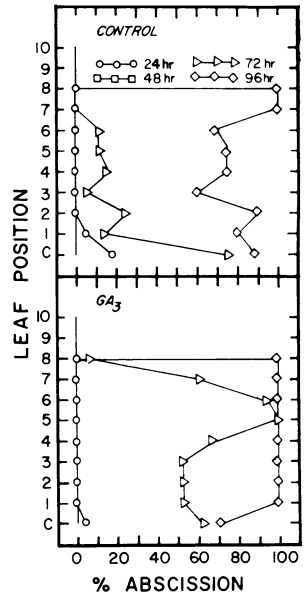

FIG. 6. Effect of 10^{-1} M GA_3 in 40% aqueous ethanol applied to stems on leaf abscission induced by 2.86 μl/l ethylene. Total per cent abscission at different positions for 3 days. Plants in chamber without ethylene lost no leaves and a small percentage of cotyledons with either GA_3 or carrier (control). Melted lanolin was applied to petioles and stem above and below treatment area to prevent surface movement of the GA_3 solution. Stems above leaf 6 were not treated.

acteristic promotion of lateral cell expansion by ethylene (7) does occur in abscission (27).

The observations discussed here suggest the following explanation or hypothesis for the interaction of GA_3 and ethylene in abscission. (*a*) Exogenous ethylene initiates abscission (Figs. 1 and 3) but suppresses cell division (7); (*b*) added GA_3 promotes cell division (4) so that the over-all progress of abscission is more prompt (4, 18, and Figs. 1 and 3); (*c*) natural leaf fall would involve both endogenous gibberellin and ethylene; (*d*) the natural abscission process should involve both cell division and cell wall breakdown and that is what has been observed (1, 4, 6, 15, 16, 27). This explanation is in harmony with the fact that differentiation of the abscission zone is often late in the course of leaf maturity (4, 15, 27), and cell division is one of the sequential anatomical stages involved (27, 28). Further, a speculative explanation for why young leaves fall first in ethylene can be offered. In young cotton plants, many leaves would not have differentiated abscission zones. Relatively high GA levels in the apical leaves would promote differentiation of abscission zones in response to ethylene (including cell division) over that in basal leaves. In older plants, most of the primary leaves would have differentiated abscission zones (reducing one need for gibberellin), and gibberellin would have less effect on ethylene-induced abscission. Both of these responses (young leaves fall first in ethylene and old leaves relatively insensitive to promotion of abscission by GA) occur (21 and Fig. 2). A critical role of the endogenous GA level may be argued further because kinetin and ABA have less effect on ethylene-induced abscission than GA_3 (text).

Carns *et al.* (8) observed the unusual process of stem abscission in cotton explants and noted that it was promoted by GA_3. Webster and Leopold (28) have examined this process in beans recently. Involved is differentiation of an abscission zone in a location where one does not normally occur. The location is shifted by ethylene and formation is prevented by auxin (28). These findings, along with the GA_3-ethylene interactions detailed here, suggest that the formation of an abscission zone occurs at an interface between different hormone sources which produces a localized environment that results in the cell division, differentiation, and other characteristics of the specialized separation tissue. From this view point the "juvenile" or growth-promoting hormones, GA and auxin, may be of equal importance with the senescence or growth-inhibiting hormones, ethylene and ABA, in controlling the sequential anatomical and histochemical changes of the abscission process.

Since GA_3 accelerates ethylene-induced abscission, it may improve the defoliating action of Ethephon (19) as auxin transport inhibitors do (20, 22). Another possible use of GA_3 in defoliation is suggested by the observation that Endothal (7-oxabicyclo-(2,2,1)-heptane-2,3-dicarboxylic acid) and Ethephon have a synergistic effect on abscission (26). At levels which caused no leaf shed alone, Endothal and Ethephon together induced abscission of leaves of bean and several woody species. Agriculturists now have the option of using any combination of conventional defoliants, Ethephon, auxin transport inhibitors, and GA_3 which will give the optimum abscission or dehiscence response.

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