Stimulation of Ethylene Evolution and Abscission in Cotton by 2-Chloroethanephosphonic Acid[1]

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Abstract. **Ethrel, a mixture of 2-chloroethanephosphonic acid and its ethyl ester, hastens abscission of leaves, debladed petioles, and flower buds of cotton plants (*Gossypium hirsutum*, L.). Both young and old leaves abscissed while still green. Application of Ethrel stimulated evolution of ethylene, and this response preceded abscission. Air concentrations of ethylene around enclosed, treated-plants were adequate to produce abscission in plants. Non-treated plants defoliated when enclosed with plants sprayed with Ethrel. The stimulation of abscission of explant petioles by Ethrel was reversed by naphthalene acetic acid. The stimulation of abscission by Ethrel was concluded to be mediated by ethylene.**

Materials that stimulate ethylene production, either as substrates or by indirect action, may be useful growth regulators (18). This proposal was based on the finding that exogenous auxins stimulate ethylene production and the resulting conclusion that ethylene may in turn contribute to the symptoms previously attributed solely to auxin (12, 19, 20, 25). The significance of auxin-induced ethylene production has since been established by a series of investigations of specific plant responses (1, 4, 5, 6, 17, 21). Abscission, growth inhibition, tissue proliferation, flowering, root growth inhibition, geotropism, fruit maturation, epinasty and leaf senescence have all been shown to involve a modification of ethylene evolution by auxin.

An opportunity to test an apparent "ethylene producing", growth regulator occurred when Amchem Products, Incorporated released an experimental chemical whose effects parallel those of exogenously applied ethylene (2). The active ingredient is 2-chloroethanephosphonic acid ($ClCH_2CH_2PO_3H_2$), but the product, named Ethrel, also contains the mono 2-chloroethyl ester (7).

Since this study was initiated, Warner and Leopold (23) have reported that Ethrel, then identified by the formulation code name Amchem 66-329, increased the ethylene production of pea stem sections. Cooke and Randall (7) reported that 2-chloroethanephosphonic acid is converted quantitatively to ethylene by a base catalyzed elimination reaction. They postulated that the conversion of the ester to ethylene in the plant is mediated by hydrolytic enzymes.

The present paper reports the effect of Ethrel on abscission and the mechanism by which the effect occurs.

Methods and Materials

Cotton grown in a greenhouse in inert media supplied with Hoagland's nutrient solution was used in all tests. For defoliation tests, potted plants were treated and then maintained in a greenhouse or placed outside and leaf fall noted periodically. In addition, the activity of the compound was determined by the cotton cotyledonary node explant bioassay which has been described previously (22).

In experiments where ethylene production was measured, plants were treated in the laboratory, enclosed in gas-tight containers and maintained at 32° in continuous light (135 ft-c). Following each ethylene determination, the plant containers were opened and thoroughly ventilated to remove all ethylene. At this time each plant was shaken gently and abscission of leaves and fruiting forms recorded.

Plants were treated by spraying with water or Ethrel in distilled water to the point of run off. Plants were allowed to dry before they were moved or enclosed. Concentrations of active ingredient were expressed as molar concentration of 2-chloroethanephosphonic acid equivalents.

Ethylene was measured with a Model 810 F&M Gas Chromatograph using a flame deionization detector, 6 feet by one-eighth inch activated alumina column, a 5cc gas sampling valve, helium carrier gas, and ambient oven temperature. Ethylene was identified by retention time and co-chromatography with ethylene. The identity of the emanation from Ethrel-treated plants was verified by complete absorption of the emanation in cold mercuric perchlorate and quantitative release by $LiCl_4$ (24). The amount

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of ethylene was determined from a standard curve based on peak area.

For convenience, the test material (formulation code Amchem 66-329) will be referred to as Ethrel.

Results

Abscission. Ethrel accelerated the defoliation of cotton (table I). Ethrel also accelerated abscission of isolated petioles of cotyledonary node explants (table II).

Ethylene Evolution. Ethrel caused a marked increase in ethylene evolution by flowering cotton plants (Fig. 1). In another experiment with larger plants (Acala 4-42 variety) air concentrations of ethylene were 65 and 15.5 ppm at 32 and 50 hr after treatment.

The question of whether the amount of ethylene produced was adequate to accelerate abscission was considered. The time course of abscission in plants treated with 9 to 10 ppm ethylene was similar to that of the plants sprayed with Ethrel (Fig. 2). However, in the ethylene treatment the abscission response was delayed because the gas was not added

Table I. *Defoliation of Cotton Plants Sprayed With Ethrel September 26, 1967*

Treatment	Abscission 2 Days	Abscission 8 Days
Greenhouse plants[1]	%	%
Control	0	2
1.4×10^{-2} M	3	86
2.8×10^{-2} M	10	90
5.5×10^{-2} M	5	50 + 48D[2]
Outside plants[3]		
Control	...	0
5.5×10^{-2} M	...	75

[1] Mature Deltapine TPSA plants, 2 reps/treatment, open bolls.
[2] Dessicated, non-abscissed leaves.
[3] Preflowering, vegetative Deltapine TPSA cotton plants, 2 per pot, 7 pots per treatment. Leaves which did not fall in 8 days were the just fully expanded leaves of intermediate age.

Table II. *Abscission of Petioles of Cotton Cotyledonary Explants Dipped for 15 Min in Ethrel*

Three week-old, Acala 4-42 seedlings cut to yield 10 mm apical stem, 25 mm petioles and basal stem. 2 reps/treatment, 10 explants per rep.

Ethrel concn.	Abscission 24 Hr	Abscission 48 Hr	72 Hr
M	%	%	%
Control	0	3	20
6.9×10^{-5}	20	90	93
6.9×10^{-4}	58	95	95
3.5×10^{-3}	38	100	...
6.9×10^{-3}	0	60	80
1.4×10^{-2}	0	48	63

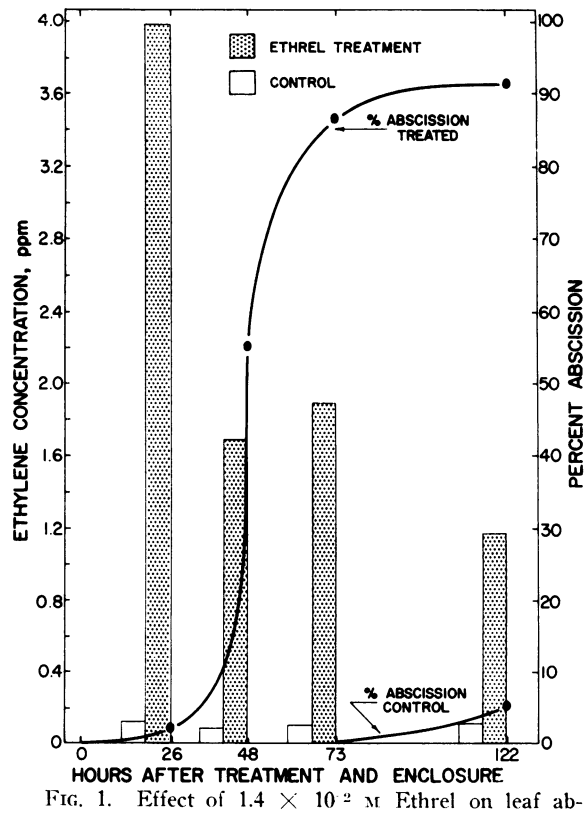

FIG. 1. Effect of 1.4×10^{-2} M Ethrel on leaf abscission and ethylene production per collection period by Stoneville 213 cotton plants. Two plants enclosed in each 12 liter bell jar which was ventilated after each ethylene assay (2 reps per treatment.)

until 27 hr after the plants were enclosed. In this experiment, 4 non-sprayed seedlings were enclosed in each bell jar (with treated plants). The abscission of leaves of the seedlings was hastened in the Ethrel-treatment containers, and the effect was paralleled by 9 ppm ethylene (Fig. 3). These results indicate that ethylene produced by the plants treated with Ethrel induced defoliation of the non-treated seedlings. Alternatively, the Ethrel might have been transferred physically to the non-treated plants. To further clarify this question, an experiment was conducted with flowering Acala 4-42 cotton plants. Three plants treated with 1.4×10^{-2} M Ethrel and 3 non-treated plants were enclosed in a large plexiglass chamber. The plants were physically separated by a polyethylene sheet, and the chamber was opened and ventilated each 24 hr. Abscission was rapid and essentially parallel in each group of plants. Abscission of flower buds, flowers and young bolls preceded that of leaves. The ethylene level after 24 hr was 16.5 ppm.

If the induction of abscission by Ethrel was caused exclusively by the ethylene produced in response to the Ethrel, then the abscission response should be blocked by exogenous auxin. Hall (10) has demonstrated that the abscission activity of ethylene is reduced or blocked completely if the plants are first

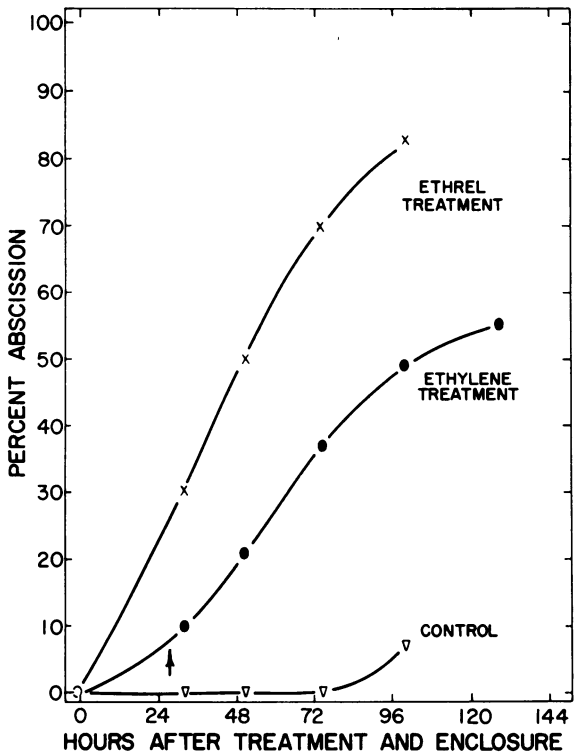

FIG. 2. Abscission of leaves of flowering Acala 4-42 cotton plants following treatment with 1.4×10^{-2} M Ethrel or 9 to 10 ppm ethylene. Ethylene treatment started 27 hr after spray application of Ethrel. Containers ventilated during each observation of abscission (2 reps per treatment).

treated with exogenous auxin. The supposition was tested with cotton explants (Fig. 4). Ethrel accelerated abscission, and auxin very clearly reversed or blocked this acceleration of abscission. As the concentration of Ethrel (and presumably ethylene) declined, the ability of auxin to delay abscission increased. These observations are consistent with the hypothesis that ethylene accounts for all of the abscission stimulating activity of Ethrel.

Since ^{14}C-labeled 2-chloroethanephosphonic acid was not available, no attempt was made to determine whether the observed ethylene arose directly from the material applied or the Ethrel stimulated the natural synthesis system. I have observed that ethylene is present in air spaces over the undiluted and diluted formulation. Ethylene was detected at about 0.2 ppm when the material sprayed on paper was enclosed in bell jars. All of the observations mentioned here were made under non-sterile conditions.

Discussion

The results here show that Ethrel will cause abscission. The ethylene produced by plants treated with Ethrel was present in amounts large enough to induce abscission. The stimulation of ethylene production preceded abscission (Fig. 1), and the time course of abscission due to ethylene and Ethrel was similar (Fig. 2 and 3). Therefore, the observed abscission is concluded to be due to the ethylene produced from or by the active ingredients in the formulation.

Other observations support the hypothesis that abscission caused by application of 2-chloroethanephosphonic acid is mediated by ethylene. Exogenous ethylene causes an extremely rapid abscission of flower buds, flowers and young bolls of cotton which precedes leaf abscission (10, 11), and the same behavior was noted with Ethrel. Further, with the exception of cotyledons, ethylene in high concentrations usually causes the abscission of the young terminal leaves of cotton before the mature or fully expanded young leaves (11, 12, 13). A similar pattern of abscission occurred in response to Ethrel (table I) where the just fully expanded leaves of intermediate age did not abscise, but all younger and older leaves did. The behavior of cotton leaves subjected to Ethrel and ultimately to ethylene generated from this substrate suggest that in cotton

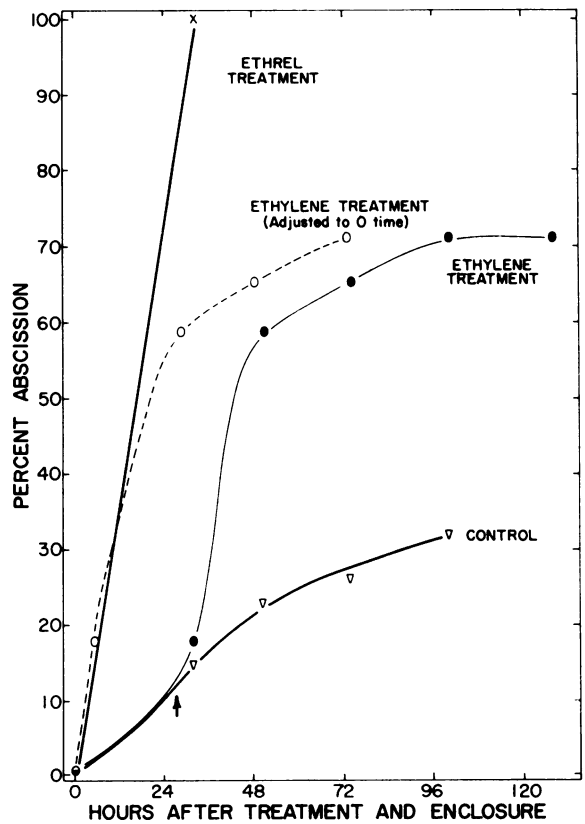

FIG. 3. Abscission of leaves and cotyledons of nontreated Acala 4-42 cotton seedlings enclosed with the various treatments outlined in Fig. 2. The plants were subjected to the atmosphere in the containers but not sprayed with Ethrel. Abscission in the control was senescing cotyledons.

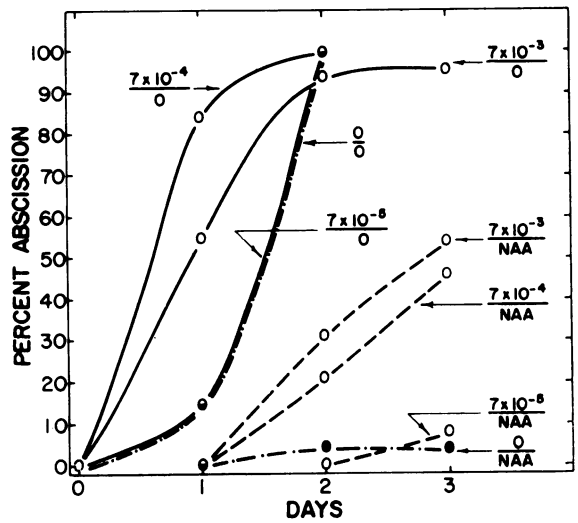

FIG. 4. Effect of Ethrel and naphthalene acetic acid on abscission of cotton cotyledonary node petioles. Curves are identified by fractional symbols which present the molar concentration of 2-chloroethanephosphonic acid equivalents over a notation of the presence or absence of NAA. NAA was supplied at 5.4×10^{-5} M. Data are the average of 2 experiments, 2 replications of each treatment per experiment. Explants were immersed in water or NAA for 15 min and then immersed in Ethrel for 15 min at the concentrations indicated.

ethylene responsiveness of intact leaves is not always directly correlated with age or senescence. Thus, cotton is an exception to the generalization that the sensitivity of intact leaves to ethylene increases with age (3, 8, 9).

Leiberman and Kunishi (15) have reported that propanal stimulates the production of ethylene by tissue slices of mature green tomatoes. Methionine, ethionine, and γ-methylthio α-ketobutyric acid are converted to ethylene by model systems (14, 16). It seems probable that several precursors to ethylene will be available for use in plant growth control. These materials appear to have an advantage over auxins which, in addition to stimulating ethylene production, produce other effects. Thus, 2,4-D stimulated ethylene synthesis and ethylene induced abscission; yet, except under specialized conditions, the "auxin-action" of auxins predominated and abscission was inhibited in spite of enhanced ethylene production (12). Ethrel was without auxin activity in the *Avena* straight growth test (23) and had the opposite effect of auxin in the cotyledonary node abscission bioassay (Fig. 4). An ethylene precursor, with no other hormonal activity, would have no action other than that of the ethylene it produces.

Ethrel and similar materials that stimulate ethylene synthesis are potentially useful in regulating any response that ethylene alone will regulate as well as some responses once considered to be caused by exogenous auxin (12, 25). The list of processes which may be regulated includes: rooting, epinasty of leaves, proliferation of tissue, growth inhibition, coloring and ripening of fruits, hydrolysis of storage materials and other metabolic changes, flowering, leaf movements, abscission, apical dominance and bud dormancy.

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