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Abscission Responses to Moisture Stress, Auxin Transport Inhibitors, and Ethephon[1]

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ABSTRACT

The three abscission-inducing agents—water stress, Ethephon, and auxin transport inhibitors—acted synergistically to promote leaf fall in cotton (*Gossypium hirsutum* L.). However, the synergism was primarily between stress and Ethephon. Auxin transport inhibitors did not promote the effect of stress alone, only promoted the effect of Ethephon in well watered plants and gave a very small promotion with stress and Ethephon together. Abscission was rapid in stressed plants treated with Ethephon and an auxin transport inhibitor, while leaves fell more slowly from well watered plants treated with Ethephon alone. This suggests that water stress or auxin transport inhibitors influence initial events in abscission; since an auxin transport inhibitor will replace the effect of stress but not Ethephon, an initial event in stress-induced abscission appears to be inhibition of auxin transport. Ethephon promoted lateral bud release, and auxin transport inhibitors did not duplicate that effect alone or promote it in combination with Ethephon.

Several lines of evidence suggest that a relationship may exist between abscission-modifying effects of water stress, auxin transport inhibitors, and ethylene in cotton. First, there is growing evidence that endogenous ethylene regulates leaf abscission (9, 18). The action of ethylene in abscission appears to be due in part to inhibition of auxin transport (4-6). Auxin transport inhibitors increase or hasten leaf abscission induced by exogenous ethylene or Ethephon (15, 16). There is some evidence that ethylene production in cotton petioles increases during water stress (13). Petioles contain levels of ethylene about 10 times higher than leaf blades (12). During water stress or following release from stress by watering, cotton plants defoliate (14). Finally, exogenous ethylene promotes the water stress-induced abscission of cotyledonary leaves (10). The evidence suggests that an increase in the synthesis of ethylene and interruption in the auxin flux serve to trigger the separation process.

The series of interrelations suggested to us that water stress, auxin transport inhibitors, and ethylene might have synergistic effects on leaf abscission. The evidence also suggested a way to investigate the mechanism of stress-induced abscission further.

[1] A preliminary report of this study appeared in Proceedings of the 1974 Beltwide Cotton Production Research Conferences, p. 33 (National Cotton Council, P.O. Box 12285, Memphis, Tenn. 38112).

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MATERIALS AND METHODS

Cotton plants (*Gossypium hirsutum*, L., c.v. Stoneville 213) were grown in a greenhouse in sand-peat moss-vermiculite mixture watered with Hoagland solution modified as described previously (17). Plants were in flowering to heavily fruited stage of growth with 12 to 20 expanded true leaves when experiments were initiated by withholding water.

Water stress was initiated by withholding water from the appropriate plants beginning on day zero. Growth regulators were applied when plants reached the desired level of stress; the time of application ranged between 16 and 24 hr before the pots were again watered. Aqueous solutions of the growth regulators were freshly prepared on the day of application to include 0.05% Tween 20 as a surfactant. Compressed air-activated glass atomizers were used to spray plants to a wet but not dripping stage. For dual treatments, plants were allowed to dry before the second growth regulator was applied. The auxin transport-inhibiting compounds applied were N-1-naphthyl phthalamate as the sodium salt (Uniroyal Chemical Co., Alanap) and 3,3a-dihydro-2-(*p*-methoxyphenyl)-8H-pyrazolo-(5, 1a) isoindol-8-one (E. I. du Pont de Nemours & Co., DPX-1840) (15). They were used interchangeably without apparent difference in our experiments. Ethylene treatment was accomplished by application of Ethephon:2-chloroethanphosphonic acid (Amchem Corp., Ethrel). Concentrations are indicated in the figures and table.

Plant water potentials were determined before dawn on the day of rewatering on the eighth true leaf with a Scholander pressure bomb (10). Abscission of leaves greater than 5 cm in diameter in response to gentle pressure on each main stem leaf was observed daily. In some experiments, plants were maintained for several weeks following the stress-growth regulator treatments and growth of lateral buds was observed.

RESULTS

The combination of the three abscission-inducing agents—water stress, Ethephon, and auxin transport inhibitor (ATI)[5]—consistently caused the greatest leaf fall, but the extent of abscission was not much greater than that resulting from the combination of water stress and Ethephon without a transport inhibitor (Fig. 1). The combination of agents acted synergistically causing about 32% more total abscission than the sum of the three applied singly. The ATI applied without Ethephon to stressed or well watered plants had no apparent effect, but it gave the marked synergism reported earlier (15) when applied with Ethephon to well watered plants (Fig. 1). These results were confirmed in several other experiments not reported in detail.

[5] Abbreviation: ATI: auxin transport inhibitor; Ψ: leaf water potential.

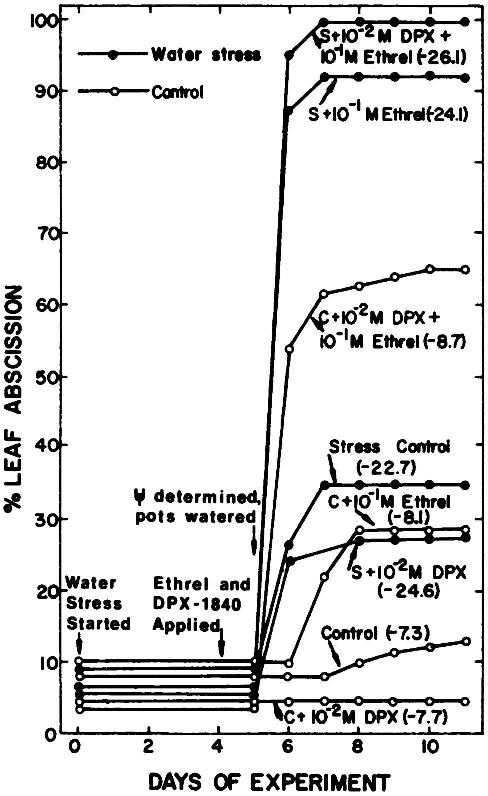

FIG. 1. Effects of DPX-1840, Ethrel, and water stress (S) alone and in combination on leaf abscission; control (C) plants were watered daily. Each treatment represents averaged data from four to six plants each with 11 to 13 leaves greater than 5 cm in diameter by day zero which served as the basis for the percentages shown. Ψ in bars at the time of rewatering are given in parentheses. Some abscission of old leaves had occurred when the experiment was initiated.

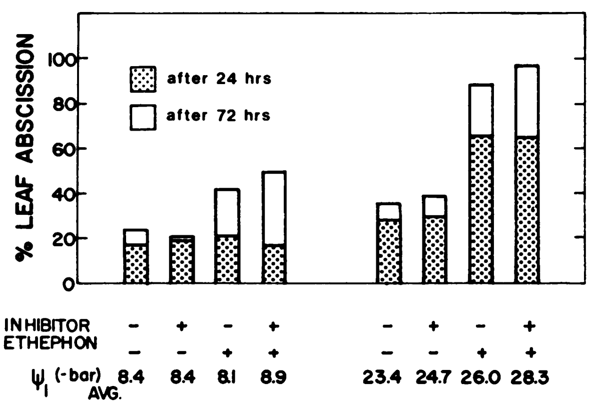

FIG. 2. Summary of three experiments in which combinations of water stress, Ethrel, and auxin transport inhibitors were evaluated as to their abscission-inducing potential. The experiments were similar in plan to Figure 1.

We found that in stressed plants, 10^{-1} M Ethephon was necessary to maximize abscission (data not given); this amount usually produces less than 50% abscission in nonstressed plants (Fig. 1). Applied with Ethephon, DPX-1840 and Alanap maximized abscission at 10^{-2} and 10^{-3} M (data not given). When ATI and Ethephon were applied at the beginning of the stress period, there was no abscission-promoting interaction between them and stress (data not given).

Abscission of leaves from plants subjected to the combination of water stress and Ethephon was very rapid (Fig. 1). Much of the abscission on stressed plants occurred in the first 24 hr after rewatering; yet, the reverse was true for nonstressed plants treated with Ethephon alone or in combination with ATI (Fig. 2). Another characteristic of the system is that with a fairly severe treatment that maximizes abscission in stressed plants (10^{-1} M Ethephon and 10^{-2} M ATI, Figs. 1 and 2), abscission maximizes at the fairly low stress levels of -10 to -12 bars (Fig. 3). Much higher levels of water stress alone are required to maximize abscission (14).

During the course of the study, the experimental treatments were observed to have a significant effect on release of lateral buds from apical dominance. Under the growing conditions employed, the plants characteristically had a single stem. Ethephon and Ethephon plus ATI promoted lateral bud growth significantly above controls (Table I). ATI alone did not promote lateral bud growth; thus, the effect of the ATI plus Ethephon treatment must have been primarily due to the Ethephon. Stressed plants always exhibited greater abscission, but less bud growth, than well watered plants comparing either Ethephon or Ethephon plus ATI treatments. Stress may have irreversibly damaged some buds thereby reducing the potential for lateral bud release. The control abscission value in experiment 1 is unusually high because in this test plants were used which had previously experienced considerable loss of old leaves (Table I).

DISCUSSION

The promotion of leaf abscission from water-stressed plants by Ethephon was similar to that described earlier (10). In addition, there appeared to be a slight synergistic promotion of leaf abscis-

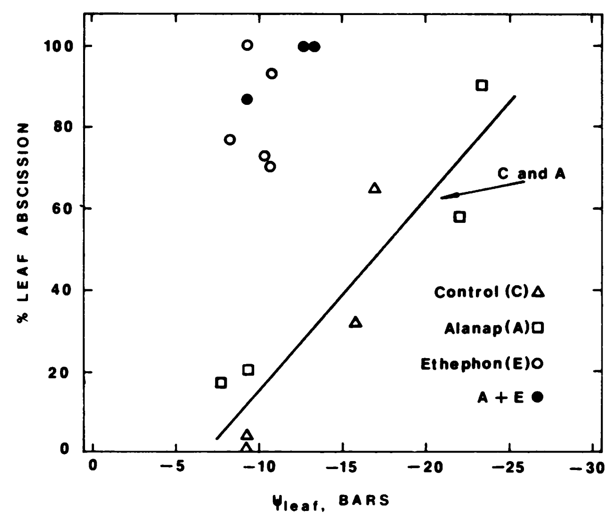

FIG. 3. Relationship of water stress to leaf abscission from control, Ethephon (10^{-1} M), Alanap (10^{-2} M) or Ethephon plus Alanap-treated plants. Ethephon was applied at 10^{-1} M, Alanap at 10^{-2} M, and abscission data are averages of two pots/treatment, two plants/pot 7 days after rewatering.

Table I. Effect of Auxin Transport Inhibitors, Ethephon and Water Stress on Apical Dominance and Lateral Bud Growth of Cotton

Experiment	ψ	Buds Released/Plant[1]			
		Control	ATI	Ethephon (10^{-1} M)	ATI & Ethephon
1 Alanap, 10^{-2}M					
watered	- 8.2	1.8 (36)	4.9 (38)	11.3 (62)	8.9 (69)
stressed [2]	-27.0	1.3 (33)	1.9 (52)	6.3 (89)	5.3 (96)
2 DPX-1840, 10^{-3}M					
stressed	-19.1	3.3 (37)	2.7 (40)	16.6 (79)	16.0 (83)

1/ The number of actively growing buds determined 30 days after rewatering. Numbers in parenthesis are average % abscission 72 hrs after rewatering.
2/ ψ averaged -27.0 bars except control which only reached -14.8 bars.

sion when auxin transport inhibitor treatments were combined with water stress and Ethephon treatments (Fig. 1). Auxin transport inhibitors alone did not promote abscission of leaves from water-stressed plants, suggesting that water stress and the inhibitors may act similarly. Since the transport inhibitors promoted abscission in well watered plants treated with Ethephon (Fig. 1), they appear to substitute at least partially for water stress in this system.

The rates of abscission also reflect on the probable roles of the three agents studied here. Following irrigation, abscission of leaves from previously water-stressed plants occurs quickly, usually within the first 24 hr, suggesting that stress prepares the leaf to respond to Ethephon (Fig. 2). Ethephon-induced abscission of leaves from well watered plants occurs more slowly, suggesting that the final abscission-achieving action of Ethephon is delayed until some preparatory event is satisfied or facilitated by auxin transport inhibitors (Fig. 1). Ethephon and ATI applied at the beginning of the water stress treatment, rather than near the end, failed to promote stress-induced abscission, which suggests a preparatory event which increases sensitivity to ethylene. Thus, both water stress and ATI seem to deal with the preparatory event(s) which is related to auxin supply, inhibition of auxin transport through the petiole and leaf veins being a critical process to that supply (4-6). Ethephon or native ethylene also promotes the final events in abscission: induction of synthesis of hydrolytic enzymes and secretion of some of these enzymes into the cell wall (1, 2, 8, 19). In the stressed or ATI-treated plants, Ethephon was able to initiate these final events more promptly. Ethephon did promote abscission in water-stressed plants, whereas the ATI did not, a result which suggests that stress reduces auxin transport more than it promotes ethylene synthesis. Davenport *et al.* (7) have recently determined that water stress does inhibit auxin transport in cotton petioles. In this same study, stress did not promote ethylene synthesis in cotyledonary and young leaf petioles, whereas it did promote ethylene production in petioles of old leaves (13) or detached leaves (3). Partial vacuum delayed or reduced water stress-induced chlorosis and abscission in cotton (11), and CO_2 competitively inhibited the effect of exogenous ethylene on leaf abscission from moisture-stressed plants (10). Both of these latter results suggest that ethylene synthesis is involved in stress-induced abscission. Our conclusion is that both processes are involved; however, inhibition of auxin transport is the primary effect and the availability of ethylene limits the degree of stress-induced abscission.

LITERATURE CITED

1. Abeles FB 1969 Abscission: role of cellulase. Plant Physiol 44: 447-452
2. Abeles FB, GR Leather 1971 Abscission: control of cellulase secretion by ethylene. Planta 97: 87-91
3. Ben-Yehoshua S, B Aloni 1974 Effect of water stress on ethylene production by detached leaves of Valencia oranges (*Cirtus sinensis* Osbeck). Plant Physiol 53: 863-865
4. Beyer EM Jr 1973 Abscission: support for a role of ethylene modification of auxin transport. Plant Physiol 52: 1-5
5. Beyer EM Jr 1975 Abscission: the initial effect of ethylene is in the leaf blade. Plant Physiol 55: 322-327
6. Beyer EM Jr, PW Morgan 1971 Abscission: the role of ethylene modification of auxin transport. Plant Physiol 48: 208-212
7. Davenport TL, PW Morgan, WR Jordan 1975 Alteration of hormone transport through cotton petioles by water stress. Plant Physiol 56: S-57
8. Horton RF, DJ Osborne 1967 Senescence, abscission, and cellulase activity in *Phaseolus vulgaris*. Nature 214: 1086-1088
9. Jackson MB, DJ Osborne 1970 Ethylene, the natural regulator of leaf abscission. Nature 225: 1019-1022
10. Jordan WR, PW Morgan, TL Davenport 1972 Water stress enhances ethylene-mediated leaf abscission in cotton. Plant Physiol 50: 756-758
11. Lipe JA, PW Morgan 1973 Ethylene, a regulator of young fruit abscission. Plant Physiol 51: 949-953
12. McAfee JA, PW Morgan 1971 Rates of production and internal levels of ethylene in the vegetative cotton plant. Plant Cell Physiol 12: 839-847
13. McMichael BL, WR Jordan, RD Powell 1972 An effect of water stress on ethylene production by intact cotton petioles. Plant Physiol 49: 658-660
14. McMichael BL, WR Jordan, RD Powell 1973 Abscission processes in cotton: induction by plant water deficit. Agron J 65: 202-204
15. Morgan PW, JI Durham 1972 Abscission: potentiating action of auxin transport inhibitors. Plant Physiol 50: 313-318
16. Morgan PW, JI Durham 1973 Morphactins enhance ethylene-induced leaf abscission. Planta 110: 91-93
17. Morgan PW, WC Hall 1963 Indoleacetic acid-oxidizing enzyme and inhibitors from light-grown cotton. Plant Physiol 38: 365-370
18. Morgan PW, DL Ketring, EM Beyer Jr, JA Lipe 1972 Functions of naturally produced ethylene in abscission, dehiscence and seed germination. *In* DJ Carr, ed, Plant Growth Substances 1970. Springer Verlag, Berlin
19. Morré DJ 1968 Cell wall dissolution and enzyme secretion during leaf abscission. Plant Physiol 43: 1545-1559