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Involvement of Ethylene in the Action of the Cotton Defoliant Thidiazuron[1]

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ABSTRACT

The effect of the defoliant thidiazuron (*N*-phenyl-*N'*-1,2,3-thiadiazol-5-ylurea) on endogenous ethylene evolution and the role of endogenous ethylene in thidiazuron-mediated leaf abscission were examined in cotton (*Gossypium hirsutum* L. cv Stoneville 519) seedlings. Treatment of 20- to 30-day-old seedlings with thidiazuron at concentrations equal to or greater than 10 micromolar resulted in leaf abscission. At a treatment concentration of 100 micromolar, nearly total abscission of the youngest leaves was observed. Following treatment, abscission of the younger leaves commenced within 48 hours and was complete by 120 hours. A large increase in ethylene evolution from leaf blades and abscission zone explants was readily detectable within 24 hours of treatment and persisted until leaf fall. Ethylene evolution from treated leaf blades was greatest 1 day posttreatment and reached levels in excess of 600 nanoliters per gram fresh weight per hour (26.7 nanomoles per gram fresh weight per hour). The increase in ethylene evolution occurred in the absence of increased ethane evolution, altered leaf water potential, or decreased chlorophyll levels. Treatment of seedlings with inhibitors of ethylene action (silver thiosulfate, hypobaric pressure) or ethylene synthesis (aminoethoxyvinylglycine) resulted in an inhibition of thidiazuron-induced defoliation. Application of exogenous ethylene or 1-aminocyclopropane-1-carboxylic acid largely restored the thidiazuron response. The results indicate that thidiazuron-induced leaf abscission is mediated, at least in part, by an increase in endogenous ethylene evolution. However, alterations of other phytohormone systems thought to be involved in regulating leaf abscission are not excluded by these studies.

The biology of abscission continues to engage the interests of botanists conducting both basic and applied research programs. Although incompletely understood at this time, the physiological events both preceding and underlying abscission seem to involve many topics currently considered to be at the forefront of phytohormone research. These include hormone synthesis and metabolism, hormone transport, tissue competence (hormone sensitivity), and hormone action (for review, see 2).

From a more practical viewpoint, the rate or extent of abscission has a primary impact on crop productivity and value. The premature abscission of developing reproductive organs (flowers and/or fruits) can have a dramatic impact on the overall yield of a crop. The abscission of mature weed seed/fruit aids in both the dispersal of that weed as well as its ability to re-establish itself in subsequent seasons.

Deliberate induction of abscission or defoliation is also of considerable importance in agriculture. Defoliation can facilitate mechanical harvesting of many crops including cotton. It has been estimated that well over 75% of the total United States cotton acreage is treated with harvest aids such as defoliants.

Thidiazuron (tradename, Dropp; Fig. 1) is currently registered as a cotton defoliant.[1] Although the defoliating activity of TDZ[2] was first reported in 1976 (3), its mode of action remains unknown. Treatment of plant tissues (including cotton) with TDZ results in a large and sustained increase in the rate of ethylene evolution (7, 17, 18). Ethylene is currently regarded as an endogenous regulator of leaf abscission in many plants, including cotton (for reviews, see 8, 13).

These considerations prompted an examination of the role of endogenous ethylene in TDZ-mediated leaf abscission. Portions of these results have been presented previously (17).

MATERIALS AND METHODS

Plant Material and Experimental Procedure. Cotton (*Gossypium hirsutum* L. cv Stoneville 519) seeds were sown in plastic pots containing vermiculite. Seedlings were raised in a growth chamber under the following conditions: 16-h photoperiod provided by a combination of high pressure sodium and metal-halide lamps (light intensity at plant height, 500 μE m^{-2} s^{-1}); day/night temperature, 30/25°C; 50% RH. Seedlings were watered daily with one-third strength, modified Hoagland solution. Seedlings were used when they were between 20 and 30 d old. In experiments involving spray treatments, the seedlings were sprayed to run-off with a 0.5% (v/v) Tween 20 solution containing the treatment chemical(s).

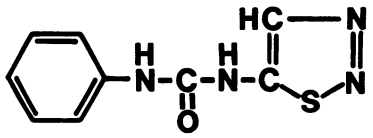
All experiments described in this paper were conducted a minimum of three times. Whenever possible, each treatment within an experiment was replicated ($n = 3$). Due to the nature of some of the experiments, replication within an experiment was not feasible. Data from typical experiments are presented.

Dose-Response and Time Course Studies. Seedlings were sprayed with varying concentrations of technical grade TDZ in surfactant solution and were returned to the growth chamber. Each day thereafter the degree of abscission was determined. Abscission at each leaf position was evaluated by deflecting the petiole (at the leaf junction) approximately 2 cm and recording the number of separations that occurred.

Time Course of Ethylene Evolution. Seedlings were sprayed with various concentrations of TDZ in surfactant solution. One and 2 d after treatment, the second true leaves and their subtending abscission zones were excised from the seedlings and were allowed to stand for 3 to 4 h to allow wound ethylene

[1] Mention of trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

[2] TDZ, thidiazuron; AVG, aminoethoxyvinylglycine; ACC, 1-aminocyclopropane-1-carboxylic acid; FID, flame ionization detector.



Thidiazuron (DROPP^R)

FIG. 1. Thidiazuron (TDZ; *N*-phenyl-*N'*-1,2,3-thiadiazol-5-ylurea).

production to subside. The abscission-zone explants consisted of a 1-cm segment of the main stem (centered around the point of petiole attachment) and 2 to 3 mm of petiole tissue. The tissues were incubated for 4 h (25°C, dark) in sealed containers. The ethylene content of the headspace was then determined by GC/FID using an activated alumina column (60–80 mesh).

Mechanism of Action Studies. Seedlings were sprayed with surfactant solution ± 100 μM TDZ. One d after treatment, groups of second true leaf blades were excised and weighed. After standing for 3 h, one group of leaf blades was incubated in sealed containers for 2 h. At that time, the headspace was analyzed by GC/FID for both ethylene and ethane accumulation. The instrument was calibrated with known standards of each gas. Discs (8 mm) were removed from the remaining leaves with a cork borer. One set of discs was used for the determination of leaf water potential, the other for Chl levels. Leaf water potential was determined using a dew point hygrometer (HR-33 Dew point microvoltmeter with a C-52 sample chamber; Wescor Inc., Logan, UT). The leaf samples were allowed to equilibrate for 7 h (23 ± 2°C) prior to taking the measurement. Chl levels were determined in 80% (v/v) acetone extracts using published calibration curves (9).

Explant Studies. Explants containing the two cotyledonary-node abscission zones were excised from cotton seedlings. The explants were surface sterilized using a 1:4 dilution of commercial bleach (5.3% (w/v) NaOCl). After 10 min in the bleach solution, the explants were rinsed 3× with sterile distilled H_2O. Subsequent manipulations were conducted aseptically. The explants were trimmed to size (as before) and were placed in 9 cm Petri dishes that contained 25 ml of sterile treatment solution. Treatment solutions were prepared in 10 mM Mes/KOH (pH 5.7) and were sterilized by passage through a 0.22-μm filter. The explants were treated for 4 h in the dark (25°C). After treatment, the explants were placed upright in a plexiglass holder which was then placed in a humid chamber and maintained in the dark (25°C). Each day groups of explants were sealed in 10-ml plastic syringes (sterile) and the ethylene evolution and percent abscission were determined. Abscission was scored using an abscissor (2).

Silver Thiosulfate Studies. Silver thiosulfate complex was prepared by slowly adding a silver nitrate solution (8 mM) to a rapidly stirring solution of sodium thiosulfate (32 mM). Tween 20 was added to a final concentration of 0.5% (v/v). Seedlings were sprayed to run-off with the surfactant solution ± Ag^+ and the solution was allowed to dry. After 4 h, the seedlings were again sprayed with the surfactant solution ± 100 μM TDZ.

Hypobaric Incubation. Seedlings were sprayed with surfactant solution ± 100 μM TDZ. One group of treated seedlings was maintained at atmospheric pressure while two other groups were placed in desiccators. The desiccators were evacuated to a final pressure of 150 mm Hg using variable vacuum gauges equipped with inlet ports. Bleed-in air was humidified using a laboratory bubbler. The bleed-in air of one desiccator was fortified with ethylene (final concentration, 5.5–9.0 μl/l) using the dilution apparatus described by Saltveit (15). The desiccators were opened daily for approximately 1 h to score for abscission.

AVG Treatment. Beginning 1 d prior to TDZ treatment (100 μM), seedlings were sprayed twice daily with a 100 μM solution of AVG. Following TDZ treatment, groups of seedlings were also sprayed twice daily with a 5 mM solution of ACC ± 100 μM AVG. Two d following TDZ treatment, groups of second true leaves were excised from the seedlings and, after standing for 4 h (25°C), the leaves were enclosed in containers. After 4 h, the ethylene content was determined by GC/FID. Total abscission was scored 3 d after TDZ treatment.

Chemicals. Technical-grade TDZ was a gift from E. Pieters of Nor-Am Agricultural Products, Inc. AVG was a gift from Dr. R. W. Bagley of HLR Sciences, Inc. All other chemicals used were reagent grade and were obtained from commercial supply houses.

RESULTS

Treatment of cotton seedlings with TDZ at concentrations of 10 μM or greater resulted in a marked stimulation of abscission of the younger leaves (Table I). Greater than 80% abscission was observed at these leaf positions at the highest TDZ concentration tested (100 μM). Cotyledons exhibited a similar, although less pronounced, response to increasing concentrations of TDZ. The oldest true leaves required a greater threshold concentration (100 μM) and exhibited little total abscission.

Following TDZ treatment, abscission of the younger leaves was easily detected by 2 d and was essentialy complete by 5 d (Table II). Both the cotyledons and the oldest true leaves exhibited a slower and less pronounced response. Because the maximum response was observed in the younger leaves, the remainder of this study was focused on the effects of TDZ treatment on these tissues.

Ethylene is thought to be an endogenous regulator of leaf abscission (8, 13). Previous studies (18) have found TDZ to be a potent stimulator of ethylene evolution from mung bean hypocotyl segments and preliminary results indicated a similar effect in cotton leaf tissues (17). For this reason, the effect of TDZ treatment on ethylene evolution from leaf blades as well as their respective abscission zone explants was examined. At TDZ concentrations greater than or equal to 1 μM, both leaf and abscis-

Table I. *Effect of Leaf Age (Position) on TDZ-Mediated Abscission in Cotton Seedlings*

Cotton seedlings were sprayed to run-off with surfactant solutions containing various concentrations of TDZ. Cumulative leaf abscission at each leaf position was determined after 7 d. Ten seedlings per treatment.

Leaf Position	Cumulative Leaf Abscission at Following TDZ Concentrations (−Log M)			
	Con[a]	6	5	4
Cotyledon	0	0	4	3
1[b]	0	0	0	3
2	0	0	3	9
3	0	0	7	8

[a] Controls. [b] First true leaves.

Table II. *Time-Course of TDZ-Mediated Leaf Abscission at Various Leaf Positions*

Cotton seedlings were sprayed to run-off with a 100 μM solution of TDZ. Cumulative leaf abscission was evaluated at daily intervals thereafter. Ten seedlings per treatment.

Leaf Position	Cumulative Leaf Abscission (Days After Treatment)						
	1	2	3	4	5	7	10
Cotyledon	0	0	0	1	2	3	5
1[a]	0	0	2	2	3	3	3
3	0	7	7	7	8	8	8

[a] First true leaves.

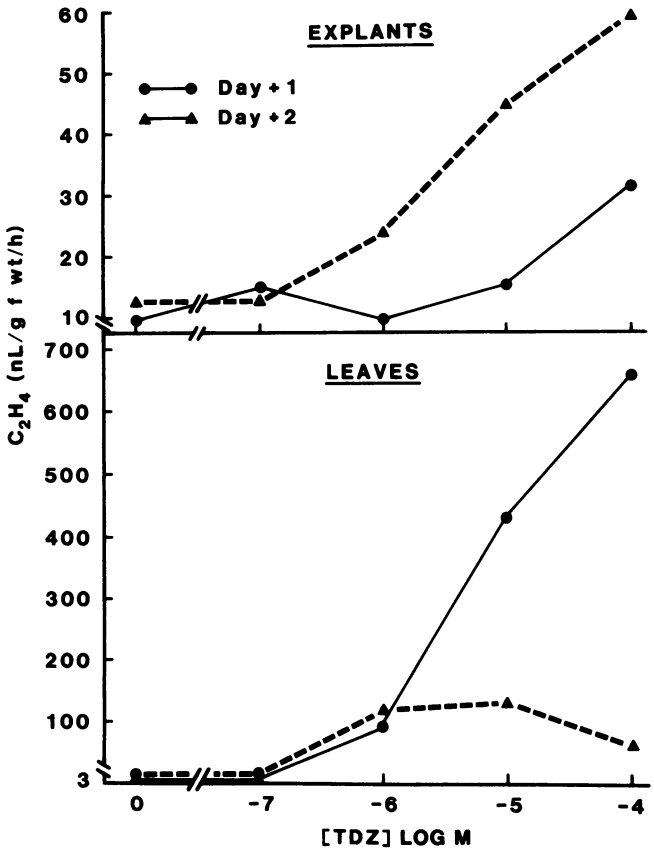


FIG. 2. Effect of various concentrations of TDZ on ethylene evolution from leaf blades and explants containing the abscission zone 1 and 2 d after treatment. (——), Ethylene evolution 1 d posttreatment; (---), ethylene evolution 2 d posttreatment.

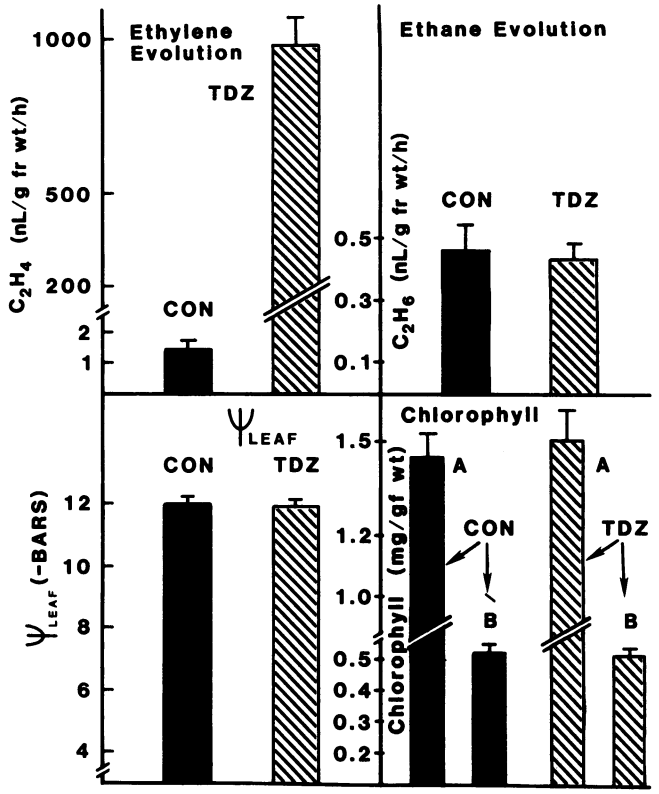


FIG. 3. Effect of 100 μM TDZ treatment on ethylene evolution (upper left), ethane evolution (upper right), leaf water potential (lower left), and Chl content (lower right) 1 d after treatment. Bars indicate SE (n = 3). Controls, solid bars; TDZ treated, hatched bars.

sion-zone explant tissues exhibited large increases in the rate of ethylene evolution (Fig. 2). Ethylene evolution from leaf blades reached a maximum (>600 nl/g fresh weight·h) 1 d after treatment and declined thereafter. Ethylene evolution from explant tissues was greatest 2 d after treatment. The determination of ethylene evolution beyond day 2 was precluded by advancing leaf abscission.

The stimulation of ethylene evolution by TDZ treatment could have resulted from one or more of the following physiological mechanisms: (a) chemical injury (wounding); (b) leaf desiccation; (c) accelerated leaf senescence; or (d) hormone-like induction. One d after TDZ treatment, cotton leaf tissues exhibited well over a 500-fold increase in ethylene evolution (Fig. 3). Ethane evolution, a physiological marker for wounding (6) was unaffected. Leaf water potential was also unaltered, a fact that eliminates desiccation as the underlying mechanism. Finally, TDZ treatment had no discernible effect on the levels of Chl *a* or *b* (hence, total Chl), suggesting that accelerated leaf senescence was not the physiological basis for this stimulation.

Isolated cotyledonary node abscission zone explants have been extensively used as a model system with which to screen for potential defoliants and to explore the physiological basis(es) for any observed defoliation. Treatment of isolated explants *in vitro* with 1 or 100 μM TDZ resulted in an elevation of ethylene evolution that persisted for 3 d (Fig. 4). However, the rate of petiole abscission in these explants was retarded. ABA was included in these tests to ensure that the explants were responsive to an abscission-inducing stimulus. Treatment with 100 μM ABA resulted in a small, but significant, increase in ethylene evolution by day 2 and complete abscission by day 3.

Having established that increased ethylene evolution preceded and accompanied TDZ-induced abscission, an attempt was made to determine if this increased ethylene release was essential for the observed response. This was done using inhibitors of ethylene action and synthesis.

Silver ions, by virtue of their ability to antagonize ethylene action, have been extensively employed to evaluate the role of ethylene in various physiological processes (4, 20). A single spray treatment with silver thiosulfate completely abolished the defoliating activity of TDZ (Table III). This antagonism was observed at all leaf positions exhibiting a TDZ response.

Incubation of plant tissues under reduced pressure (hypobaric storage) has also been found to retard or eliminate many processes thought to be regulated by endogenous ethylene (for review, see 5). Incubation of TDZ-treated seedlings under reduced pressure in a flow-through system inhibited TDZ-mediated abscission by 70% (Table IV). The inclusion of exogenous ethylene in the bleed-in air largely restored this response.

AVG is a potent inhibitor of ethylene evolution from most higher plants (see 10). Daily foliar treatment of TDZ-treated seedlings with surfactant solution containing 100 μM AVG inhibited ethylene evolution by 50% and also reduced TDZ mediated abscission to the same extent (Fig. 5). When 5 mm ACC was included in the daily foliar treatment solution, both of these responses were completely restored. Daily foliar treatment with ACC alone was ineffective in stimulating abscission at this leaf position although it had a marked effect on the rate of ethylene evolution.

DISCUSSION

The results presented clearly demonstrate that an increase in ethylene evolution both precedes and accompanies TDZ induced leaf abscission (compare Table II and Fig. 2). A marked increase

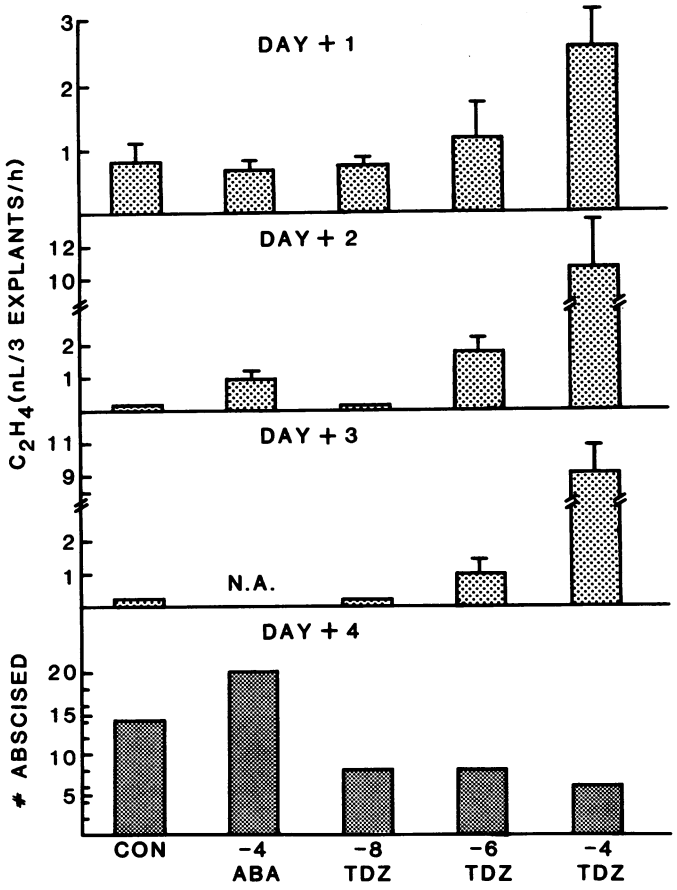


FIG. 4. Effect of various concentrations of TDZ or ABA (100 μM) on ethylene evolution 1, 2, and 3 d posttreatment (upper three panels) and total abscission (lower panel) when applied directly to isolated, cotyledonary node abscission zone explants. TDZ concentrations are in log M (*i.e.* −4 = 100 μM, etc.). Day + 1 refers to 1 d posttreatment. Bars indicate SE (n = 10 explants).

Table III. *Effect of Silver Thiosulfate on TDZ-Induced Leaf Abscission in Cotton Seedlings*

Seedlings were sprayed to run-off with a 0.5% (v/v) Tween 20 solution plus or minus silver thiosulfate ([Ag^+], 4 mM). Four h later, these seedlings were again sprayed to run-off with the surfactant solution plus or minus 100 μM TDZ. Cumulative abscission for each leaf position was evaluated after 3 d.

Treatment	Total Abscission at Leaf Position[a]			
	1[b]	2	3	4
Control	0	0	0	0
Silver thiosulfate	0	0	0	0
TDZ	0	4	10	10
Silver thiosulfate + TDZ	0	0	0	0

[a] Ten seedlings per treatment. [b] First true leaves.

in ethylene evolution is evident 24 h after TDZ treatment and abscission becomes detectable by 48 h. The experimental protocol used in these studies precluded the exact determination of the lag phase in TDZ-induced ethylene production. However, increased ethylene evolution from mung bean hypocotyl segments has been observed within 90 min of TDZ treatment (18). Leaf abscission in cotton seedlings as grown for these studies can be observed within 24 h following application of exogenous ethylene (Suttle, unpublished results). Therefore, endogenous

Table IV. *Effect of Hypobaric Storage on TDZ-Induced Leaf Abscission in Cotton Seedlings*

Seedlings were treated with 100 μM TDZ (controls, surfactant only). Groups of seedlings were maintained at atmospheric pressure or under hypobaric (final pressure, 150 mm Hg) conditions in a flow-through system. In one hypobaric chamber, ethylene was added to the flow-through air to a final concentration between 5.5 and 9 μl/l (v/v). Abscission of the second true leaves was scored 3 d posttreatment.

Treatment	Total Leaf Abscission[a]
Control	0
TDZ	10
TDZ/hypobaric	3
TDZ/hypobaric + ethylene	8

[a] Ten seedlings per treatment.

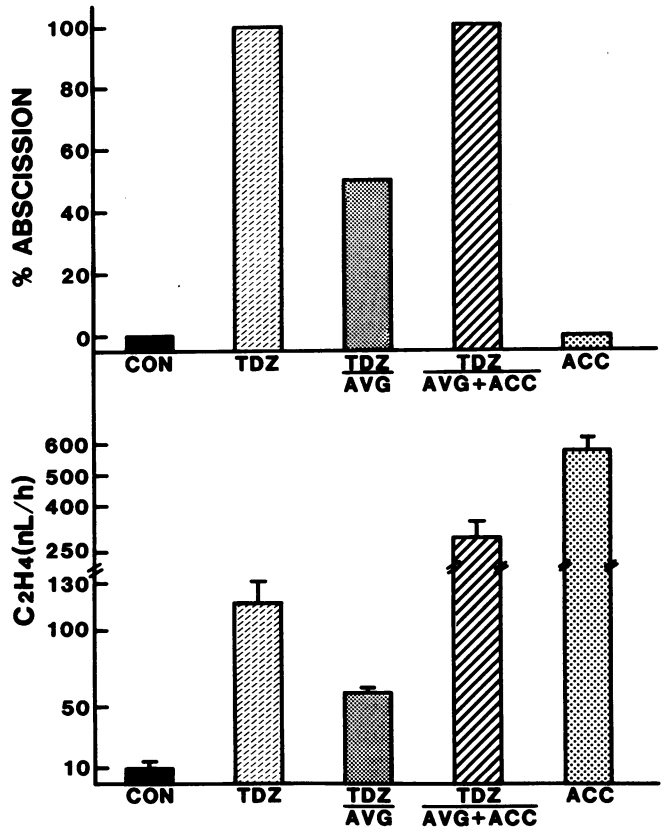


FIG. 5. Effect of TDZ (100 μM): TDZ and AVG (both 100 μM); TDZ, AVG, and ACC (5 mM); or ACC alone (5 mM) on per cent abscission (upper) and ethylene evolution (lower) from cotton seedlings. Per cent abscission (10 seedlings/group) was scored 3 d posttreatment. Ethylene evolution was determined 2 d posttreatment. Bars indicate SE (n = 3).

ethylene would have sufficient time to induce the necessary biochemical changes that underlie abscission following TDZ treatment. Further support for the hypothesis that TDZ action is mediated, at least in part, by endogenous ethylene can be derived from the inhibitor studies described herein. Inhibitors of ethylene action (Tables III and IV) or synthesis (Fig. 5) reduce TDZ-mediated abscission and application of ethylene or ACC reverses this response. On the whole, these studies clearly indicate that an increase in endogenous ethylene evolution is an essential prerequisite for TDZ action in this system.

Exogenous ACC, while effective in enhancing ethylene evolution, elicited no leaf abscission (Fig. 5). Treatment with ACC has been shown to induce partial defoliation of other crops such as *Vitus* (11). To our knowledge its efficacy in cotton has not been

reported. During the course of several experiments, it was found that twice-daily treatment of cotton seedlings with 5 mM ACC resulted in little to no abscission of expanded leaves but did promote nearly complete abscission of the youngest leaves (Suttle, unpublished results). It is possible that poor penetration of exogeneous ACC through the more fully developed cuticle of the older leaves resulted in insufficient production and accumulation of ethylene within the leaf's interior. This small increase in internal ethylene levels, while insufficient to induce abscission on its own could still reverse the AVG inhibition since internal ethylene levels were already substantially elevated by the preceding TDZ treatment (Fig. 5). In this scenario, the large increase in ethylene evolution elicited by ACC treatment (Fig. 5) would have little impact on internal ethylene levels as the production of the gas would be limited to the outer cell layers(s) of the leaf. In addition, the diffusion of this newly produced gas would be directed primarily in an outward fashion away from the leaf's interior where ethylene is presumed to act. More detailed studies comparing the efficacy of several ethylene releasing or generating compounds (including ACC) have found both qualitative and quantitative differences in the observed abilities of these agents to affect physiological processes (including abscission) known to be responsive to ethylene itself (11). Thus, the degree of penetration/uptake of the material as well as the duration of ethylene release can be as important as the total amount of ethylene released.

Alternatively, it can be argued that the levels or activities of other hormone systems or other biochemical factors were altered by TDZ treatment and that these modifications, together with enhanced ethylene evolution resulted in leaf abscission. Such an interpretation would be consistent with current hypotheses (2, 13) that regard hormone balance as the critical factor in the regulation of leaf abscission. Studies aimed at identifying effects of TDZ treatment on other hormone systems are currently underway and may possibly shed light on this matter.

Other registered defoliants appear to act as contact herbicides whose defoliating activity is the end result of stress ethylene (1). Because their activity results from direct or contact action, recommended application rates for these defoliants are quite high (about 1–3 kg/ha). Recommended field rates for TDZ are approximately 1/10 of these, and TDZ treatment generally results in the abscission of green, fully turgid leaves (so called 'green drop'). Following TDZ treatment, large elevations in ethylene evolution can be detected prior to any measurable indication of wounding or desiccation (Fig. 3). Similar results were found in bean (*Phaseolus*) leaves following TDZ treatment (7). Thus, the stimulation of ethylene evolution in cotton seedlings by TDZ appears to be the result of hormone-like activity of TDZ itself. Similar conclusions were made concerning TDZ action in mung bean hypocotyls (18). In fact, TDZ is quite effective in supporting the growth of cytokinin-dependent callus cultures (12) and also exerts other cytokinin-like activities as well (19). While not extensively studied in leaf tissues, cytokinins are potent stimulators of ethylene evolution from etiolated plant tissues (21).

Treatment of isolated citrus pistil explants with TDZ results in little or no change in ethylene evolution but greatly stimulates stylar abscission (16). In contrast, treatment of isolated cotton petiole explants with TDZ resulted in an elevation of ethylene evolution while retarding the rate of petiole abscission (Fig. 4). This seemingly anomalous behavior of TDZ in these two model systems can be resolved by the contrasting effects of cytokinins in these two test systems. Cytokinin-like compounds have been found to be potent abscission stimulators in citrus floral explants (16) while cytokinin treatment (depending on the site of application) can retard abscission in petiole explants (14). The efficacy of TDZ as a defoliant should not be appreciably affected by this direct effect on the abscission zone since, under field conditions, the bulk of the spray treatment would be intercepted by the leaf tissues.

The use of this sterile petiole explant system permitted the daily evaluation of ethylene evolution without the complications of microbial ethylene release. In this system, 100 μM ABA treatment consistently resulted in a small but significant increase in ethylene evolution that preceded petiole abscission (Fig. 4). Preliminary experiments have indicated that sustained ethylene evolution and action are necessary aspects of ABA action in these explants.

A long-standing goal in research on agricultural chemicals continues to be to reduce unnecessary environmental impact while maintaining or increasing efficacy. As the field application rates as a chemical are reduced, performance generally becomes less certain and more susceptible to environmental vagaries. Low temperatures immediately following TDZ treatment have been correlated with decreased field performance. Once the mechanism(s) of action of a particular compound is understood, it is then possible to evaluate the influence of the environment on the component process(es). The results of these investigations should lead to more consistent performance of the chemical in the field and greater utility to the grower hence to the public in general.

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LITERATURE CITED

1. ABELES FB 1966 Mechanism of action of abscission accelerators. Physiol Plant 20: 442–454
2. ADDICOTT FT 1982 Abscission. University of California Press, Berkeley
3. ARNDT F, R RUSCH, HV STILLFRIED 1976 SN 49537, A new cotton defoliant. Plant Physiol 57: S-99
4. BEYER EM 1976 A potent inhibitor of ethylene action in plants. Plant Physiol 58: 268–271
5. DILLEY DR, WJ CARPENTER, SP BURG 1975 Principles and application of hypobaric storage of cut flowers. Acta Hortic 41: 249–268
6. Elstner EF, JR Konze 1976 Effect of point freezing on ethylene and ethane production by sugar beet leaf discs. Nature 263: 351–352
7. ELSTNER EF, G KELLER, I PARADIES 1983 Contrasting effects of the cotton defoliant thidiazuron and aminoethoxyvinylglycine, an inhibitor of ethylene formation, on stomatal aperature and on ethylene formation in bean (*Phaseolus vulgaris*) leaves. Ber Dtsch Bot Ges 96: 459–467
8. JACKSON MB, DJ OSBORNE 1970 Ethylene, the natural regulator of leaf abscission. Nature 225: 1019–1022
9. KIRK JTO 1968 Studies on the dependence of chlorophyll synthesis on protein synthesis in *Euglena gracilis*, together with a nomogram for determination of chlorophyll concentration. Planta 78: 200–207
10. LIEBERMAN M 1979 Biosynthesis and action of ethylene. Annu Rev Plant Physiol 30: 533–591
11. LURSSEN K 1982 Manipulation of crop growth by ethylene and some implications of the mode of generation. *In* JS McLaren, ed, Chemical manipulations of Crop Growth and Development. Butterworth Scientific Publishers, London, pp 67–78
12. MOK MC, DWS MOK, DJ ARMSTRONG, K SHUDO, Y ISOGAI, T OKAMOTO 1982 Cytokinin activity of *N*-phenyl-*N'*-1,2,3-thiadiazol-5-ylurea (thidiazuron). Phytochemistry 21: 1509–1511
13. MORGAN PW 1984 Is ethylene the natural regulator of abscission? *In* Y Fuchs, E Chalutz, eds, Ethylene: Biochemical, Physiological and Applied Aspects. Martinus Nijhoff/Dr. W Junk Publishers, The Hague, pp 231–240
14. OSBORNE DJ, SE MOSS 1963 Effect of kinetin on senescence and abscission in explants of *Phaseolus vulgaris*. Nature 200: 1299–1301
15. SALTVEIT ME 1978 Simple apparatus for diluting and dispensing trace concentrations of ethylene in air. HortScience 13: 249–251
16. SIPES DL, JW EINSET 1983 Cytokinin stimulation of abscission in lemon pistil explants. J Plant Growth Regul 2: 73–80
17. SUTTLE JC 1983 Effect of the defoliant thidiazuron on ethylene production. Plant Physiol 72: S-121
18. SUTTLE JC 1984 Effect of the defoliant thidiazuron on ethylene evolution from mung bean hypocotyl segments. Plant Physiol 75: 902–907
19. THOMAS JC, RFH KATTERMAN 1983 Thidiazuron effects on soybean callus and radish cytokinin bioassays. In Vitro 19 (Part II): 265 (Abstr)
20. VEEN H 1983 Silver thiosulphate: an experimental tool in plant science. Sci Hortic 20: 211–224
21. YU Y, SF YANG, J CORSE, JA KUHNLE, S HUA 1981 Structures of cytokinins influence synergistic production of ethylene. Phytochemistry 20: 1191–1195