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Cytokinin-Induced Ethylene Biosynthesis in Nonsenescing Cotton Leaves

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ABSTRACT

The influence of cytokinins on ethylene production was examined using cotton leaf tissues. Treatment of intact cotton (*Gossypium hirsutum* L. cv LG 102) seedlings with both natural and synthetic cytokinins resulted in an increase in ethylene production by excised leaves. The effectiveness of the cytokinins tested was as follows: thidiazuron ≫ BA ≫ isopentyladenine ≥ zeatin ≫ kinetin. Using 100 micromolar thidiazuron (TDZ), an initial increase in ethylene production was observed 7 to 8 hours post-treatment, reached a maximum by 24 hours and then declined. Inhibitors of 1-aminocyclopropane-1-carboxylic acid (ACC) synthesis and its oxidation to ethylene reduced ethylene production 24 hours post-treatment; however, by 48 hours only inhibitors of ACC oxidation were effective. The increase in ethylene production was accompanied by a massive accumulation of ACC and its acid-labile conjugate. TDZ treatment resulted in a significant increase in the capacity of tissues to oxidize ACC to ethylene. Endogenous levels of methionine remained constant following TDZ treatment. It was concluded that the stimulation of ethylene production in cotton leaves following cytokinin treatment was the result of an increase in both the formation and oxidation of ACC.

Previous studies in this laboratory have dealt with the physiological bases of defoliant action in crop plants. In particular, the effects of the defoliant thidiazuron (*N*-phenyl-*N'*-1,2,3-thiadiazol-5-ylurea) on cotton seedlings have been extensively examined (14). TDZ[1] is unique among currently registered defoliants in that it possesses a high degree of intrinsic cytokinin-like activity in a range of bioassay systems (11, 16).

Following foliar treatment with TDZ, cotton leaf tissues exhibit a massive and sustained increase in ethylene production (14). This stimulation of ethylene production was found to occur in the absence of any measurable changes in leaf senescence, chemical injury (wounding) or leaf water potential (14). Hence, it was concluded that TDZ exerted its effects on ethylene biosynthesis via its cytokinin-like activity.

Although ethylene production in many plant tissues, including leaves, can be influenced by a range of plant growth substances, there has been almost nothing reported in the literature concerning the effects of cytokinin-like compounds on ethylene production from intact, turgid leaf tissues (for reviews, see Lieberman [8], Yang and Hoffman [19]). In this report, the effects of a variety of cytokinins on ethylene production from cotton leaves are described and data concerning the biochemical bases for the observed responses are presented.

[1] Abbreviations: TDZ, thidiazuron; ACC, 1-aminocyclopropane-1-carboxylic acid; α-AIB, α-amino-isobutyric acid; AOA, aminooxy acetic acid; AVG, aminoethoxyvinylglycine; BA, 6-benzylaminopurine; EFE, ethylene-forming enzyme (ACC oxidase); IPA, $N^6(\Delta^2$-isopentenyl)adenine; MACC, malonyl ACC.

MATERIALS AND METHODS

Plant Material and Experimental Procedure. Cotton (*Gossypium hirsutum* L. cv LG 102), bean (*Phaseolus vulgaris* L. cv Black Valentine), sunflower (*Helianthus annuus* L. cv NK 265), and corn (*Zea mays* L. cv PX 9144) were grown in a growth chamber as described previously (14). Seedlings to be studied were used between 17 and 30 d after sowing. In all cases, intact seedlings were sprayed to run-off with an aqueous solution (0.5% v/v, Tween 20) of the cytokinin or auxin to be examined and were returned to the growth chamber. Further manipulations were carried out using leaves or leaf discs (8 mm diameter) excised from the treated seedlings. Care was exercised to select leaves of uniform physiological status. All experiments described in this paper were conducted a minimum of three times. Each treatment within an experiment was replicated ($n = 2$ or 3). Data from typical experiments are presented.

Cytokinin and Species Comparisons. All cytokinins examined were initially prepared as 0.1 M stock solutions in DMSO. Dilutions were made by dissolving the appropriate amount of stock solution in a 0.5% (v/v) Tween 20 solution. Leaves were treated by spraying both sides to run-off with the appropriate solutions. Controls were sprayed with surfactant alone. Treated leaves were excised from the seedlings 18 h later and were enclosed in glass jars (volume ≈ 500 mL). After a 3 to 4 h dark incubation period (27 ± 1°C), the ethylene content of the headspace was determined by GC/flame ionization detector using an activated alumina column. The species comparison was conducted in a similar fashion except the excised corn leaves were incubated in a smaller vial (volume ≈ 55 mL).

Dose-Response and Time Course Studies. Intact cotton seedlings were sprayed with various concentrations of TDZ in surfactant solution as before. The treated leaves were excised 18 or 42 h later and ethylene evolution was determined as described above. Two types of leaves were used in these studies: fully mature and completely expanded leaves and leaves that had reached approximately 75% of mature leaf area (immature leaves). The time-course of ethylene production was studied by excising immature leaves at various times following 100 μM TDZ treatment. After a 60 min dark incubation period (27 ± 1°C) ethylene was determined as before.

Calcium, Lanthanum and Inhibitor Studies. Seedlings were treated with a 100 μM TDZ solution and 18 h later immature leaves were excised. Leaf discs (8 mm diameter) were removed from the treated leaves using a cork borer and were then floated on a solution containing the compound of interest (*i.e.* calcium, AVG, etc.) prepared in 10 mM Mes/KOH (pH 5.7; 50 μg/mL chloramphenicol). After a 2 h (calcium, lanthanum studies) or 3

h (inhibitor studies) preincubation on the indicated solution, leaf discs were enclosed in a flask containing the same treatment solution for 4 h (27 ± 1°C). Ethylene accumulation was then determined as before.

Endogenous Levels of Free and Conjugated ACC. Immature leaves were sprayed to run-off with a surfactant solution ± 100 μM TDZ. At various times thereafter, the treated leaves were excised and the rate of ethylene evolution was determined by GC following a 1 h dark incubation in sealed containers (27 ± 1°C). Groups of 15 discs (8 mm diameter, about 0.1 g fresh weight) were removed from these leaves and were homogenized in 80% (v/v) aqueous ethanol. Free and conjugated ACC were determined as described previously (13). An internal standard of 10 nmol ACC was included in these assays to correct for any interfering materials in the extracts.

Uptake and Oxidation of Exogenous ACC. Immature leaves were treated with 1 or 100 μM TDZ in surfactant solution. Treated leaves were excised 18 or 42 h later and groups of 10 discs (8 mm diameter; about 0.09 g fresh weight) were removed using a cork borer. After standing in a humid chamber for 2 to 3 h the discs were placed in flasks containing 5 mL of 10 mM Mes/KOH (pH 5.7 + 50 μg/mL chloramphenicol) ± 5 mM ACC. In order to more easily follow uptake and metabolism, [^{14}C]ACC (0.5 μCi/flask) was included in those treatment solutions containing ACC. The flasks were sealed and after a 4 h dark incubation period (27 ± 1°C) the ethylene content of the headspace was determined by GC. EFE activity was determined by the amount of ethylene formed in the presence of 5 mM ACC. These same leaf discs were then removed from the flasks, blotted dry, and then rinsed with ice-cold distilled H_2O for 5 min. The discs were then extracted with 80% aqueous ethanol and the extracts were clarified by centrifugation (10,000*g*). The radioactivity in these extracts was taken as a measure of ACC uptake. TLC (6) of aliquots of these extracts was used to determine the extent of ACC conjugation during the treatment period.

Endogenous Levels of Methionine, ACC, and Conjugated ACC. All treatments and manipulations were essentially identical to those described above for the determination of endogenous ACC except that 5 mM mercaptoethanol was included in the 80% aqueous ethanolic extraction medium in order to minimize the oxidation of methionine. The clarified 80% aqueous ethanolic extracts were divided into three portions: one portion for free ACC, one for conjugated ACC, and one for methionine. Free and acid-labile, conjugated ACC were determined as before. The aliquot to be used for methionine quantitation was further clarified by passage through a 0.5 μM filter. After drying under a N_2 stream (35°C), the aliquot was redissolved in a 0.2 N lithium citrate buffer (pH 2.2). Methionine levels were determined using a Beckman[2] model 119 amino acid analyzer. Verification of the suspected methionine peak was accomplished using an internal standard of methionine.

Chemicals. Technical-grade TDZ (*N*-phenyl-*N'*-1,2,3-thiadiazol-5-ylurea) was a gift from Dr. E. Pieters (Nor-Am Agricultural Products, Inc.). AVG was a gift of Dr. R. W. Bagley (HLR Science, Inc.). [2,3-^{14}C]-1-Aminocyclopropane-1-carboxylic acid (80 mCi/mmol) was obtained from Research Products International Corp. All other chemicals used were reagent grade and were obtained from commercial supply houses.

RESULTS

Preliminary experiments demonstrated that cytokinin application to excised leaf tissues (*i.e.* leaf discs) elicited only a marginal increase in subsequent ethylene evolution (not shown). In contrast, foliar treatment of intact seedlings was found to result in a greater and more consistent response. Therefore, in all experiments described in this paper, intact seedlings were treated with the test compounds by foliar spray. Subsequent manipulations (*i.e.* inhibitor treatments, etc.) were performed on leaves or leaf tissues excised from treated seedlings.

The effect of a variety of synthetic and naturally occurring cytokinins was examined in both mature (fully expanded) and young (incompletely expanded) cotton leaves. While all cytokinins tested stimulated ethylene production in leaves of both ages, the degree of stimulation varied greatly (Table I). Of the compounds tested, TDZ was the most active. The effectiveness of the cytokinins tested was as follows: TDZ ≫ BA ≫ IPA ≥ zeatin ≫ kinetin. With the exception of kinetin, younger leaves exhibited the greatest response.

The specificity of this response to cytokinins was examined in four species using the two most potent cytokinins (TDZ and BA) and also the auxin 2,4-D. Of the three dicots examined, both cotton and sunflower leaf tissues exhibited a measurable response to both cytokinins and to 2,4-D (Fig. 1). Bean (cv Black Valentine) seedlings responded to 2,4-D but showed a smaller response to both cytokinins. Corn, the only monocot examined, produced the least amount of ethylene but it too exhibited a marginal response to both TDZ and 2,4-D treatment.

Treatment of cotton seedlings with TDZ concentrations of 3 × 10^{-7} M or greater resulted in a dose-dependent increase in ethylene evolution (Fig. 2). One day after treatment, no saturation of this response was observed at TDZ concentrations up to 100 μM. At this treatment level ethylene production 24 h post-treatment was stimulated by over 440- and 149-fold in young and mature leaves, respectively. At treatment concentrations of 1 μM or less, maximum stimulation of ethylene production occurred 48 h post-treatment. Above 1 μM, the maximum stimulation was observed 24 h post-treatment. Due to their degree of responsiveness, leaves used in the remainder of this study were between 50 and 75% fully expanded.

Calcium salts have been shown to enhance the physiological effects of cytokinins in a range of bioassay systems. When discs, isolated from treated leaves, were floated on buffered solutions containing various concentrations of $CaCl_2$, no effect of external Ca was observed (Table II). The inclusion of lanthanum chloride (a potential Ca antagonist) did not reduce the cytokinin-induced ethylene production.

The effect of TDZ on ethylene evolution could be detected

Table I. *Effects of Various Cytokinins on Ethylene Evolution by Cotton Leaves*

Cotton seedlings were sprayed to run-off with 0.5% (v/v) Tween 20 solutions containing the indicated cytokinin (100 μM). The treated leaves were excised from the seedlings 20 h after treatment and were enclosed in sealed containers. Ethylene levels were determined after 4 h enclosure (dark; 27 ± 1°C). Mature leaves refer to fully expanded leaves while immature leaves refer to leaves having reached between 50 to 75% of full expansion.

Treatment	Ethylene Evolution	
	Immature leaves	Mature leaves
	nl/g fresh wt·h	
None	1.63 ± 0.25[a]	1.04 ± 0.35
Thidiazuron	432.07 ± 41.37	298.99 ± 17.66
BA	230.42 ± 51.58	142.82 ± 28.49
Kinetin	14.18 ± 3.29	14.90 ± 6.32
Zeatin	65.20 ± 11.16	50.75 ± 13.10
IPA	79.34 ± 8.44	44.64 ± 5.89

[a] Values presented are means ± SEM (n = 3).

[2] 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.

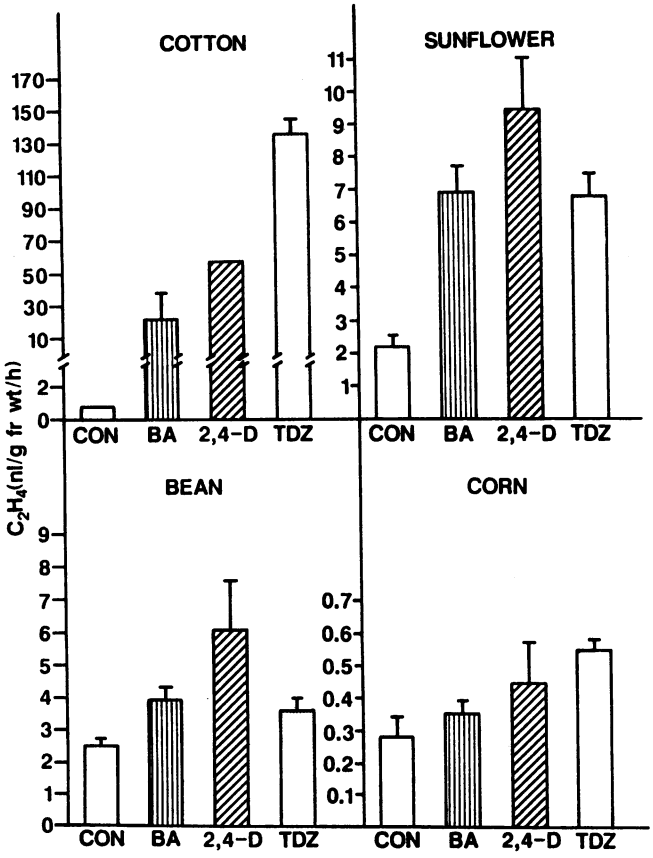

FIG. 1. Effect of BA, TDZ, or 2,4-D (all 100 μM) on ethylene production by cotton (upper left); sunflower (upper right); bean (lower left); and corn (lower right) 24 h after treatment. Bars indicate SE.

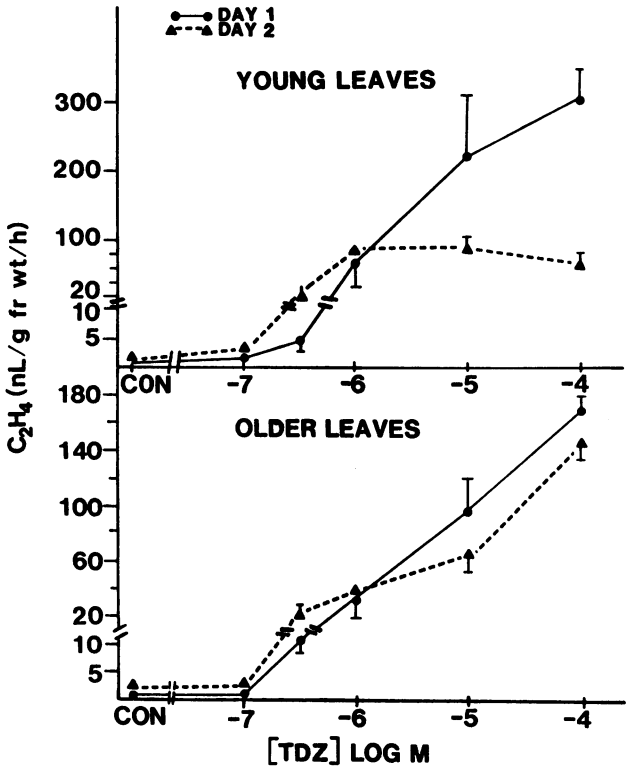

FIG. 2. The effect of various concentrations of TDZ on the rate of ethylene production 24 h (——) and 48 h (- - -) following treatment. Upper panel: young, incompletely expanded leaves; lower panel: fully mature leaves. Bars indicate SE.

within 8 h of treatment (Table III). By 12 h post-treatment the stimulation was even more apparent. In contrast, the auxin 2,4-D stimulated ethylene production within 4 h of application (not shown).

Attention was next turned to the biochemical bases for the observed response. Evidence gathered to date indicates that methionine is the major precursor of ethylene in all higher plants (8). Following administration of [^{14}C]methionine to discs isolated from TDZ treated leaves, radioactivity was found in ethylene and its immediate precursor ACC (not shown). These results suggested that the well-known biosynthetic pathway: methionine → *S*-adenosylmethionine → ACC → ethylene was operative in cytokinin treated leaves.

The *in situ* operation of the methionine pathway in cytokinin treated leaf tissues was confirmed by the results of inhibitor studies. Inhibitors of ACC synthase (AVG, AOA) reduced ethylene production when administered 1 d after cytokinin treatment (Table IV). Two days after treatment these same inhibitors were without effect. Compounds known to interfere with the oxidation of ACC to ethylene ($CoCl_2$, α-AIB) were effective on both days.

Both the formation and utilization of ACC are considered to be major regulatory points in ethylene biosynthesis (19). The effects of TDZ treatment on these steps were examined next.

As described above, a small increase in ethylene production could be measured 7 h following TDZ treatment (Fig. 3). During this period, no measurable change in either free or conjugated ACC was observed. Twenty-four h post-treatment ethylene evolution by treated leaves was over 200 times control. This increase was accompanied by a 48- and 15-fold increase in free and conjugated ACC, respectively. By 31 h post-treatment ethylene evolution had declined while endogenous levels of free and conjugated ACC continued to rise.

Table II. *Influence of Calcium or Lanthanum on TDZ-induced Ethylene Evolution*

Cotton leaves were sprayed on both sides to run-off with a 100 μM TDZ solution in 0.5% (v/v) Tween 20. After 18 h, leaf discs (8 mm diameter) were excised from the treated leaves and groups of five discs were placed in 25 ml flask that contained 3.5 ml of the chloride salts of either Ca or La at the indicated concentrations. The flasks were sealed 2 h after the addition of the leaf discs. Ethylene accumulation was determined after 4 h.

Treatment	Concentration	Ethylene Evolution	Control
	mM	*nl/h·5 discs*	%
None[a]		14.67 ± 2.42[b]	100
$CaCl_2$	0.1	14.51 ± 2.69	99
	1.0	15.71 ± 2.47	107
	10.0	12.97 ± 2.77	88
$LaCl_3$	10.0	18.08 ± 4.95	123
$LaCl_3$ + $CaCl_2$	10.0 (both)	14.89 ± 5.47	102

[a] Leaf discs prepared from leaves not treated with TDZ produced 0.14 nl ethylene. [b] Values presented are means ± SEM (n = 3).

The effect of TDZ-treatment on the last step of ethylene biosynthesis (*i.e.* ACC oxidation) was examined by providing excised tissues with a saturating amount of ACC. This methodology provides two types of information: (a) ethylene production in the presence of excess ACC is a measure of the total capacity of the EFE system(s) and (b) the difference in ethylene production in the presence and absence of ACC indicates the degree of saturation of this system by endogenous substrate. Exogenous ACC was readily taken up by control and treated leaf tissues (Fig. 4). Treatment with 1 μM TDZ resulted in a 3- and 1.5-fold

Table III. *Time Course of Ethylene Evolution from Excised Cotton Leaves following TDZ Treatment*

Leaves on intact cotton seedlings were treated with TDZ (100 μM) in a surfactant solution. At various times thereafter, leaves were excised from the seedlings and were enclosed for 1 h. Ethylene evolution was determined by GC.

Collection Period	Ethylene Evolution	
	Control	TDZ
h[a]	*nl/g fresh wt·h*	
4–5	1.00 ± 0.06[b]	1.29 ± 0.27
7–8	0.65 ± 0.18	1.34 ± 0.18
11–12	0.86 ± 0.47	7.78 ± 1.88

[a] Time elapsed from treatment. [b] Mean ± SE (n = 3).

Table IV. *Effects of Various Inhibitors on TDZ-induced Ethylene Evolution from Cotton Leaves 1 and 2 Days after Treatment*

Cotton leaves were sprayed on both sides to run-off with a solution of 100 μM TDZ in 0.5% (v/v) Tween 20. One and two days after treatment, the treated leaves were removed from the seedlings and discs (8 mm diameter) were removed with a cork borer and placed in Petri dishes containing the inhibitor at the concentrations indicated. After 3 h of incubation in the dark, groups of 10 discs were transferred to flasks containing 1 ml of the inhibitor solution. The flasks were sealed, incubated in the dark (27 ± 1°C), and the ethylene content of the flasks was determined after 4 h.

Treatment	Concentration	Ethylene Evolution	
		Day + 1	Day + 2
	mM	*nl/10 discs·4 h*	
None		99.05 ± 17.08[a]	15.53 ± 4.75
AVG	0.1	30.81 ± 2.87	20.41 ± 2.33
AOA	1.0	34.78 ± 2.98	13.01 ± 2.98
$CoCl_2$	1.0	1.12 ± 0.12	1.22 ± 0.00
α AIB	10.0	34.12 ± 5.33	4.55 ± 1.15

[a] Values presented are means ± SEM (n = 3).

increase in total EFE activity 1 and 2 d post-treatment. The large response to exogenous ACC by these tissues suggested that endogenous levels of this substrate were in no way saturating. Treatment with 100 μM TDZ resulted in a 5.5-fold increase in total EFE activity 1 d post-treatment. By 2 d post-treatment total EFE activity had declined below control levels and the enzyme system appeared to be saturated. That ethylene production by these tissues in the absence of exogenous ACC was still well above control rates most likely resulted from the much higher endogenous levels of ACC in these tissues at this time (Fig. 3). Extraction and fractionation of these leaf tissues following these assays revealed no differences in the capacity of these tissues to malonylate ACC; in all tissues 2 to 3% of the exogenous ACC taken up was converted to MACC.

Unlike many fruit tissues such as ripening apple (19), carbon flux through the ethylene pathway in vegetative tissues (petals, leaves) utilizes only a small percentage of the free methionine pool(s) present in the plant tissue (8, 15). However, the fact that a stoichiometric relationship exists between methionine utilization and ethylene, ACC and MACC formation coupled with the massive synthesis of these compounds following TDZ treatment prompted an investigation of the changes in methionine levels following TDZ treatment. Ignoring the diversion of methionine carbon to ethylene itself, the combined amounts of ACC and malonylate ACC accumulated 1 and 2 d post-treatment amounted to over 13 and 18 times the endogenous levels of methionine present in these tissues (Table V). In spite of this massive utilization of methionine, endogenous levels of free methionine remained remarkably constant.

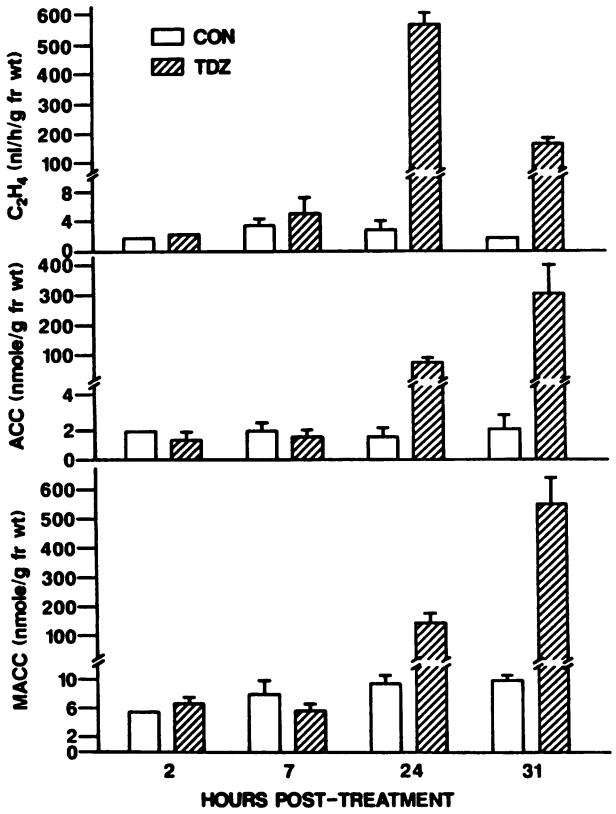

FIG. 3. Effect of 100 μM TDZ on ethylene production (upper panel), free ACC (middle panel), and acid-labile, conjugated ACC (lower panel) at various times after treatment. Bars indicate SE.

DISCUSSION

The data presented herein demonstrate that treatment of previously nonstressed cotton leaves with a variety of cytokinins results in a marked stimulation of ethylene production. The synthetic cytokinins BA and TDZ were especially active in this regard (Table I). With TDZ, the maximum stimulation (440 × control) was observed 24 h after treatment (Fig. 2). By 48 h after 100 μM TDZ treatment, rates of ethylene production had diminished somewhat but were still well above control values. Not all species examined exhibited this response to cytokinins (Fig. 2). Of those tested, cotton and, to a much less extent, sunflower leaves exhibited the greatest sensitivity. At present, it is not clear to what extent differences in uptake and/or metabolism contributed to the observed efficacies of the various cytokinins studied. The failure to observe a TDZ response within 24 h in bean leaves is not at variance with previously published information (2). These authors did not observe any effects of TDZ treatment on ethylene production until after 4 d post-treatment. In cotton, TDZ was far more effective than the auxin 2,4-D in stimulating ethylene production while in sunflower the reverse was true. Cytokinins have been shown to enhance ethylene production in root (12), epicotyl (3), hypocotyl (7, 13) and leaf tissues (2, 9, 14). In these cases the cytokinins studied were much less effective than auxins and the maximum stimulation observed was generally small (less than 10 × control). During the course of comprehensive studies on hormonal relations during water stress, Wright (18) observed that BA markedly enhanced the magnitude and prolonged the duration of water stress-induced ethylene production.

The application of kinetin to etiolated pea seedlings resulted in an increase in ethylene production that commenced between 5 and 9 h following application of the cytokinin (3). Subsequent

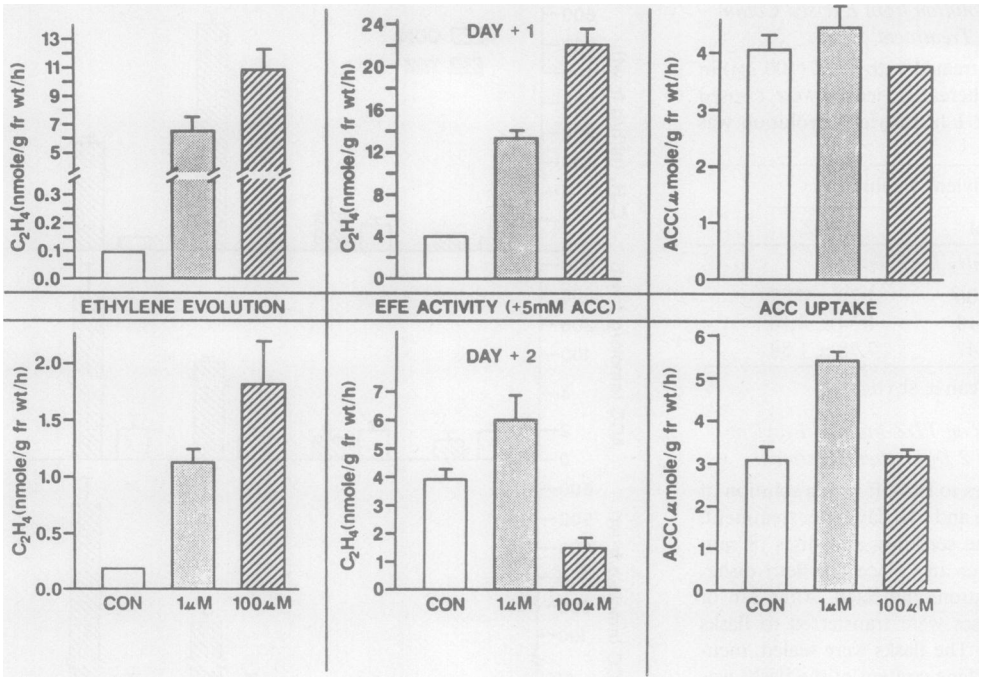

FIG. 4. Effect of 1 or 100 μM TDZ on ethylene production (left), EFE activity (middle), and ACC uptake (right). Upper panels: 24 h post-treatment; lower panels: 48 h post-treatment. Bars indicate SE.

Table V. *Effect of TDZ on the Rate of Ethylene Evolution and on the Endogenous Levels of ACC, MACC, and Methionine*

Cotton leaves were sprayed to run-off with 100 μM TDZ in 0.5% (v/v) Tween 20. One or 2 d after treatment, intact leaves were enclosed for 1 h to determine the rate of ethylene evolution. After this, leaf discs were removed and the endogenous contents of methionine, ACC, and MACC were determined after homogenation.

Group	Day	Ethylene	ACC	MACC	ACC + MACC	Methionine
		nmol/g fresh wt·h			*nmol/g fresh wt*	
Control	1	0.04	2.18	10.41	12.59	37.63
TDZ	1	13.01	400.49	70.65	471.14	35.22
Control	2	0.03	2.73	5.56	8.29	46.68
TDZ	2	1.67	525.22	241.60	766.82	40.75

studies found a 6 h lag period in kinetin-induced ethylene production in etiolated mung bean hypocotyl segments (7). Previous studies in this laboratory have found a readily measurable increase in ethylene production from etiolated mung bean hypocotyl segments within 2 h of TDZ treatment (13). In cotton leaves, no increase in ethylene evolution could be detected until 7 to 8 h following TDZ treatment (Table III). Since the lag period is a reflection of the cumulative delays in the penetration of an exogenous substance, its primary interaction with a cellular constituent (receptor) and the time required for the alteration in cell functioning, these differences in lag periods may simply reflect the experiment protocol(s) used.

In etiolated mung bean hypocotyl segments, a marked synergism was found between kinetin and calcium ions (7). Other studies using this same tissue found only a marginal effect of calcium ions on TDZ-induced ethylene production (13). This type of marginal response to external calcium was also found in the present study (Table II). In addition, the inclusion of the potent calcium antagonist lanthanum (17) in the incubation medium did not reduce the cytokinin effect. Whether these differences relate to the cytokinin used, the tissue under study or to the incubation conditions employed is not clear. Due to the presumed importance of calcium in plant biology, this point deserves further study.

As is the case with auxins (5), the increase in ethylene production following TDZ treatment of etiolated tissue segments was attributed to an increase in ACC synthase activity (13). No effect of TDZ treatment on EFE was observed. The cytokinin-dependent increase in stress ethylene was also ascribed to an increase in ACC synthesis (10). Here again, no effect on EFE activity was found. Evidence has been provided in this study that suggests that the stimulation of ethylene production in cotton leaves following TDZ treatment is due to increases in both ACC availability and EFE activity.

At the higher treatment levels (≥10 μM), TDZ application results in two distinct phases of ethylene production. Initially (*i.e.* between 8–24 h post-treatment) there is a massive increase in ethylene production in treated tissues (Fig. 2). During this time inhibitors of ACC synthesis (AVG, AOA) are effective in reducing ethylene production (Table IV). The initial increase in ethylene production (7–8 h post-treatment) is not accompanied by an increase in endogenous ACC (Fig. 3). Provision of exogenous ACC results in a dramatic increase in ethylene production in both control and in TDZ-treated leaves (Fig. 4). Together these results suggest that during this period ACC is limiting, its continued synthesis is required and that initially it is metabolized as rapidly as it is formed. Thus, the initial increase in ethylene production following TDZ treatment is the result of increased ACC availability. By 48 h post-treatment ACC is apparently no longer limiting as judged by the observations that: (a) AVG and AOA are without effect and (b) provision of exogenous ACC to tissue treated with 100 μM TDZ has no impact on ethylene production.

EFE activity can be assayed via the provision of saturating

levels of ACC. Using this technique it was found that 24 h following treatment, EFE activity was stimulated by 3- and 5-fold in tissues treated with 1 or 100 M TDZ, respectively (Fig. 4). One day later (48 h post-treatment), EFE activity in tissues previously treated with 1 μM TDZ was still above control levels while in tissues treated with 100 μM TDZ, EFE activity had declined below control values. This loss of EFE activity undoubtedly contributes to the decline in ethylene evolution coupled with the sustained increases in ACC and MACC observed 48 h post-treatment (Fig. 3). Ethylene itself has been found to increase EFE activity in leaf tissues (1). In the absence of TDZ, ethylene treatment resulted in roughly a 2-fold increase in EFE activity in cotton leaves (JC Suttle, unpublished data). Therefore it is possible that at least a part of the increase in EFE activity observed following TDZ treatment is the result of TDZ-induced ethylene production. This possibility is being explored.

Recent evidence suggests that the formation of MACC from ACC can act to regulate ethylene production in certain tissues (6). The massive accumulation of acid-labile, conjugated ACC in these tissues following TDZ treatment (Fig. 3) attests to their possession of this metabolic shunt. Despite this fact, very little (2–3%) of the [^{14}C]ACC taken up by these tissues during the EFE determinations was converted to MACC (not shown). Since exogenous ACC was readily metabolized to ethylene (Fig. 4), it is likely that the two enzyme systems reside in different cellular compartments and that, in the short term (<4 h), exogenous ACC is not available to the conjugating enzyme.

Finally, it should be noted that methionine is a critical amino acid serving as a carbon source for many biochemical pathways including polyamine, phospholipid, nucleic acid biosynthesis and pectin modification (methylation). It has been suggested that, under normal conditions, the bulk of methionine turnover is involved in these reactions (4). The massive diversion of methionine to ethylene, ACC, and MACC synthesis following TDZ treatment (Table V) could have a dramatic effect on many of these critical metabolic pathways. For this reason, TDZ may prove extremely useful to those concerned with the regulation and partitioning of methionine flux in higher plants. Furthermore, it is possible that one or more of these metabolic alterations, together with the demonstrated role of ethylene (14), may play a role in TDZ-induced cotton defoliation.

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