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Induction of Leaf Abscission in Cotton Is a Common Effect of Urea- and Adenine-Type Cytokinins

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ABSTRACT

Cytokinins of the urea and adenine type induced leaf abscission in young cotton (*Gossypium hirsutum*) plants in the following order of activity: *N*-phenyl-*N'*-1,2,3-thiadiazol-5-ylurea (thidiazuron) ≫ *N*-phenyl-*N'*-(2-chloro-4-pyridyl)urea > isopentenyladenine ≥ 6-benzyladenine > zeatin = dihydrozeatin > kinetin. It is suggested that ethylene production is implicated in this response because it was stimulated by the compounds in cotton leaf discs with nearly the same effectiveness. Moreover, similar to thidiazuron (JC Suttle [1985] Plant Physiol 78: 272–276), isopentenyladenine-induced defoliation was inhibited by aminoethoxyvinylglycine, and the effect was restored by 1-aminocyclopropane-1-carboxylic acid.

The phytohormonal cytokinins are N^6-substituted adenine compounds that have been implicated in the regulation of many physiological processes including cell division and expansion, seed germination, flowering, and senescence (*e.g.* ref. 7). In addition to these natural cytokinins, synthetic compounds of different chemical classes demonstrated cytokinin-like activities (5, 9). Among them the N^6-substituted adenine derivatives, *e.g.* Kn[1] and BA, and the heterocyclic ureas, *e.g.* TDZ and 4 PU-30, are the most effective substances known (9, 10, 19). In particular, TDZ and its derivatives displayed qualitatively similar biological properties to purine cytokinins in a range of bioassays including cytokinin-dependent ethylene production systems (19, 23). The question is still open as to whether the biosynthesis and/or metabolism of cytokinins are influenced or a common site of action with endogenous cytokinins exists (11).

As an additional physiological effect, TDZ induced abscission of young, nonsenescing, and mature cotton leaves (2). In this function, the compound is used in agriculture as a cotton defoliant to facilitate mechanical and manual harvesting (13). Results indicated that the effect was mediated by an increase in endogenous ethylene (16). This phytohormone is currently regarded as an important regulator of leaf abscission (6, 13, 15). However, TDZ-induced defoliation seems to be restricted to plants of the Malvaceae (11).

[1] Abbreviations: Kn, 6-furfurylaminopurine (kinetin); ACC, 1-aminocyclopropane-1-carboxylic acid; MACC, *N*-malonyl-1-aminocyclopropane-1-carboxylic acid; AVG, aminoethoxyvinylglycine; DZ, DL-dihydrozeatin; IP, N^6-(Δ^2-isopentenyl)adenine; 4 PU-30, *N*-phenyl-*N'*-(2-chloro-4-pyridyl)urea; TDZ, *N*-phenyl-*N'*-1,2,3-thiadiazole-5-ylurea (thidiazuron); Z, zeatin.

In contrast, purine-type cytokinins usually delay leaf senescence and abscission (*e.g.* Ref. 21). However, in exceptional cases such as in explants of *Phaseolus* (14) and leaves of *Streptocarpus* (20), promotion of the abscission process probably caused by stimulated ethylene (1) has been observed also.

In cotton, ethylene production was increased by thidiazuron as well as by natural and synthetic cytokinins of adenine type (17). However, little information has been given in the literature on the potency of adenine-type cytokinins in defoliating cotton. The only indication for such an activity has been provided for BA (24). In this communication, it is demonstrated that induction of leaf abscission in cotton is a common effect of cytokinin-active heterocyclic ureas and N^6-substituted adenines. The data are compared with the effectiveness of the compounds in stimulating ethylene formation of cotton leaf discs *in vitro*.

MATERIALS AND METHODS

Chemicals

The following cytokinin-active compounds were used: TDZ (Dropp, SN 49537; Ref. 2) and 4 PU-30 (18). Cytokinins of the adenine type, benzimidazole, and *N,N'*-diphenylurea were obtained from Sigma, ACC was from Calbiochem (La Jolla, CA), and AVG was from Fluka AG (Buchs, Switzerland).

Defoliation Experiments

Cotton plants (*Gossypium hirsutum* L. cv Stoneville 825, cv Coker 310, cv Acala; *Gossypium barbadense* L. cv Pima 55) were grown with a nutrient-supplemented peat-based substrate in plastic pots (diameter 12.5 cm, 500 mL, five plants/pot) under controlled greenhouse conditions (light/dark: 14/10 h provided by additional illumination with radium HRLV lamps, approximately 180 μmol m^{-2} s^{-1}, at 25/19°C and 50–70% relative humidity). Plants of the fifth leaf stage were sprayed uniformly with 2.5 mL/pot with an aqueous solution containing the cytokinin-active compound prepared in Tween 85 (polyoxyethylene-sorbitan trioleate, 2% w/w final concentration). In control treatments, the cytokinin-active ingredient was omitted. In each treatment four replicate pots were used. Six days after application, abscission of the five true leaves was determined by deflecting the petiole (at the leaf blade junction) approximately 2 cm and counting the number of abscised leaves (according to the procedures in Ref. 16). The degree of abscission was calculated in percentage of the total number of true leaves. Abscised blades were sampled from selected trials and weighed, the leaf area was

measured, and the blades were immediately frozen in solid CO_2. After powdering under liquid N_2, Chl contents were measured in 80% (v/v) acetone extracts according to methods described previously (4). In the experiments with AVG treatment, cotton plants of the fifth leaf stage were sprayed uniformly with 2.5 mL/pot of an aqueous solution containing 1 or 3 mg AVG and 1% w/w Tween 85 1 d before treatment with IP (3 mg/pot, 2% w/w Tween 85). Simultaneously, groups of plants were also sprayed with 3 mg/pot ACC together with IP. In control treatments, aqueous solutions containing Tween 85 in corresponding concentrations were applied.

Ethylene Formation and ACC and MACC Levels in Leaf Discs

Cotton plants (*Gossypium hirsutum* L. cv Stoneville 825) were raised to the fifth leaf stage as described above. Discs (0.5 cm in diameter) were cut from blades of the second and third true leaves with a corkborer. Discs were floated for about 2 h in Petri dishes containing double-distilled water so that ethylene produced from the excision process could dissipate. Twenty randomized leaf discs were then placed adaxial side down on filter paper in a Petri dish (5 cm in diameter) moistened with 1 mL of the test solution. Solutions of chemical compounds used were prepared in acetone or in ethanol (100 times concentrated) and diluted with 10 mmol/L Mes/KOH (pH 6.1) in double-distilled water. Measurement of ethylene production and ACC and MACC contents of the leaf discs were carried out as described previously (3). The leaf discs were incubated in darkness at 25°C for 16 h. Then the filter papers with the leaf discs were placed in plastic tubes that were sealed with rubber caps. After incubation in darkness at 25°C for 6 h, a 1 mL gas sample of head space was withdrawn, and ethylene was measured with a gas chromatograph. The leaf discs from each assay were weighed, powdered under liquid N_2, and extracted in 70% ethanol. After passage through a C_{18}-reversed-phase prepacked column (SEP-PAK; Waters, Königstein, FRG), the ACC content of the extract was assayed by converting it to ethylene (8). Ethylene was measured by gas chromatography. MACC was quantified by hydrolyzing it to ACC by treatment with 2 N HCl at 110°C for 4 h. The concentration of ACC was similarly assayed (8). The amount of MACC was determined by subtracting the value of ACC in the nonhydrolyzed sample from that in the hydrolyzed sample. For each treatment, four replicates were used and mean values ± SE are given. All experiments were repeated at least twice with reproducible effects and a representative result shown.

RESULTS AND DISCUSSION

Foliar treatment of young cotton plants at the fifth leaf stage with TDZ resulted in a dose-dependent abscission of the true leaves (Fig. 1). At low concentrations the leaves dropped in a green stage without exhibiting visible damage. This is caused by a premature activation of abscission zone tissue between the plant stem and the petioles (Schering Information, Dropp Cotton Defoliant, third edition, 1981). As previously observed (16), abscised leaves did not exhibit altered

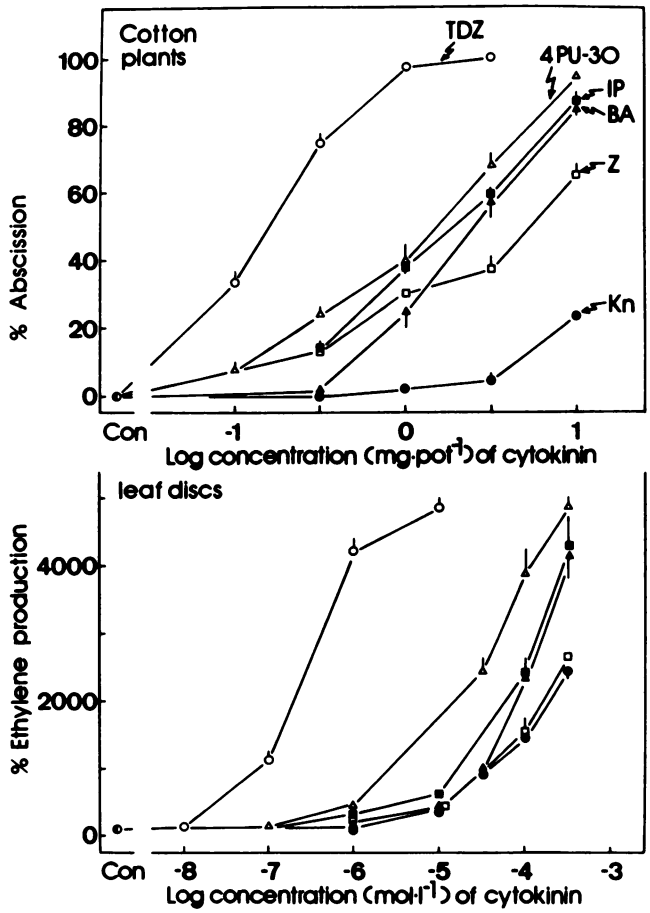

Figure 1. Influence of various cytokinins on leaf abscission in cotton plants (*G. hirsutum* L. cv Stoneville 825) at the fifth leaf stage (top panel) and on ethylene formation of cotton leaf discs (bottom panel). ○—○, TDZ; △—△, 4 PU-30; ■—■, IP; ▲—▲, BA; □—□, Z; ●—●, Kn. Six days after treatment, abscission was calculated in percentage to the total number of true leaves. Concentrations are expressed in log mg/pot (*i.e.* 1 = 10 mg/pot). Vertical bars represent SE of the mean (four replicate pots with five plants per pot). Leaf discs were treated *in vitro* and ethylene was measured after 16 h. Bars indicate SE of the mean (four replicates). Control value ± SE representing 100% was 0.042 ± 0.006 nmol C_2H_4 × g fresh weight^{-1}.

water potential and Chl contents. Likewise, ethane evolution (a physiological parameter for wounding) was not affected (16). Hence, TDZ did not act like contact herbicides via chemical wounding, desiccation, or accelerated leaf senescence (13, 15). The same phenomenon occurred when leaf abscission in cotton was induced by treatment with increasing concentrations of the synthetic or naturally occurring cytokinins 4 PU-30, IP, BA, Z, DZ, and Kn (Fig. 1). In abscised leaves, fresh weight and Chl content were not affected (data not shown). However, at the highest concentration applied (1 mg/pot in the case of TDZ, 10 mg/pot for all others), the leaf surface exhibited chlorotic and partially necrotic spots accompanied by symptoms of desiccation. Of the cytokinins tested, the heterocyclic ureas 4 PU-30 and, particularly, TDZ were more effective in mediating leaf abscission than the adenine derivatives (Fig. 1). The order of activity was as follows: TDZ

≫ 4 PU-30 > IP ≥ BA > Z > Kn. DZ applied only at a concentration of 3 mg/pot, induced abscission similar to Z (41.2 ± 6.5% abscission for DZ, 36.5 ± 3.7% for Z). BA has recently been shown also to promote leaf shedding in cotton, being less active than TDZ (24). Besides their activity in the cotton cv Stoneville 825, TDZ, IP, and BA induced leaf abscission with the same efficiency in plants of the cv Coker 310 and Acala as well as in *G. barbadense* L. cv Pima 55 (data not shown).

In the case of TDZ, it was concluded that the compound exerted its effect by stimulating ethylene production (16). Hence, the degree of leaf abscission mediated by the cytokinins was compared with their ability to evoke ethylene formation in leaf discs of cotton. Ethylene was stimulated by the cytokinins in the following order: TDZ ≫ 4 PU-30 > IP ≥ BA > Z ≥ Kn (Fig. 1). Endogenous ACC and MACC levels were elevated to a lesser extent (Table I). However, ACC was increased by the compounds with nearly the same order of activity as ethylene (Table I). This supports previous results of a cytokinin-induced, ACC-dependent ethylene formation (17). With the exception of Kn, which was comparatively more effective in increasing ethylene than in defoliating, a close correlation between both cytokinin-stimulated activities was observed ($r = 0.88$, $P < 0.01$; calculated from data presented in Fig. 1 between the effects of 10^{-4} mol/L of cytokinins, including Kn, on ethylene stimulation and of 3 mg/pot on leaf abscission). This relationship was also obtained when compounds with less cytokinin-like activity such as benzimidazole and *N,N'*-diphenylurea (9, 12) were used. They affected ethylene formation in cotton leaf discs only slightly and did not induce leaf abscission in cotton plants (data not shown).

It is widely accepted that ethylene is an important regulatory signal for abscission (*e.g.* 6, 13, 15). Thus, the increase in ethylene formation elicited by the applied compounds including the most active cytokinins known (9) appears to be involved in the induction of leaf abscission in cotton. This assumption was confirmed in experiments attempting to abolish abscission effects of IP (a representative of adenine-type cytokinins) by application of AVG, an inhibitor of ACC-synthase (22). Pretreatment of cotton plants at the fifth leaf stage with increasing concentrations of AVG 1 d before IP application gradually reduced the IP-induced abscission response (Fig. 2). The effects of AVG on abscission could be completely reversed by additional treatment with ACC, the direct precursor of ethylene in the biosynthetic pathway (Fig. 2). In this context, AVG was also able to neutralize IP-stimulated ethylene production as demonstrated in cotton leaf discs treated *in vitro* (Table II). Similar results were obtained in corresponding trials with the heterocyclic urea TDZ (16).

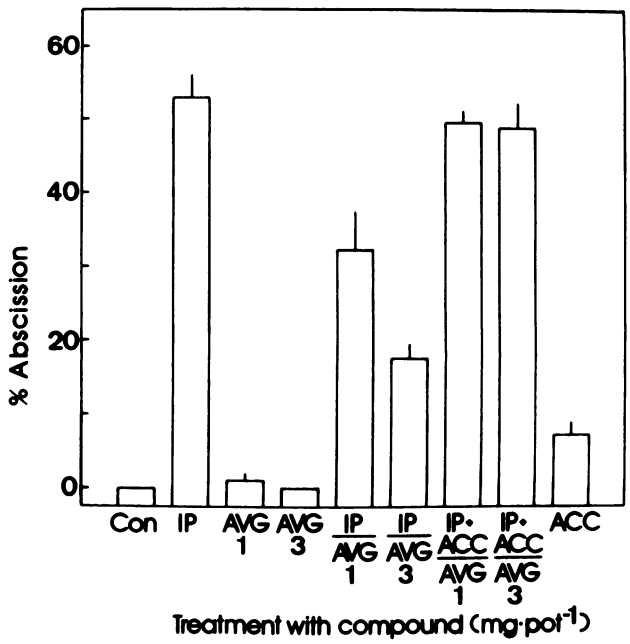

Figure 2. Influence of AVG and ACC on IP-induced leaf abscission in cotton plants (*G. hirsutum* L. cv Stoneville 825) at the fifth leaf stage. Treatment with IP (3 mg/pot); 1 d before AVG alone (1 or 3 mg/pot); IP (3 mg/pot) and 1 d before AVG (1 or 3 mg/pot); IP together with ACC (both 3 mg/pot) and 1 d before AVG (1 or 3 mg/pot); or ACC (3 mg/pot) alone. Six days after first treatment abscission was calculated in percentage to the total number of true leaves. Vertical bars represent SE of the mean (four replicate pots with five plants per pot).

In conclusion, there seems to exist no qualitative difference

Table I. *Influence of Various Cytokinins on Production of Ethylene and Content of ACC and MACC in Cotton Leaf Discs (G. hirsutum L. cv Stoneville 825) Treated in Vitro*

Data are means ± SE (four replicates) compiled from an experimental series. Values in brackets are stimulation in percent of control.

Compound	Concentration	Ethylene Formation	ACC Content	MACC Content
	mol × L⁻¹	*nmol × g fresh wt⁻¹ × h⁻¹*	*nmol × g fresh wt⁻¹*	*nmol × g fresh wt⁻¹*
Control		0.054 ± 0.005 (100)	0.26 ± 0.06 (100)	6.66 ± 0.19 (100)
TDZ	10^{-4}	2.51 ± 0.18 (4648)	3.98 ± 0.26 (1531)	29.69 ± 0.53 (446)
4 PU-30	10^{-4}	1.94 ± 0.32 (3593)	3.92 ± 0.18 (1508)	21.10 ± 0.66 (317)
Control		0.041 ± 0.005 (100)	0.30 ± 0.13 (100)	5.92 ± 0.46 (100)
IP	10^{-4}	0.979 ± 0.09 (2388)	2.21 ± 0.24 (737)	22.17 ± 0.96 (375)
Z	10^{-4}	0.648 ± 0.11 (1580)	0.82 ± 0.05 (273)	17.87 ± 0.55 (302)
Control		0.032 ± 0.008 (100)	0.22 ± 0.09 (100)	3.60 ± 0.38 (100)
Kn	10^{-4}	0.475 ± 0.04 (1484)	0.78 ± 0.09 (356)	8.23 ± 0.44 (229)
BA	10^{-4}	0.742 ± 0.02 (2319)	1.17 ± 0.19 (532)	6.49 ± 0.36 (180)

Table II. *Influence of AVG on IP-Stimulated Ethylene Production of Cotton Leaf Discs (G. hirsutum L. cv Stoneville 825) Treated In Vitro*

The compounds were applied simultaneously. Data are means ± SE (five replicates). Values in brackets are ethylene formation in percent of control.

Compound	Concentration	Ethylene Production
	$mol \times L^{-1}$	$nmol \times g\ fresh\ wt^{-1} \times h^{-1}$
Control		0.043 ± 0.002 (100)
IP	10^{-4}	1.087 ± 0.040 (2528)
IP	10^{-4} + AVG, 10^{-5}	0.431 ± 0.011 (1002)
IP	10^{-4} + AVG, 10^{-4}	0.079 ± 0.003 (184)
AVG	10^{-5}	0.020 ± 0.001 (47)
AVG	10^{-4}	0.005 ± 0.001 (12)

between heterocyclic urea- and adenine-type cytokinins in mediating leaf abscission in cotton. It is suggested that this effect is common for cytokinin-active compounds in this species. The greater quantitative effect of the heterocyclic ureas might be explained by more favorable penetration and/or metabolic properties or, alternatively, by higher target sensitivity (11).

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