# Effects of Kinetin Formulations on Allelochemicals and Agronomic Traits of Cotton

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Twelve candidate plant growth regulator formulations were applied twice at two levels to fruiting cotton (Gossypium hirsutum L.). Leaves and squares were collected for analysis of allelochemicals (gossypol, tannin, anthocyanin, flavonoids) at 3 and 5 weeks after the first treatment. The plots were machine harvested one time to determine yield. Seeds were delinted and analyzed for agronomic traits and gossypol. Leaf gossypol and square gossypol were the categories most frequently increased by the bioregulators. Kinetin, and kinetin plus CaCl<sub>2</sub> or Na<sub>2</sub>SeO<sub>3</sub>, and mepiquat chloride (PIX) alone or with a commercial cytokinin preparation (Foliar Triggrr) all increased gossypol and one or more of the other allelochemicals significantly. A sugar-amino acid fraction isolated from Foliar Triggrr increased cotton yield by 26% when applied as a foliar dressing at 2.88 mol/ha, as well as increasing gossypol. These results suggest that plants under stress may respond positively to nutrient foliar applications, giving both increased allelochemicals and improved yield.

# INTRODUCTION

Bioregulators (natural and synthetic) are being researched extensively to increase agricultural production. They are normally applied directly to crop plants to alter growth patterns, improve quality, increase yields, or facilitate harvesting. They are understood to act, directly or indirectly, by modifying gene action. They have also been found to have effects on pests of the crop plants, either by affecting the pests' metabolism or by the eliciting of disease and insect resistance factors (allelochemicals) in the plant. The latter may involve activation of defense genes, in turn activating enzymes to produce the allelochemicals.

The use of bioregulators on cotton (Gossypium hirsutum L.) has been investigated extensively, and the literature has been cited in several of our previous papers (Hedin et al., 1984, 1988a,b; McCarty et al., 1987; Mc-Carty and Hedin, 1989). The focus of our present study is kinetin, which is understood to influence many aspects of plant growth and development (Weaver, 1972; Elliott, 1982), and some kinetin formulations. Preliminary results from several field tests showed that kinetin and two commercial kinetin formulations tended to increase yield of cotton, pest resistance, and four allelochemicals: gossypol, condensed tannins, flavonoids, and anthocyanins (Hedin et al., 1988a; McCarty et al., 1987; McCarty and Hedin, 1989).

For the present study, 12 bioregulator preparations were applied at two levels twice to fruiting cotton. Plant tissues were collected for analysis of allelochemicals at 3 and 5 weeks after the first treatment. The plots were machine harvested one time to determine yields. Properties of the lint and seed were determined. The objectives of this study were to determine whether the candidate growth regulators affected yield and levels of allelochemicals and to discern the mechanisms if possible.

## MATERIALS AND METHODS

1989 Bioregulator Field Test. The commercial cotton cultivar Deltapine 50, well adapted for the study area, was grown in 1989 at Mississippi State University. The cotton was planted April 29 in single row (38 in. by 42 ft) plots. Insects were controlled all season with Fenvalerate and Cythion RTu. Twelve plant growth regulator formulations (see the listing of the procurement source elsewhere under Materials and Methods) were applied at three rates (zero, low, high) on July 10 and July 24. Each compound was handled as a separate randomized complete block experiment with five replications.

The timing of applications and rates were in general those recommended by previous investigators or the provider (Hedin et al., 1988a,b). Two rates, with the second application generally 3-fold higher, were used to improve the likelihood that a response would be elicited. Each compound was weighed and dissolved in 5–10 mL of H<sub>2</sub>O. One milliliter each of Span 80 and Tween 80 were then added. The solutions were made up to 1.25 L with water and stored at 4 °C until use. They were applied with a  $CO_2$ -pressurized backpack sprayer delivering 203 L/ha at 207 kPa pressure. Plant material (terminal leaves and squares) was collected on July 31 and August 14 and placed in the freezer (-20 °C) until processed.

The plots were machine harvested one time for yield determination on September 30. Prior to machine harvest, 25 open bolls were hand harvested from each plot, weighed, and ginned to determine boll size, lint percentage, and seed index. Seed index is the weight in grams of 100 fuzzy seeds. The lint percentage determined was used in calculating lint yields.

**Procurement of Bioregulators.** Kinetin (6-furfuryladenine) and IAA (indole-3-acetic acid) were procured from Sigma Chemical Co., St Louis, MO. Urea, calcium chloride, sodium selenite ( $Na_2SeO_3$ ), thioglycolic acid (TGA), and vanillic acid (VA) were obtained from ICN Nutritional Biochemicals, Cleveland, Oh. PIX (mepiquat chloride) was obtained from BASF, Ludwigshafen, W. Germany. Foliar Triggrr (FT; active ingredient: kinetin, 0.012%) wasobtained from Westbridge Agricultural Products, San Diego, CA.

Fractionation of Foliar Triggrr (FT). FT, a dark liquid commercial preparation with some particulate material (22% total solids), was mixed with 50% aqueous acetonitrile at the rate of 200 mL of FT to 3200 mL of 50% aqueous acetonitrile to yield on precipitation and subsequent filtration 2.93% of a black precipitate and 19.05% of a yellow filtrate that was a solid semicrystalline product after freeze-drying (yields were calculated as the percent of the total liquid). The filtrate fraction (5 g) was redissolved in 50% aqueous acetonitrile and chromatographed with the same solvent on a Sephadex G-50-1505  $\times$  70 cm column, collecting 9-10 fractions of 200 mL each. Nearly all of the total solids could be accounted for in the fractions collected; about 2% of which was proteinaceous (initial fraction), then 48% as sugars and amino acids, and finally 47% as urea. The identity of the urea was confirmed by MS, NMR, IR, and cochromatography (TLC) with an authentic sample.

Table I. Effect of	<b>Bioregulators</b> on	Allelochemical	Levels in Cotton	Terminal Leaves	. Percent of Dry	V Weight <sup>4,b</sup>
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			goss	ypol	tannins		anthocyanin		flavonoids	
compd	g/ha	mol/h <b>a</b>	date 1	date 2	date 1	date 2	date 1	date 2	date 1	date 2
control	0	0	0.37	0.25	21.43	14.46	0.19	0.17	3.93	3.31
kinetin	3.7	0.017	0.36	0.27	22.93*	14.60*	0.18	0.19	3.92	3.48
	21.6	0.100	0.36	0.24	22.94*	12.39**	0.21	0.18	3.82	3.42
kinetin + IAA	3.7, 3.0	0.017, 0.017	0.33	0.25	21.20	13.11	0.20	0.18	4.00	3.33
	21.6, 17.6	0.100, 0.100	0.37	0.26	23.09**	13.47	0.21	0.18	3.95	3.68**
kinetin + CaCl <sub>2</sub>	3.7, 111.0	0.017, 1.000	0.37	0.25	22.49**	12.35**	0.21	0.20	3.88	3.42
	21.1, 347.4	0.100, 3.130	0.34**	0.26	20.47**	13.10	0.19	0.17	3.85	3.44
kinetin + Na <sub>2</sub> SeO <sub>3</sub>	3.7, 173.0	0.017, 1.000	0.38	0.26	20.96	15.80**	0.23**	0.18	3.93	3.61**
	21.6, 541.5	0.100, 3.130	0.34**	0.29**	17.59**	14.03	0.23**	0.21**	3.96	3.56**
kinetin + TGA	3.7, <b>9</b> 2.0	0.017, 1.000	0.37	0.26	24.27**	12.97	0.20	0.17	3.72	3.46
	21.6, 288.0	0.100, 3.130	0.38	0.26	24.06**	13.48	0.20	0.16	3.78	3.39
kinetin + VA	3.7, 84.0	0.017, 0.500	0.35	0.25	23.66**	13.57	0.20	0.16	3.73	3.32
	21.6, 262.9	0.100, 1.565	0.36	0.26	23.31**	14.22	0.19	0.16	3.70	3.32
FT	0.065	0.0003	0.38	0.26	22.43	13.41	0.19	0.17	3.86	3.61
	0.214	0.0010	0.40	0.26	22.91**	13.92	0.20	0.18	3.95	3.62
urea	60.0	1.00	0.38	0.25	21.86	14.13	0.19	0.16	3.85	3.39
	187.6	3.13	0.36	0.22	21.10	12.95	0.17	0.16	3.83	3.33
FT sugars, amino acids	187.6	1.00	0.37	0.24	22.16	13.58	0.19	0.16	3.96	3.40
-	519.1	2.88	0.38	0.25	22.16	13.67	0.21	0.19	4.02	3.31
PIX	14.9	0.100	0.38	0.26	20.96	15.80**	0.23	0.18	3.93	3.61
	46.7	0.313	0.34	0.29	17.59**	14.03	0.23	0.21	3.96	3.56
PIX + FT	14.9, 0.065 <sup>c</sup>	0.100, 0.0003	0.38	0.26	19.51**	13.24	0.22	0.19	4.00	3.49
	46.7, 0.214	0.313, 0.0010	0.34	0.28	17.94**	14.17	0.22	0.19	3.87	3.50
PIX + urea	14.9, 60.0	0.100, 1.00	0.35	0.26	20.94	14.18	0.21	0.18	3.95	3.41
	46.7, 187.6	0.313, 3.13	0.38	0.27	19.35**	16.04**	0.22	0.19	3.91	3.45

<sup>a</sup> \* and \*\* indicate significantly different from the zero rate at the 5 and 1% levels, respectively. <sup>b</sup> Applications of growth regulators were made on July 10 and 24. Plant tissues were collected on July 31 and August 14. <sup>c</sup> 0.065 g of stated active ingredient (kinetin).

			goss	ypol	tan	nins	antho	cyanin	flavo	noids
compd	g/ha	mol/ha	date 1	date 2	date 1	date 2	date 1	date 2	date 1	date 2
control	0	0	0.30	0.35	15.18	14.05	0.14	0.15	2.16	2.01
kinetin	3.7	0.017	0.29	0.41**	14.99	14.07	0.14	0.15	2.18	1.96
	21.6	0.100	0.29	0.37	15.86	13.28	0.14	0.14	2.08	2.08
kinetin + IAA	3.7, 3.0	0.017, 0.017	0.30	0.38	15.46	12.64	0.14	0.13	2.33	2.24
	21.6, 17.6	0.100, 0.100	0.30	0.37	15.13	13.49	0.13	0.15	2.23	2.42
kinetin + $CaCl_2$	3.7, 111.0	0.017, 1.000	0.32	0.35	14.60	14.21	0.15	0.16	2.30	2.07
	21.1, 347.4	0.100, 3.130	0.29	0.38	14.39	14.22	0.14	0.14	2.13	1.98
kinetin + Na <sub>2</sub> SeO <sub>3</sub>	3.7, 173.0	0.017, 1.000	0.30	0.32	16.22**	13.25	0.14	0.15	2.19	1.86
	21.6, 541.5	0.100, 3.130	0.35**	0.37	15.44	12.32**	0.14	0.17	2.05	2.00
kinetin + TGA	3.7, 92.0	0.17, 1.000	0.29	0.33	15.40	13.60	0.14	0.15	2.15	2.16
	21.6, 288.0	0.100, 3.130	0.28	0.34	15.45	14.10	0.13	0.15	2.04**	2.26**
kinetin + VA	3.7, 84.0	0.017, 0.500	0.28	0.33	14.77	13.7 <del>9</del>	0.14	0.15	1.98**	2.08
	21.6, 262.9	0.100, 1.563	0.28	0.34	14.57	14.60	0.14	0.15	2.10	2.14**
FT	0.065	0.0003	0.30	0.35	14.63	13.53	0.14	0.15	2.09	2.08
	0.214	0.0010	0.30	0.36	15.5 <del>9</del>	14.80	0.14	0.16	2.11	2.12
urea	60.0	1.00	0.31	0.31**	15.10	14.34	0.14	0.14	2.05	2.13
	187.6	3.13	0.29	0.35	15.40	12.48	0.14	0.15	2.10	2.35 * *
FT sugars, amino acids	87.6	1.00	0.31	0.36	15.33	13.43	0.14	0.14	2.16	2.26**
	519.1	2.88	0.30	0.33	15.11	13.57**	0.14	0.16	2.13	2.09
PIX	14.9	0.100	0.30	0.32	16.22**	13.25	0.14	0.15	2.19	1.86**
	46.7	0.313	0.35**	0.37	15.44	12.32	0.14	0.17	2.05**	2.00
PIX + FT	14.9, 0.065°	0.100, 0.0003	0.31	0.35	1 <b>4.9</b> 3	14.43	0.13	0.14	2.16	1.89
	46.7, 0.214	0.313, 0.0010	0.34**	0.34	16.54**	15.54**	0.13	0.16	2.07	1.82**
PIX + urea	14.9, 60.0	0.100, 1.00	0.31	0.37	15.37	13.79	0.14	0.15	2.30	2.07
	46.7, 187.6	0.313, 3.13	0.32	0.41	15.53	12.84**	0.14	0.14	2.19	2.01

Table II.	Effect of Bioregulators on	Allelochemical Levels in Co	Cotton Squares (Buds), Percent of Dry Weight <sup>4,b</sup>	
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<sup>a</sup> \* and \*\* indicate significantly different from the zero rate at the 5 and 1% levels, respectively. <sup>b</sup> Applications of growth regulators were made on July 10 and July 24. Plant tissues were collected on July 31 and August 14. <sup>c</sup> 0.065 g of stated active ingredient (kinetin).

**Analysis of Allelochemicals.** Plant tissue [ca. 25 terminal leaves and 25 squares (buds)] from each replication was collected, freeze-dried, and ground prior to allelochemical analysis. Analysis of allelochemicals (gossypol, tannin, anthocyanin, flavonoid) was conducted following the procedures described by Hedin et al. (1988a).

Statistical Procedures. Data obtained from the various analyses and measurements were subjected to the analysis of variance, and LSD values were calculated according to SAS (1985).

#### RESULTS

The contents of allelochemicals in leaves and squares found as a result of the various bioregulator treatments are given in Tables I (leaves) and II (squares). As previously stated, applications of candidate growth regulators were made on July 10 and July 24. Plant tissues were collected on July 31 and August 14. The levels and in-season variations of levels were similar to those in previous years. The nomenclature, trivial names, and sources of procurement of the bioregulators are listed under Materials and Methods. The percent change of the allelochemicals as affected by the bioregulators from the zero treatment is given in Table III. The effect of the bioregulators on agronomic traits is given in Table IV.

Table III shows that increases of gossypol by at least

Table III. Percent Change of Allelochemicals from the Zero Treatment<sup>a</sup>

	date 1		date 2		date 1		date 2	
compd	lev 1	lev 2	lev 1	lev 2	lev 1	lev 2	lev 1	lev 2
			Gos	sypol				
kinetin	-5.3	+10.5	+11.8	+5.9	-3.3	-3.3	+17.2	+5.7
kinetin + IAA	+5.3	+10.5	+5.9	+5.9	0	0	+8.6	+5.7
kinetin + CaCl <sub>2</sub>	+10.5	0	+17.6	0	+6.6	-3.3	0	+8.6
kinetin + $Na_2SeO_3$	+21.0	+21.0	+5.9	+23.5	0	+16.6	-8.6	+5.
kinetin + TGA	+5.3	+5.3	0	-5.9	-3.3	-6.6	-5.7	-2.9
kinetin + $VA$	+5.3	0	-5.9	-5.9	-6.6	-6.6	-5.7	-2.9
FT	0	+5.3	0	+5.9	0.0	0	0	+2.9
	0		-5.9		-	-6.6	-11.4	+2
urea		-10.5		-5.9	+3.3			
FT sugars, AA	0	+10.5	-5.9	+17.6	+3.3	0	+2.9	-8.0
PIX	+21.0	+21.0	+5.9	+23.5	0	+16.6	-8.6	+5.'
PIX + FT	+15.8	+15.8	+11.8	+11.8	+3.3	+10.0	0	-2.9
PIX + urea	+10.5	+15.8	+5.9	+11.8	+3.3	+6.6	+5.7	+17.
			Tai	nnins				
kinetin	+7.0	+7.1	+5.4	-8.0	-1.2	+4.5	+0.1	-5.8
kinetin + IAA	-1.1	+7.8	-1.6	+0.19	+1.8	-0.3	-10.0	-4.
IX + urea	-5.4	+2.7	+4.9	+8.0	0	0	0	-6.
kinetin + CaCl <sub>2</sub>	+5.0	-4.5	-5.2	-2.7	-3.8	-5.2	+1.1	+1.
kinetin + $Na_2SeO_3$	-2.2	-18.0	+17.3	+4.2	+6.9	+1.7	-5.6	-12.
kinetin + TGA	+13.3	+12.4	-2.3	+0.1	+1.5	+1.8	-3.2	+0.4
kinetin + $VA$	+10.5	+8.8	+0.8	+5.6	-2.7	-4.0	-2.1	+3.
					-3.5	+2.7	-3.7	
FT	+4.7	+6.9	-0.4	+3.4				+5.
urea	+2.0	-1.1	+4.9	-2.4	-0.5	+1.5	+2.1	-11.
FT sugars, AA	+3.4	+3.4	+5.6	+1.6	+0.9	-0.5	-4.4	-3.
PIX	-2.2	-18.0	+17.3	+4.2	+6.9	+1.7	-5.6	-12.
PIX + FT	-9.0	-16.4	-1.6	+5.3	-1.6	+8.9	+2.7	+10.
PIX + urea	-2.3	-9.8	+3.4	+19.1	+1.2	+2.3	-1.8	-8.0
			Anth	ocyanin				
kinetin	-2.7	-2.7	+8.0	-4.0	0	0	0	-6.
kinetin + IAA	-10.8	0	0	+4.0	0	-7.1	-13.3	0
kinetin + $CaCl_2$	0	-8.1	ŏ	+4.1	+7.1	0	+6.7	-6.
kinetin + $Na_2SeO_3$	+2.7	-8.1	+4.0	+4.0	0	ŏ	0	+13.
kinetin + $TGA$	0	+2.7	+4.0	+4.0	ŏ	-7.1	ŏ	0
	-5.4	-2.7	0		ŏ	0	ŏ	ŏ
kinetin + VA				+4.0			0	+6.
FT	+2.7	+8.1	+4.0	+4.0	0	0		
urea	+2.7	-2.7	0	-12.0	0	0	-6.7	0
FT sugars, AA	0	+2.7	-4.0	0	0	0	-6.7	+6.
PIX	+2.7	-10.8	+4.0	+16.0	0	0	0	+13.
PIX + FT	+2.7	-8.1	+4.0	+12.0	-7.1	-7.1	-6.7	+6.
			Flav	onoids				
kinetin	-0.3	-2.8	+5.1	+3.3	+0.9	-3.7	-2.5	+3.
kinetin + IAA	+1.8	+0.5	+0.6	+11.1	+7.8	+3.2	+11.5	+20.
kinetin + CaCl <sub>2</sub>	-1.3	-2.0	+3.3	+3.9	+6.4	-1.4	+3.0	-1.
kinetin + $Na_2SeO_3$	0	+0.8	+9.1	+7.6	+1.4	-5.1	-7.5	-0.
kinetin + TGA	-5.3	-3.8	+4.5	+2.4	-0.5	-5.2	+7.5	+12.
kinetin + VA	-5.1	-5.8	+0.3	+0.3	-8.3	-2.8	+3.5	+6.
FT	-1.8		+0.3			-2.8	+3.5	+5.
		+0.5		+0.4	-3.2			
urea	-2.0	-2.5	+2.4	+0.6	-5.1	-2.8	+6.0	+17.
FT sugars, AA	+0.8	+2.3	+2.7	0	0	-1.4	+12.5	+4.
PIX	0	+0.8	+9.1	+7.6	+1.4	-5.1	-7.5	-0.
PIX + FT	+1.8	-1.5	+5.4	+5.7	0	-4.1	-6.0	-9.
PIX + urea	+0.5	-0.5	+3.0	+4.2	+6.4	+1.4	+3.0	0

" "+" indicates an increase over the zero level; "-" indicates a decrease from the zero level.

10% (statistically significant at the 5% level) from the zero treatment were observed at one or both levels at either collection data in leaves or squares as a result of the following treatments: kinetin, kinetin + CaCl<sub>2</sub>, kinetin + Na<sub>2</sub>SeO<sub>3</sub>, the FT sugars and amino acids, PIX, PIX + FT, and PIX + urea. Similar significant increases of tannins were obtained with kinetin + Na<sub>2</sub>SeO<sub>4</sub>, kinetin + TGA, and kinetin + VA. Similar increases (10%) of anthocyanins were found as the result of treatments with urea, PIX, and PIX + FT. Finally, similar increases (10%) of flavonoids were found as the result of treatments with kinetin + IAA, kinetin + TGA, and kinetin + CaCl<sub>2</sub>. In a few instances, significant decreases were observed.

Table IV shows that significant increases in yield occurred only following treatments with PIX + urea and the FT fraction containing sugars and amino acids. Significant decreases were found following treatments with kinetin and kinetin + IAA,  $CaCl_2$ , and  $Na_2SeO_3$ . There were several statistically significant changes in other agronomic traits (lint %, boll size, seed index) as indicated in Table IV.

PIX has been extensively tested on cotton and is currently being used commercially. Its major effect, internode shortening, is visibly apparent, resulting in a more compact, darker green plant. Its effect on yield has varied from season to season with both increases and decreases observed, evidently because of differences in environment (Zummo et al., 1984; Mulrooney, et al., 1985; Hedin et al., 1984, 1988a,b). Increases in allelochemicals have also been found, and increased insect resistance has also been reported (Hedin et al., 1984, 1988a,b). In the present test, gossypol and anthocyanins were increased, but the effect on tannins and flavonoids was mixed. Yield was unaffected.

Table IV. Effect of Bioregulators on Agronomic Traits of Deltapine 50 Cottons

		rate, g of ai/acre	lint yield, lb/acre	lint, %	boll size, g	seed index
control		0	1082	38.1	5.7	10.4
kinetin		1.5	890*	38.4	5.9	10.3
		8.8	1039	37.8	5.7	10.3
	LSD 0.05		75	ns	ns	ns
kinetin + IAA		1.5/1.2	1159	37.7	5.7	19.4
		8.8/6.9	943*	37.5	6.0*	10.4
	LSD 0.05	/	75	ns	0.3	ns
kinetin + $Na_2SeO_3$		1.5/70.0	895*	38.0	5.4	10.0
Ameenii + 1142,5003		8.7/219.1	914*	37.8	5.5	9.9*
	LSD 0.05	0.17 210.1	90	ns	ns	0.4
kinetin + TGA		1.5/68.0	958*	38.2	5.8	10.2
killetili † 1 GA		8.7/116.6	1031	38.5	5.6	9.9*
	LSD 0.05	0.1/110.0	1031	ns	ns	ns
kinetin + VA	LOD 0.00	1.5/68.0	1116	38.4	5.7	10.3
Killetill   VA		8.7/212.8	1195	38.4	6.0	10.3
	LSD 0.05	0.7/212.0				
FT	LSD 0.05	0.026	ns 1081	ns	ns 5 C	ns
F 1				38.5	5.6	10.4
		0.079	1006	38.3	5.7	10.2
	LSD 0.05	ns	ns	ns	ns	ns
urea		24.3	1028	38.4	5.8	10.5
		79.9	972	38.8	5.9	10.2
	LSD 0.05	ns	ns	ns	ns	ns
FT sugars, AA		75.9	1201	38.7	6.0	10.5
		237.6	1367*	39.5*	6.0	11.2
	LSD 0.05		177	0.8	ns	ns
PIX		6.0	1121*	37.5	6.1*	11.0 <sup>,</sup>
		18.9	999	36.1*	6.0*	11.1
	LSD 0.05		120	1.1	0.3	0.5
PIX + FT		6.0/0.026	1146	37.2*	5.9	10.9
		18.0/0.079	1071	37.2	6.2*	10.8
	LSD 0.05	•	ns	0.6	0.3	0.3
PIX + urea		6.0/24.3	1049	37.7	5.7	10.7
		18.9/75.9	1226*	37.4	6.0	11.2*
	LSD 0.05	'	120	ns	ns	0.5

<sup>a</sup> \* indicates significantly different from the zero rate at the 5% level.

Kinetin and Burst (a commercial cytokinin preparation) have been found to slightly increase yield, pest resistance, and flavonoid in 1986 (Hedin et al., 1988a), and they decreased gossypol but increased anthocyanin and tannin in 1988 tests (McCarty and Hedin, 1989). In the present test, kinetin increased gossypol but did not statistically affect yield. Another cytokinin preparation, Foliar Triggrr (FT), containing 0.012% kinetin, and stated to promote yield through improved root growth, markedly increased leaf gossypol and flavonoids, apparently at the expense of bud gossypol and flavonoids, which were reduced, but yield was not affected in the 1988 tests (McCarty and Hedin, 1989).

Because kinetin, kinetin riboside, and Burst had a negative effect on leaf gossypol and no effect on flavonoids in the 1988 tests (McCarty and Hedin, 1989), it appeared that other constituents (99.9% of the total solids in the FT preparation) were responsible for the increases of leaf gossypol and flavonoids. Consequently, isolation work was carried out as a part of the present work (see Materials and Methods). It was found that the total solids of the FT preparation could mostly be accounted for by a fraction consisting at least partially of amino acids and sugars (48%) and urea (47%).

Accordingly, the present test (Tables I–IV) was organized to include urea (test 8), the sugar-amino acid fraction (test 9), PIX + FT (test 11), and urea + PIX (test 12). As in the previous test, PIX + FT increased gossypol significantly in several categories and anthocyanins and tannin to a lesser extent. These allelochemicals were increased to a lesser extent with FT alone. PIX + urea generally increased allelochemicals and yield, while urea alone decreased allelochemicals, suggestive that urea did not contribute to the increases of allelochemicals and yield. The sugar-amino acid fraction generally increased only gossypol (in addition to yield).

### DISCUSSION

Bioregulator effects have been demonstrated with naturally occurring and synthetic bioregulators and also with many commonly occurring inorganic and organic compounds. There is some evidence that cytokinins influence many aspects of plant growth and development including germination, cell division, cell enlargement, cell and organ differentiation, apical dominance, photosynthesis, nutrient translocation, flowering, fruit set, fruit growth, and plant senescence (Weaver, 1972: Elliott, 1982). Seed or foliar treatments with ammonium nitrate, urea, hydroxyurea, Hg<sup>2+</sup>, Cu<sup>2+</sup>, NaN<sub>3</sub>, cysteine, cystine, thioglycolic acid, sodium selenite, (p-chloromercuri)benzoate, lithium sulfate, calcium chloride, barium chloride, sodium sulfite, and glutathione are some examples of such compounds (Keen and Bruegger, 1977; Orion et al., 1980; Sinha and Hait, 1982; Hait and Sinha, 1986, 1987). They further suggest that these compounds can sensitize plants so that their defensive responses to pathogens are more intense. There is also evidence that commonly occurring inorganic compounds such as calcium salts can potentiate the action of natural plant growth regulators such as kinetin (Haberlach et al., 1978).

In the present tests, kinetin increased gossypol, but not yield. Attempts to synergize kinetin with several inorganic or organic compounds were generally unsuccessful except for  $Na_2SeO_3$ , which appeared to contribute to increased gossypol to a level greater than that elicited by kinetin alone but at the expense of yield, which was significantly decreased. This was apparently due to toxicity of

Na<sub>2</sub>SeO<sub>3</sub>, perhaps through interference with sulfur metabolism, because the plants showed visible effects.

The increased vield that occurred as the result of the application of the FT sugar-amino acid fraction and PIX + urea (PIX along did not increase yield) presumably may be explained in terms of the effect of a foliar nutrient (nitrogen) application, although the level applied was lower than the commercial practice. The season was initially very wet, leaching away the nitrogen side dressing. As the plants approached "squaring", the season turned dry, perhaps stressing them. Thus, a foliar "nutrient" application might be expected to improve vield. The vield increases of 11 and 26% at the two levels with the FT sugars and amino acids (probably a growth regulator effect because the amounts applied were too low to expect a nutrient effect) could be of both scientific and commercial importance if the effectiveness can be demonstrated in future years.

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**Registry No.** IAA, 87-51-4; TGA, 68-11-1; VA, 121-34-6; PIX, 24307-26-4; CaCl<sub>2</sub>, 10043-52-4; Na<sub>2</sub>SeO<sub>3</sub>, 10102-18-8; urea, 57-13-6; gossypol, 303-45-7; kinetin, 525-79-1; kinetin with IAA, 8073-67-4; kinetin with CaCl<sub>2</sub>, 130933-97-0; kinetin with Na<sub>2</sub>SeO<sub>3</sub>, 130933-98-1; kinetin with TGA, 130933-99-2; kinetin with VA, 130934-00-8.