Plant Bioregulator Induced Increases in the Protein Content of Cotton Plant Tissues

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Two applications at two rates of 16 synthetic and naturally occurring plant bioregulators were sprayed on growing cotton, *Gossypium hirsutum* (L.), to evaluate their effects on protein levels and on yield. Three of the compounds, chlormequat chloride [(2-chloroethyl)trimethylammonium chloride], mepiquat chloride (1,1-dimethylpiperidinium chloride), and V-3183 [*N*-(2-chloro-4-pyridyl)-*N'*-phenylurea], significantly (17–50%) increased the protein content of leaves and squares (buds) harvested 4 weeks after the first spraying. This confirmed a 1982 test in which mepiquat chloride had increased the protein content by 14%. However, the yield of seed cotton was significantly decreased when mepiquat chloride and V-3183 were applied, particularly at the higher treatment levels, but it was not decreased when chlormequat chloride was applied. The major impact of the work may be that there is the potential for bioregulators to increase the protein content of other crops, perhaps forages and crops for human consumption, without a sacrifice of yield.

Plant hormones have an important role in the growth and developmental processes of plants. The endogenous hormones as well as externally applied bioregulators may also affect insect populations feeding on these plants by inducing changes in the composition of the plants. Gibberellic acid, for example, elicits increased terpene biosynthesis in citrus (*Citrus sp.*), thus decreasing attack by fruit flies (*Anasterpha sp.*) (Greaney, 1978; Coggins et al., 1969).

In cotton, *Gossypium hirsutum* L., termination of late-season fruiting has been achieved with potassium 3,4-dichloroisothiazole-5-carboxylate, thus depriving the pink bollworm [*Pectinophora gossypiella* (Saunders)] of food and oviposition sites (Kittock et al., 1975; Kittock and Arle, 1977). The effects of the plant bioregulator mepiquat chloride (1,1-dimethylpiperidinium chloride) on cotton have been widely studied. Its reported effects include increased leaf thickness, shorter internodes, reduced plant height, increased boll retention, reduced boll rot, and increased yields and earliness (Bader and Niles, 1986; Willard, 1979; York, 1983). Zummo et al. (1983) reported less plant damage, decreased bollworm [*Heliothis zea* (Boddie)] growth, and 10–20% increased terpenoids, tannins, and astringency (biological tannin). When mepiquat chloride was applied to cotton in work at this location (Hedin et al., 1984; Graham et al., 1987; Jenkins et al., 1987), it caused internode shortening but it did not elicit an increase in resistance in cotton to the tobacco budworm [*Heliothis virescens* (Fab.)]. Also, changes in content of four known allelochemicals, sometimes alternately described as allelopathic chemicals (condensed tannins, gossypol, anthocyanins, and flavonoids), were minimal. An unexpected finding was the increase in content of several nutritional factors including protein (14%) that may be related to greater growth of tobacco budworm larvae feeding on cotton tissues (Hedin et al., 1984). No particular notice was taken of this increase at the time because there was abundant rainfall during the test year resulting in lush growth.

A search of the literature did not reveal any reports about the increase of cotton plant protein when bioregulators were applied. However, Yokoyama (1984) reported a 68% increase in protein when he applied DCPTA (2-(diethylamino)ethyl 3,4-dichlorophenyl ether) to soybeans. Rittig (1987) reported that increases of protein in alfalfa can be induced by the bioregulator mepiquat chloride.

In the present work, a number of plant bioregulators were applied to growing cotton to determine their effects on levels of nutrients including protein, on any induced plant resistance to the tobacco budworm, and on yield. Two applications of 16 bioregulators were applied at two rates to growing cotton in statistically designed field plots. Plots were duly infested, plant tissues were harvested and analyzed, and cotton was harvested to determine yields. The information from these tests is the basis of this report.

MATERIALS AND METHODS

1986 Bioregulator Field Test. The cultivar Stoneville 213 (most widely grown commercial variety in the mid-south and the most widely used as a standard in research tests) was grown in two environments in 1986 at Mississippi State, MS. The cotton was planted on 30 April in single row (1 × 12.8 m) plots in a two-planted one-skip row pattern on a marietta sandy loam (fine-loamy, siliceous thermic Fluvaquentic Eutrochrepts) soil. Thus, each bioregulator required 0.109 acre (0.044 ha) for six replicates at each level (low, high, control) with and without insecticide. Insects were controlled all season with guthion and fenvalerate in environment one. Environment two (the other half of the plots) had an artifically induced infestation of tobacco budworms. Plots were infested weekly, beginning 15 July, for 5 weeks with 8–10 first instar larvae per 30-cm row (Jenkins et al., 1982).

Sixteen plant bioregulators (see Figure 1 for structures and Table I for nomenclature and rates) were applied at two rates (low, high) on 7 July and 21 July. Controls were also included. The timing of applications and rates were in general those recommended by previous investigators or by the provider (see references below). Two rates with the second 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 specified solvent. Portions of 1 mL each of Span 80 and Tween 80 were then added. The solutions were made up to 3 L with water and stored at 4 °C until used. They were applied with a CO_2-pressurized backpack sprayer delivering 203 L ha^{-1} at 207 kPa pressure. Each compound in

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Table I. Trivial Names, Nomenclature, Procurement Source, Recommended Treatment, and Application Rates and Solvents for 16 Bioregulators (See Figure 1 for Structures)

no.	trivial name(s); source	systematic name	recommended treatment	rates, g a.i.[a] ha^{-1} (solvents)
1	chlormequat chloride, cycocel, CCC; Sigma Chemical Co., St. Louis, MO	(2-chloroethyl)trimethylammonium chloride	cotton; 80 mL of 50% a.i./ha = 60 g/0.039 acre (Virk et al. (1984))	13.5, 40.5 (H_2O)
2	BAS-105 00 W, LAB 13338; BASF, Ludwigshafen, West Germany	4-chloro-5-(dimethylamino)-2-phenylpyridazine-3-one	2.24 kg a.i./ha = 36 g/plot (Ory et al. (1984))	67.4, 202.4 (H_2O)
3	BAS-109 00 W; BASF	*all-cis*-8-(4-chlorophenyl)-3,4,8-triaza-tetracyclo[4.3.1.$0^{2,5}$.$0^{7,9}$]dec-3-ene	cotton; 243 mg/acre = 1.4 mg/plot (Mulrooney (1984))	2.8, 8.4 (H_2O)
4	mepiquat chloride, PIX; BASF	1,1-dimethyl-piperidinium chloride	cotton: 1 part 4% a.i./acre = 1.6 g/46% Tech/plot (BASF)	50.0, 150.0 (H_2O)
5	Dinoseb; Sigma	2-*sec*-butyl-4,6-dinitrophenol	10–50 ppm = 0.1 g of 50 ppm/plot (Campbell et al. (1984))	6.7, 20.2 (5% aq acetone)
6	Burst, Cyotgen; Burst Agritech, Overland Park, KS	mixture of cytokinins including zeatins	cotton: 1 pint Tech/A. corn: $^1/_2$ pint Tech/A = 8.78 mL/plot (Burst)	561.9, 1123.8 (H_2O)
7	XE-1019, S-3307; Chevron Chemical Co., Memphis, TN	(*E*)-(*p*-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol	13 g Tech/A = 0.48 g/plot (Chevron)	320.0, 640.0 (H_2O)
8	V-2307; Velsicol Chemical Corp., Rosemont, IL	3-chlorobenzyl 3,6-dichloro-2-methoxybenzoate	0.5–2.0 oz/acre = 0.5–2.2 g/plot (Velsicol)	16.0, 48.0 (H_2O)
9	DCPTA; Dr. Henry Yokoyama, USDA, Pasadena, CA	2-(diethylamino)ethyl 3,4-dichlorophenyl ether	80 ppm = 0.16 g of 80 ppm/plot (Yokoyama (1984))	4.0, 12.1 (H_2O)
10	Glyphosine; Sigma	*N*,*N*-bis(phosphonomethyl)glycine	4 lb a.i./acre, 72 g/plot (Nickell (1984))	33.7, 202.4 (H_2O)
11	Velsicol-3183, KT-30, 4PU-30; Velsicol	*N*-(2-chloro-4-pyridyl-*N*′ phenylurea	0.5–2.0 oz/acre = 0.5–2.0 g/plot (Velsicol)	16.0, 48.0 (H_2O)
12	gibberellic acid, GA_3; Sigma	*ent*-3,10,13-trihydroxy-20-norgibberella-1,16-diene-7,19-dioic acid 19,10-lactone	25 ppm = 50 mg/plot (Williams (1984))	4.0, 13.5 (5% aq EtOH)
13	IAA; Sigma	indole-3-acetic acid		4.0, 13.5 (5% aq EtOH)
14	Kinetin; Sigma	6-furfurylaminopurine		4.0, 13.5 (1% aq HCl)
15	Arabinogalactan; Sigma	α-D-arabinopyranosyl-(3→6)-α-D-galactopyranoside		67.4, 202.4 (H_2O)
16	Treflan, trifluralin; Elanco Products Co., Indianapolis, IN	α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine	$^1/_2$ lb Tech/A = 2.7 mL Tech/plot (Elanco)	276.5, 835.4 (H_2O)

[a] Active ingredient

each environment was handled as a separate randomized complete block experiment with six replications. The plots were machine-harvested one time for yield determinations on 30 September. There was no apparent difference in ripening between controls and tests.

Procurement of Bioregulators. The trivial names, nomenclature, sources of procurement, and recommended treatments including literature references, and rates of application are given in Table I, and the structures are given in Figure 1.

Analysis of Leaf, Square, and Seed Protein. Terminal leaf and square (bud) tissues were collected on 4 August, frozen, freeze-dehydrated, and ground prior to analysis. Seeds were obtained by ginning of the harvested cotton and cleaned by treatment with H_2SO_4. Total protein (dry-weight basis) was determined by the Kjeldahl procedure according to AOAC Method 2.049 (Horwitz, 1975). Percent protein was calculated from percent nitrogen × 6.25.

Statistical Procedures. Data obtained from the analysis of the protein samples were subjected to the analysis of variance, and means were separated with Duncan's new multiple-range test. Data obtained from the determination of yield were subjected to analysis of variance, and LSD values were calculated.

RESULTS AND DISCUSSION

The protein content of terminal leaves and squares (buds) from cotton plants treated two times with two levels

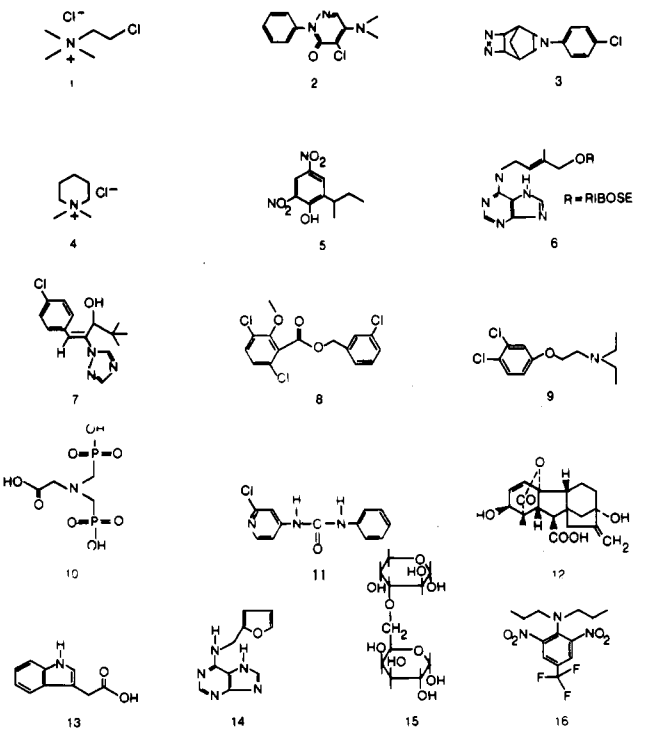

Figure 1. Structures for 16 bioregulators sprayed on cotton. See Table I for trivial names, nomenclature, recommended treatments, and rates and solvents.

Table II. Protein Content of Leaves and Squares (Buds) of Bioregulator-Treated Cotton Plants[a]

		protein,[b] %					
		leaves			squares		
no.	sample	0	L	H	0	L	H
1	CCC	23.1	27.5	28.8	16.5	20.6	20.0
2	BAS-105		21.3	21.6		17.3	17.2
3	BAS-109		21.9	21.9		17.7	17.8
4	PIX	23.2	30.4	29.8	17.2	20.2	21.2
5	Dinoseb		22.3	21.9		18.1	18.1
6	Burst		21.6	21.9		18.4	18.8
7	XE-1019		21.9	20.9		18.4	17.2
8	V-2307	22.2	21.3	21.9	17.5	17.5	18.0
9	DCPTA		21.3	21.3		17.2	17.2
10	Glyphosine		22.2	21.6		27.7	17.2
11	V-3183	23.9	30.4	29.8	17.6	21.1	22.2
12	GA_3		21.3	21.3		17.2	17.0
13	IAA		22.5	22.0		18.3	18.4
14	Kinetin		21.9	21.6	17.3	17.0	17.8
15	Arabinogalactan	21.6	20.9	21.6	17.3	17.8	17.8
16	Treflan		22.3	23.4		17.2	18.1

[a] Rates: 0 = control, L = low, H = high. See Materials and Methods for actual amounts. [b] Protein analyses (dry-weight basis) were performed in duplicate in first survey, and only four of the controls were analyzed. Later, four replicates of all levels of CCC, PIX, and V-3183 were analyzed for protein to permit statistical analysis by Duncan's new multiple-range test.

of 16 bioregulators and controls is presented in Table II. Protein analyses were performed in duplicate in the first survey, and only four of 16 controls were analyzed. Later, four replicates of chlormequat chloride, mepiquat chloride, and V-3183 treated whorl samples were analyzed for protein to permit statistical analysis by Duncan's multiple-range test. In Table II, the protein values obtained for these three bioregulators are, in fact, the averages of the four replicates. Protein values were not increased in either terminal leaves or squares as the result of treatment with any of the other 13 bioregulators. One of the 13, DCPTA, had been reported to increase the protein content of soybeans by 68% when applied at 80 ppm (Yokoyama, 1984). On the basis of their report in which 80 ppm was reported to give a stronger increase than 120 ppm, levels of 30 and 90 ppm were used in the present cotton test.

Table III gives the protein content for leaves, squares, and seed and yield of seed cotton with appropriate statistical documentation for plants that were treated with the bioregulators chlormequat chloride, mepiquat chloride, and V-3183. The yield of lint cotton from seed cotton is approximately 38–40%. The protein content of leaves and squares was significantly increased (17–50%) by all three bioregulators. However, there was no accompanying increase in seed protein although a slight trend was apparent. In a similar test in 1986 conducted at this location (Hedin et al., 1986) with these bioregulators on corn, there was no increase in the protein content of corn whorls of corn treated with these three or any of the other bioregulators. A number of effects of bioregulators have been reported for several small-grain crops and sugar cane, but they do not seem to include increases in tissue protein (Jung, 1984; Nickell, 1984). With tall fescue, 14 days after the application of mefluidide, percent cellulose was decreased and reproductive development was inhibited, while percent sugar and crude protein were increased. Dry-matter yield was decreased at 21 days, but not regrowth after 71 days (Glenn et al., 1980). Increases in protein have been reported with soybean (Yokoyama, 1984) and alfalfa (Rittig, 1987).

The yield of seed cotton treated with chlormequat chloride is not significantly (statistically) decreased although there appears to be a slight downward trend (8–13%). However, the yields of seed cotton were significantly decreased in the infested plants treated with mepiquat chloride (16–34%) and V-3183 (17–59%). At least with chloromequat chloride, the increased protein content (19–25%) does not appear to be achieved totally at the expense of yield: L, 0–3%; H, 8–13%.

Table III. Effect of Three Bioregulators on Protein Content of Cotton Leaves, Squares, and Seed and on Seed Cotton Yield

		protein,[a] %			seed cotton yield,[b] kg/ha	
bioregulator	level	leaves	squares	seed	unin-fested	infested
chlormequat chloride	0	23.1 b	16.5 b	24.2 a	3532	3068
	L	27.5 a	20.6 a	24.7 a	3543	2987
	H	28.8 a	20.0 a	24.7 a	3252	2662
LSD					NS	NS
PIX	0	23.2 b	17.2 b	23.5 a	3727	3327
	L	31.2 a	20.2 a	24.1 a	3156	2598
	H	34.7 a	21.2 a	24.3 a	2472	2417
LSD					1337	330
V-3183	0	23.9 b	17.6 b	23.8 a	3055	2185
	L	30.4 a	21.1 a	24.4 a	2532	1285
	H	29.8 a	22.2 a	24.3 a	1864	898
LSD					523	396

[a] Percent protein; dry-weight basis, 5% probability level, Duncan's new multiple-range test. [b] Analysis of variance.

Therefore, there is the reasonable expectation that somewhat lower treatment levels or other adjustments could sustain the increased protein content without a concomitant yield decrease.

It can be inferred from the reports of Glenn et al. (1980), Yokoyama (1984), Rittig (1987), and others that the increases in the protein content observed in the leaves and buds of bioregulator treated plants can be explained in terms of retarded growth (smaller plants with more concentrated cell nitrogen content) and delayed senescence with longer retention of nitrogen in the leaves before translocation into generative organs. To provide further information, whole cotton plants ready for harvest that had been treated with the high level of chlormequat chloride or mepiquat chloride were collected and weighed. Nontreated plants were also collected from the experimental plots and weighed. Internode lengths were recorded. These plants were available as a part of 1987 tests that were being carried out in a manner identical with the 1986 tests but were being compared with other bioregulators.

The results were as follows. High level, chlormequat chloride, average weight (g/plant): 199.5 ± 10.8; control,

196.8 ± 9.6. Internode lengths, chlormequat chloride: 3.91 ± 0.98 cm; control, 5.62 ± 1.16 cm. Mepiquat chloride, high level, average weight (g/plant): 216.0 ± 10.1; control, 228.1 ± 12.6. Internode lengths, mepiquat chloride: 4.12 ± 0.76 cm; control, 5.44 ± 0.78 cm.

This study indicates that the biomasses of chlormequat chloride treated and control plants were approximately equal. The biomass of mepiquat chloride treated plants was slightly decreased. Therefore, these and the earlier yield tests suggest that the increases in protein of chlormequat chloride treated plants appear to be real and should not be attributed to growth retardation. Evidence that the plants received these treatments was provided by the shortening of the internode distances. There was no observable difference in ripening at harvest time.

While the results are interesting in their own right, the major impact of the work may be that there is the potential for bioregulators to increase the protein content of other crops, perhaps forages for animals and food crops for human consumption, without a sacrifice of yield.

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