Effects of Foliar Applications of Carbohydrates on the Yield of Cotton (*Gossypium hirsutum*) Lint

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Various plant growth hormones and bioregulators have been reported to increase the yield of cotton *Gossypium hirsutum* L. lint when applied foliarly in field tests. However, while positive effects with these compounds were often observed in individual years by us, multiyear results were not significant. Initial results with a commercial formulation, Foliar Triggrr, FT (Westbridge Agricultural Products, San Diego, CA), in which the stated active ingredient was cytokinin, led us to investigate the composition. Over a several year period (1989−1995), application of a succession of sugar-containing fractions to the cotton plants gave consistent small increases but not statistically significant lint yield increases. The composition of the active carbohydrate fraction upon acid hydrolysis was a mixture of mainly xylose, mannose, galactose, and glucose. Equal molar mixtures of sugars (man, gal, glc, suc) and ι-, κ-, and λ-carrageenans (linear sulfated polysaccharides) gave small consistent increases. The commercial application of this lead with carbohydrates may be in the utilization of an economical sugar-processing byproduct.

Keywords: *Cotton yields; Gossypium hirsutum L.; carbohydrates; Foliar Triggrr*

INTRODUCTION

Naturally occurring and synthetic bioregulators have an important role in the growth, developmental processes, and yield of plants. They may also induce the biosynthesis of allelochemicals, secondary plant constituents that may protect the plant against infection and injury by phytophagous pests. Consequently, the chemistry and action of these bioregulators is of interest.

In recent years, we have evaluated a number of kinetin-based commercial plant growth regulators for their effects on cotton plants. In a study at Mississippi State during 1986−1992, five commercial plant growth regulators and urea were evaluated as foliar sprays on growing cotton plants for their effects on yield. Of the five tested at the levels recommended by the providers, the stated activities of three (Burst, Burst Agritech, Overland Park, KS; Foliar Triggrr, FT, Westbridge Agricultural Products, San Diego, CA; and Maxon, Terra International, Blytheville, AR) were attributed by their providers to cytokinins. FPG-5 (Baldridge Bio-Research, Inc., Cherry Fork, OK) contained cytokinins, IAA, GA, and several inorganic micronutrients, and PG-IV (Microflo, Lakeland, FL) contained IBA, GA, and micronutrients but no cytokinins. FPG-5 and Foliar Triggrr gave small consistent increases in one or more years and significant increases in yield in 1992. Urea had a consistent negative effect on yield. Formulations containing IAA/IBA, GA, and inorganic micronutrients also failed to increase yields (Hedin and McCarty, 1994a).

In a series of tests conducted from 1986 to 1992, the synthetic cytokinin kinetin was evaluated along with kinetin riboside, IAA, and GA. The effects of these plant growth hormones were near zero over the 6-yr period, although, in some individual tests, statistically significant differences in yield were obtained (Hedin and McCarty, 1991, 1994b).

Our attention was drawn to one of the commercial plant growth regulators, FT, which gave small consistent increases in yields in field tests annually commencing in 1989, and in 1992 gave statistically significant increases in the yield of cotton lint of 15.5 and 12.6% at 8 and 16 oz/acre, respectively (528 and 1056 mL/ha). While the several cytokinin constituents and preparations performed poorly for us as previously stated (Hedin and McCarty, 1991, 1994a,b), we assume that geographical, climatic, and procedural differences may have been contributing factors to the successful tests of FT by Parker et al. (1988). However, because we were not able to show yield increases with cytokinins, we hypothesized that, alternatively, some unknown component(s) was responsible. This possibility was indirectly supported by the report of Parker et al. (1988) that described FT as "the product of a fermentation process that is then combined with extracts from a variety of plants which contain naturally occurring growth stimulating substances".

In our initial investigations, we found that liquid FT consisted of a black suspension (5% of solids) that could be precipitated by treatment with 20 volumes of acetonitrile/water 1/1. The resulting amber filtrate gave an amorphous crystalline mass upon freeze drying. Chromatography of the filtrate on a Sephadex LH-20 column with methanol as the eluant provided a fraction that gave a significant yield increase in 1989 field tests of 10 and 26% at 0.2 and 0.5 kg/ha, respectively, and small consistent increases in other years. This report describes our further efforts to fractionate FT with the objective of identifying some other constituent(s) that could consistently increase lint yields.

MATERIALS AND METHODS

1992−1995 Bioregulator Field Tests. The commercial cotton cultivars Deltapine 50 (DPL-50) and DES-119, well adapted for the study area, were grown each year on the North Farm at Mississippi State University. The cotton was planted about May 1 in single-row (0.97 × 12.8 m^2; width × length) plots. Insects were controlled all season with fenvalerate (DuPont Agricultural Products, Wilmington, DE) and malathion (American Cyanamid, Princeton, NJ). The growth regulator formulations were applied at three rates (zero, low, and high;

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2.7−8.1 mol/ha) to plants whose squares were "match head" (2−4 mm) in size on about 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 the provider or as tested previously (Hedin and McCarty, 1991). Normally, two rates, with the second application generally 3-fold higher, were used to improve the likelihood that a reponse would be elicited. Each compound or formulation was weighed or measured and dissolved in 5−20 mL of H_2O. One milliliter of Tween 80 was then added. The solutions were made up to 1.25 L with water just before use, and they were applied with a CO_2-pressurized backpack sprayer delivering 203 L/ha at 207 kPa of pressure.

The plots were machine harvested one time for yield determination when cotton was fully open (about 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 and Other Test Components. FT is a commercial preparation in which cytokinin (0.012%) is the stated active ingredient. The carbohydrates xylose, arabinose, mannose, galactose, glucose, sucrose, inositol, *n*-acetylglucosamine, glucuronic acid, galacturonic acid, glucosamine, and ι-,κ-,-and λ-carrageenans were obtained from Fluka Chemical Corporation, Ronkonkoma, NY. Tween 80 was obtained from Sigma Chemical Co., St. Louis, MO.

Fractionation of Foliar Triggrr. FT (**1**) was procured in 1 gal lots (3.785 L), and 500 mL was diluted with 3600 mL of acetonitrile/water 1/1, stirred, and filtered to give 2.9% of a black residue (**2**) and 19.1% of an amber crystalline material (**3**) upon freeze drying. In sequence, several chromatographic separations were employed. To a 5 × 70 cm Sephadex LH-20 (Sigma Chemical Co., St. Louis, MO) column equilibrated with 80% aqueous methanol, approximately 7 g of the freeze-dried amber solids **3** was applied (see Table 1 for identification and analysis of fractions). Approximately 200-mL fractions were collected, and the output was monitored by chromatography on silica gel thin layer chromatography (TLC) plates and subsequent spraying with a mixture of 0.2 g of naphthoresorcinol (Fluka Chemical Co., Ronkonkoma, NY) in 100 mL of ethanol and 5 mL of H_3PO_4. Upon heating, the carbohydrate **6** fraction, which gave a blue color (31.6%), was generally separated from the longer retained fraction **7** (53.9%) containing mostly urea, which gave a red color (the yields of **4** and **5** were very low and therefore not investigated further).

Fraction **3** was also subjected to large-scale preparative paper (Whatman 3MM) chromatography employing the solvent mixture (*n*-butanol/acetic acid/water 8/1/1) in cylindrical jars to give multigram quantities, each of bands **9**, **10**, **11**, and **12** that were located by UV inspection and spraying with the reagents described above.

Additional amounts of fraction **6** (enriched in carbohydrates), were obtained from repetitive LH-20 (5 × 20 cm) chromatography, freeze dried, and subjected to silica gel chromatography (5 × 20 cm Baker Silica Gel, 60−200 mesh, Baker Analyzed Reagents, Phillipsburg, NJ) employing 100% ethanol and increasing water/ethanol mixtures. Fraction **6** was then subjected to Dowex-50-X-4-(H^+) (2 × 6 cm) chromatography employing as eluants water, 10% NH_4OH, and 5% HCl, again to obtain multigram quantities for field testing.

Finally, the carbohydrate-enriched fraction **18** from the Dowex-50-X-4 separation was chromatographed on a Dowex-1-X-8 column (Baker Analyzed Reagents, Phillipsburg, NJ), Cl^- form, 3.5 × 37 cm, 100−200 mesh, by elution in water, and fractions were monitored by silica gel TLC plates that were sprayed with naphthoresorcinol and Ehrlich's reagent to detect carbohydrates and urea.

Analytical Procedures. Association of Official Analytical Chemists (AOAC) methods (Horwitz, 1975; Helrich, 1990) were used for the following analyses: ash, 942.05; nitrogen, 2.049; and sulfur, 3.057. Glycosyl compositions were determined via hydrolysis with 2 mol/L trifluoroacetic acid and subsequent preparation and analysis of their alditol acetates (Sturgeon, 1990). Glycosyl linkage analyses were performed using the Hakamori methylation procedure, all as described by York et al. (1985) and Sturgeon (1990).

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

RESULTS AND DISCUSSION

Results of our studies on the chemistry and effects on yield of FT fractions are given in Tables 1 and 2. Table 3 lists the results of glycosyl composition and linkage analyses. Table 4 lists the results of several field tests in which standard carbohydrate formulations were applied as foliar sprays to cotton. Statistically significant field tests were obtained with only two isolates: fraction **1** and fraction **6**.

Table 1 lists the ash, nitrogen, sulfur, and carbohydrate contents of fractions generated from FT by sequential separations using Sephadex LH-20, preparative paper, silica gel, Dowex-50, and Dowex-1 chromatography. Details pertaining to the procedures are given under Materials and Methods and in the footnotes to the tables.

LH-20 chromatography led to the isolation and subsequent identification of a fraction with a high content of urea present in the FT liquid. Fraction **7**, which constituted 41.2% of chromatographed fraction **3**, gave a nitrogen analysis of 44.8% (urea contains 46.65% nitrogen). The presence of urea was confirmed by H^1-NMR, solid probe MS, and TLC chromatography. The nitrogen analyses for **1** and **3** (Table 1) indicated that as much as 50% of the FT solids was urea. Treatment with urease revealed that **1** and **3** contained 48.8 and 45.7% urea, respectively, and that the nonurea nitrogen contents of **1** and **3** were 2.5 and 1.8%, respectively. The presence of significant amounts of protein was discounted because LH-20 fractions **4** and **5** accounted for less than 1% of **3**, and because, on dialysis of **3**, the yield of nondialyzables from **3** was 1.25%. Analyses of **1** and **3** for crude fat were 0.3 and 0.1%, respectively, and they contained 0.0 and 0.2% crude fiber. Therefore, approximately 70% of the solids of **1** and **3** could be accounted for by ash, nitrogen, and sulfur, while the remaining 30% was presumed to be a mixture of water soluble components including about 5% of acid hydrolyzable carbohydrates (Table 1). Analyses were focused on the carbohydrate fractions which gave consistent small increases with regard to yields of lint in field tests. Nitrogen was routinely analyzed because of the high urea content, and sulfur because of early information and subsequent chromatographic evidence that sulfated carbohydrates could be present. The isolated fractions are numbered through **26**. Those that were enriched in carbohydrates were subjected to a sequence of chromatographic separations.

Table 2 lists observed yield effects of the carbohydrate fractions. They include preparations **3**, **6**, **11**, **15**, **18**, and **24**. Generally, the fractions that were low in carbohydrates had no apparent effect on yield or a negative effect and are not reported.

LH-20 chromatography with acetonitrile/water 1/1 of **3** yielded three major fractions: **6**, **7**, and **8**. The carbohydrates were concentrated in **6**, while **7** was high in nitrogen, as previously discussed.

Fraction **3** was also subjected to large-scale preparative paper chromatography. Multigram quantities of **9**, **10**, **11**, and **12** were obtained for field tests that were

Table 1. Ash, Nitrogen, Sulfur, and Carbohydrate Contents of Isolates of FT in Percent of Dry Weight

no.	description	yield	ash	nitrogen	sulfur	ara	xyl	man	gal	glc	total sugars
1	FT, liquid	100.0	18.7	23.8	5.1			0.3	2.4	1.6	4.3
2	FT; ACN/W ppt[a]	5.2						tr	tr	tr	tr
3	FT; ACN/W filtrate	94.8	19.7	24.5	5.7			0.4	2.6	1.8	4.8
	LH-20 chromatography[b] of **3**	100.0									
4	A	0.2									
5	B	0.6									
6	C	44.0	8.4	18.0	3.8		0.4	0.9	5.9	3.2	10.4
7	D	41.2	8.2	44.8	2.5			0.3	1.9	1.1	3.3
8	E	13.9	12.9	14.6	1.7						
	paper chromatography[c] of **3**	100.0									
9	R_f 0.0 - 0.1	19.3	21.3	4.6	6.6	0.4	1.4	0.6	10.6	10.5	23.5
10	0.1 - 0.3	14.6	17.7	7.4	0.3	0.2	0.6	1.8	23.8	17.5	43.9
11	0.3 - 0.7	10.7	23.2	10.1	1.2	0.5	1.2	11.7	1.6	7.3	22.4
12	0.7 - 1.0	55.2	39.5	39.4	0.1	0.2	1.4	0.2	0.2	0.7	2.8
	silica gel chromatography[d] of **6**	100.0									
13	100 ethanol	8.2	3.9	13.1	0.3	tr	tr	1.8	5.5	2.7	12.6
14	ethanol/water 9/1	48.3	7.1	20.7	0.4	tr	tr	4.5	13.1	8.1	25.7
15	ethanol/water 8/2	39.4	10.2	7.7	1.8			2.3	11.2	6.5	19.0
16	ethanol/water 7/3	17.0	51.7	3.2	11.3		tr	0.3	1.4	1.4	3.1
17	methanol/water 1/1	5.1	45.5	5.3	16.3						
	Dowex 50-X-4 chromatography[e] of **6**	100.0									
18	H_2O	40.6	1.5	27.9	3.1		tr	1.6	11.6	6.0	18.2
19	10% NH_4OH	9.0	0.4	43.2	tr			0.2	1.6	1.4	3.2
20	H_2O	1.5									
21	5% HCl	31.3	0.6	24.7	tr			0.1	0.1	0.1	0.3
22	H_2O	17.6	11.1	22.3	tr						
	Dowex 1-X-8 chromatography[f] of **18**	100.0									
23	H_2O **A**	1.5						tr	tr	tr	tr
24	H_2O **B**	33.1	1.1	9.8	0.3	0.1	tr	3.5	24.5	14.4	42.5
25	H_2O **C**	15.4	0.4	21.3	0.1	tr	tr	1.2	8.1	4.6	13.9
26	H_2O **D**	50.0	50.3	44.5	0.0			0.2	2.1	1.4	3.7

[a] 20 volumes of acetonitrile/water 1/1 per volume of commercial Foliar Triggrr (FT) liquid. [b] 5 × 70 cm^2 Sephadex LH-20 column eluted with acetonitrile/water 1/1, 2−3 L. [c] Whatman 3MM chromatography paper, 20 g/100 sheets, 30 × 50 cm^2, stapled to form cylinders, *n*-butanol/acetic acid/water 8/1/1, visualized by end spraying with 0.2% naphthoresorcinol. [d] 5 × 21 cm^2 Baker Silica gel, 60−200 mesh, 200 mL fractions. [e] 2 × 6 cm^2 Dowex-50-X-4, H^+, 100 mesh, 20 mL fractions. [f] 3.5 × 37 cm^2 Dowex 1-X-8, Cl^- 100−200 mesh, 200 mL fractions.

Table 2. Effects of Foliar Applications of FT on the Yield of Cotton Lint

			mol/ha[b]		yield,[c] avg % change	
no.	treatment[a]	years	low	high	low	high
1[d]	FT commercial liquid	1989−1992	0.0003 (K)	0.0010 (K)	+4.2 (6)	+3.1 (6)
3	FT ACN/W filtrate	1991−1995	8.1	+4.4 (6)	+5.2 (6)	
6[d]	LH-20, Fr. **C**	1989−1995	2.7	8.1	+5.3 (11)	+8.1 (11)
11	cellulose PC	1994	2.7	5.4	+6.3 (2)	+8.6 (2)
15	silica gel, **C**	1993	2.7	8.1	+14.7 (2)	+17.0 (2)
18	Dowex-50 **A**	1994	2.7	8.1	+9.1 (2)	+9.0 (2)
24	Dowex-1 **B**	1994−1995	2.7	8.1	+7.9 (2)	+12.4 (2)

[a] See Table 1 for details of fractionation scheme. [b] Two applications at two levels; at pinhead squaring and two weeks later. [c] Numbers in parentheses indicate number of tests. [d] Yields were statistically significant (LSD 0.05) in 1989 and 1992 only.

Table 3. Glycosyl Composition and Glycosyl Linkages in FT Isolate 6

	composition, %								
sugar[a]	before hydrolysis	after hydrolysis	relative content	terminal linkage	relative content, %	singly linked	relative content, %	doubly linked	relative content, %
xyl	0.3	0.4	4.6	xylose	0.8	2-gal	3.1	3,6-man	0.7
man	3.2	0.9	11.0	mannose	tr	3-gal	6.4	2,4-gal	1.4
gal	0.8	5.9	56.7	galactose	28.0	6-gal	4.8	4,6-gal	2.7
glu	*1.2*	*3.2*	*30.7*	glucose	*14.0*	2-glc	1.7	2,3-glc	tr
	5.5	10.4	100.0		42.8	3-glc	2.5	3,4-glc	2.2
						4-glc	22.0	4,6-glc	1.5
						6-glc	*5.0*	2,3-gal	2.6
							45.5		11.1

[a] Traces of arabinose and fucose were found in some isolates.

carried out in 1994. While **9**, **10**, and **11** all were enriched in carbohydrates, only **11** appeared to enhance yields (Table 2).

The use of silica gel and Dowex-50 chromatography for separating the residual urea from the carbohydrates was then evaluated. When **6** was chromatographed through a silica gel column, the first three fractions **13**, **14**, and **15** were enriched in carbohydrates, but only **15** appeared to increase the yield of lint. The urea was only partially separated from **15**, being concentrated in **14**. Sulfur was high in **16** and **17**. However, these two fractions accounted for only about 20% of **6**, and the high ash content suggested that the sulfur in these two fractions was mostly inorganic as opposed to that in **9**,

Table 4. Effects of Foliar Applications of Standard Compounds on Yield of Cotton Lint

		mol/ha[a]		yield, avg % change[b]	
treatment	years	low	high	low	high
man, gal, glc, suc; 1/1/1/1	1991−1995	2.7	8.1	+4.5 (5)	+8.2 (5)
glc−borate[c]	1993	2.7	8.1	+0.6 (2)	+3.0 (2)
glcNAc, glc A, gal A, glc N; 1/1/1/1	1995	2.7	8.1	−3.3 (2)	−8.2 (2)
ι-, κ-, λ-carrageenans; 1/1/1	1995	2.7	8.1	12.2 (2)	8.9 (2)

[a] Two applications at two levels at pinhead squaring and two weeks later. [b] Numbers in parentheses indicate number of tests. [c] Aqueous boric acid adjusted to pH 6.3, add a threefold quantity of glc, adjust the pH to 4.8.

which appeared to contain some sulfated carbohydrates.

Fraction **6** was also subjected to Dowex-50 chromatography. The carbohydrates were not bound to Dowex-50 in the H^+ form, so 40% of **6** was recovered by elution with water. Urea was again a substantial contaminant. However, **18** was the only fraction of this set (**18**−**22**) that appeared to have lint yield enhancing properties in 1994 and 1995.

Finally, Dowex 1-X-8 in the Cl^- form was employed in an attempt to separate residual urea from the carbohydrates. Chromatography of **18** with water appeared to separate the two species **24** and **26** on the basis of analysis of the eluates using naphthoresorcinol and Ehrlich's reagents as diagnostic sprays on TLC plates. However, nitrogen and carbohydrate analyses showed that while some further separation was achieved, neither fraction was free of the other. Field tests in 1994 and 1995 again appeared to show some enhancement in lint yield by the carbohydrate fraction **24**. The urea-containing fraction **26** was not active, nor had urea enhanced yields in 1989, 1990, and 1991 when applied at a range of concentrations (Hedin and McCarty, 1994a).

Several analyses were performed to identify or otherwise describe the carbohydrates that were present in FT **6** and subsequent isolates. Table 3 presents the glycosyl composition before and after acid hydrolysis via the alditol acetate procedure and also gives the terminal, singly-linked, and doubly-linked glycosyl species (Sturgeon, 1990; York et al., 1985). Four constituent sugars (xylose, mannose, galactose, and glycosyl) were routinely found, and occasionally traces of fucose and arabinose were present. Mannose apparently decreased upon hydrolysis; but we have no explanation for this anomoly. Glycosyl linkage analysis revealed only small amounts of xylose and mannose and that 42.8% were terminal sugars, 45.5% singly-linked sugars, and 11.1% doubly-linked sugars with gal-β-1−4-glc (28.0−22.0%) as a conceivable major species. This analysis is supported by the silica gel TLC evidence that showed that the major species were mostly mono- and disaccharides with only traces of trisaccharides and other oligosaccharides present.

Silica gel TLC also indicated that small amounts of sulfated carbohydrates were present. From repetitive paper chromatography of FT **3**, quantities of a slow moving band, R_f 0.0−0.1 (**9**), were isolated. This band cochromatographed with gal-6-SO_4 and glc-6-SO_4 gave essentially equal amounts of galactose and glucose on acid hydrolysis and analyzed for 6.6% S. This analysis for sulfur suggested that isolate **9** could consist of approximately 50% of a mixture of glc-6-SO_4 and gal-6-SO_4, with the most prevalent contaminants being ash (21.3%) and urea (10.0%). Solid probe EI-MS and CI-CH_4-MS analyses of isolate **9** for these species were inconclusive, however, with only m/z 97 (C_5H_5S) found present in both standards and isolates. Isolate **9** was not a yield promoter in the field tests.

Isolate **6** was also analyzed by HPLC using a Dionex PA-1 strong ion exchange column with a pulsed amperometric detector (Dionex Corp., Sunnyvale, CA). The column was initially equilibrated at pH 12 with NaOH. After injection, sodium acetate was introduced, gradually increasing from 0.0 to 0.5 M. No species larger than disaccharides were evident in an analysis that had the capability of eluting tri- and tetrasaccharides (private communication, D. L. Hendrix, USDA, Phoenix, AZ).

The major component sugars were consistently D-mannose, D-galactose, and D-glucose. Small amounts of arabinose, xylose, and fucose were also identified. Therefore, some mixtures of these major sugars plus sucrose were evaluated in field tests at equal molar concentrations: The average apparent increase during the period 1991−1995 was 3.1% (Table 4). An equal molar mixture of *N*-acetyl D-glucosamine, D-glucuronic acid, D-galacturonic acid, and D-glucosamine appeared to have a slightly positive effect in one test conducted in 1995 (7.6%), but it had a strong negative effect (−19.0%) in a second test in 1995. A glucose−borate complex which was prepared and tested in 1993 had no evident effect. A mixture of ι-, κ-, and λ-carrageenans (linear sulfated polysaccharides) appeared to have a somewhat positive effect (9.5%) in two 1995 field tests. These carrageenans were the only commercially available sulfated polysaccharides that could be procured in quantity for testing.

In summary, the carbohydrate fraction from FT, mostly mono- and disaccharides based on their chromatographic behavior, when applied as a foliar spray tended to increase the yield of cotton lint. A search of the literature failed to identify previous evidence of this effect with carbohydrates. Foliar applications of methanol had positive effects in some field tests (van Iersel et al., 1995).

This series of tests from 1989 to 1995 provided no statistically significant results. However, they did show consistent small increases of lint yield when carbohydrate fractions were applied foliarly. The field plots were only 0.0062 ha, which is standard at this location for field testing of various effects. Statistical significance may have been less difficult to achieve with larger plots. Nevertheless, further testing with these particular isolates as reported herein does not seem likely to be productive. If the carbohydrate lead were to be pursued in further tests, low-cost byproducts from sugar processing might be investigated.

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