Effects of Bioregulators on Flavonoids, Insect Resistance, and Yield of Seed Cotton

Paul A. Hedin,* Johnie N. Jenkins, Alonzo C. Thompson, Jack C. McCarty, Jr., David H. Smith, William L. Parrott, and Raymond L. Shepherd

Two applications at three rates of 16 natural and commercial plant growth bioregulators were sprayed on growing cotton *Gossypium hirsutum* L. to evaluate their effects on levels of nutrients and allelochemicals, on any induced plant resistance to the tobacco budworm *Heliothis virescens* Fab., and on yield of seed cotton. In uninfested cotton, Pix, BAS-105, and V-3183 significantly decreased yield, while gibberellic acid (GA) increased yield. In infested cotton, PIX, BAS-105, BAS-109, V-2307, and V-3183 significantly decreased yield, while kinetin significantly increased yield. V-2307 and CCC decreased flavonoids by 19% each in leaves and squares, respectively, while arabinogalactan increased flavonoids by 60% in squares. BAS-109 increased leaf tannins by 27%. V-2307 decreased leaf anthocyanins by 53% while GA and V-3183 increased leaf anthocyanins by 28 and 39%, respectively. There appeared to be a trend that flavonoids generally were increased where yields were increased. Two cytokinins, Kinetin and Burst, tended to increase yield, pest resistance, and flavonoids.

Flavonoids are important allelochemicals (secondary plant constituents giving either the plant or the pest an adaptive advantage) for normal plant growth, development, and defense against infection and injury by phytophagous pests. Plant flavonoids affect the behavior, development, and growth of a number of insects (Hedin and Waage, 1986). Flavonoids isolated from cotton (*Gossypium hirsutum* L.) buds that are feeding stimulants for the boll weevil, *Anthonomus grandis* Boheman, include quercetin, quercetin 7-*O*-glucoside, and quercetin 3′-*O*-glucoside (Hedin et al., 1968). Flavone glycosides and aglycons in the cotton plant are also larval growth inhibitors for *Heliothis zea* Boddie, the cotton bollworm, *Heliothis virescens* Fab., the tobacco budworm, and *Pectinophora gossypiella* Saunders, the pink bollworm (Chan et al., 1978a). Flavonoid polymers (proanthocyanidins) also have been implicated as insect resistance factors in cotton (Chan et al., 1978b; Hedin et al., 1983a,b).

Cyanidin 3-β-glucoside has recently been shown to be an important factor of resistance in cotton leaves to the feeding of tobacco budworm in the field (Hedin et al., 1983a,b). The reported effectiveness of gossypol was confirmed, but the condensed tannins (proanthocyanidins) in terminal leaves were not correlated with resistance. Paradoxically, these three compounds when incorporated in laboratory diets are equally toxic to tobacco budworm larvae (Hedin et al., 1983a,b).

There is increasing evidence for multiple factor contributions to plant resistance. In laboratory feeding studies, it has been shown that growth of the tobacco budworm is retarded by a number of compounds isolated from the cotton plant including gossypol and related compounds, several flavonoids, catechin, the tannins, cyanidin, delphinidin, and their glycosides (Bell and Stipanovic, 1977; Hedin et al., 1981, 1983a,b). Field studies have shown that varieties high in several components possess the greatest insect resistance (Hedin et al., 1983a,b). Thus, there is the potential to breed for cultivars high in several components, the biosynthesis of which may be controlled by separate genes. Improved gene-splicing technology should eventually be applicable to these objectives. Multiple-factor resistance should be less vulnerable to the development of biotypes of insects and may be less costly to the plant in terms of energy diverted to biosynthesis of defensive compounds.

The mechanisms by which insects react to, or interact with, plant chemicals including flavonoids are multiple. Compounds with established antibiotic properties can also be shown to act in another situation by a nonpreference mechanism. With multicatechol systems such as condensed tannin, cross-linked and insoluble proteins are produced. In addition, these catechols may serve to bind metal ions needed for enzymic activity (Singleton and Kratzer, 1969). Masking of phenolic groups in condensed tannin by methylation with diazomethane destroys all antibiotic activity against *H. virescens* (Chan et al., 1978b).

In structure–activity work, Elliger et al. (1980) have shown that the activity of flavonoids against *H. zea* depends upon the presence of *o*-dihydroxyphenyl groups, and not just the total number of hydroxyl groups. A group of 40 flavonoids was examined for antigrowth activity toward *H. zea* and evaluated with respect to structural features affecting activity (Elliger et al., 1980). It was found that *o*-dihydroxylation in either the A or the B ring was necessary for growth inhibition of *H. zea*, that higher activity was seen with increasing polarity, that the functional group of the C ring was not significant, and that the position of the B ring (C-2 in flavones, C-3 in isoflavones) was not critical.

In a recent study (Waage et al., 1985), we tested a group of 63 flavonoids for antibacterial activity with *Pseudomonas maltophilia* Hugh and Ryschenkow and *Enterobacter cloacae* (Jordan) Hormaeche and Edwards to determine structural characteristics responsible for activity or inactivity of the flavonoids. These bacteria were used because they had been isolated from the gut of the cotton bollworm and also because compounds found toxic to bacteria are often toxic to insects, hence a probable predictor of plant resistance to insects. Among flavone and flavonol aglycons, those possessing 3′,4′- and/or 3,5-dihydroxyl groups were most active. Upon glycosidation, greater toxicity was obtained from 3-*O*- than from 7-*O*-glycosides and with rhamnose rather than glucose as the sugar.

Plant growth regulators have an important role in the growth and developmental processes of plants. In cotton,

Crop Science Research Laboratory, U.S. Department of Agriculture—Agricultural Research Service, Mississippi State, Mississippi 39762-5367.

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

trivial name(s) (source)	systematic nomenclature	recommended treatment[a]	rate, g a.i. ha^{-1} (solvent)
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-phenylpyridazin-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-triazatetracyclo[4.3.1.$0^{2,5}0^{7,9}$]dec-3-ene	cotton: 243 mg/acre = 1.4 mg/plot [Mulrooney (1984)]	2.8, 6.7 (H_2O)
4 Mepiquat chloride, PIX (BASF)	1,1-dimethylpiperidinium chloride	cotton: 1 pint of 4% a.i./acre = 1.6 g/46% a.i. per 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% aqueous acetone)
6 Burst, Cytogen (Burst Agritech, Overland Park, KS)	mixture of cytokinins including zeatins	cotton: 1 pint tech/acre corn: $^1/_2$ pint/acre = 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/acre = 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 of 25 ppm/plot [Williams (1984)]	4.0, 13.5 (5% aqueous EtOH)
13 IAA (Sigma)	indole-3-acetic acid		4.0, 13.5 (5% aqueous EtOH)
14 Kinetin (Sigma)	6-(furfurylamino)purine		4.0, 13.5 (1% aqueous 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/acre = 2.7 mL tech/plot [Elanco]	276.5, 836.4 (H_2O)

[a] Tech = technical preparation.

termination of late-season fruiting has been achieved with potassium 3,4-dichloroisothiazole-5-carboxylate, thus depriving the pink bollworm of food and oviposition sites (Kittock et al., 1975). Of perhaps greater importance would be the control of insects during the growing season. Plant growth regulators have been shown to increase the biosynthesis of certain secondary plant constituents that in turn decrease plant attack by insects. Gibberellic acid, for example, elicits increased terpene biosynthesis in citrus (*Citrus sp.*), thus decreasing attack by fruit flies (*Anastrepha sp.*) (Coggins et al., 1969; Greany, 1978).

The effects of the plant growth regulator mepiquat chloride (1,1-dimethylpiperidinium chloride) on cotton have been widely studied. Its reported effects include increased leaf thickness, shorter internodes that reduced plant height, increased boll retention, reduced boll rot through canopy improvements, and increased yields and earliness (Willard, 1979; York, 1983; Bader and Niles, 1986).

There have been recent reports about the effects of PIX on insect pests of cotton (York, 1983; Bader and Niles, 1986). Zummo et al. (1983) reported less plant damage, decreased bollworm growth, and a 10–20% increase in terpenoids, tannins, and astringency (biological tannin) in a Texas field plot test. Graham et al. (1987) did not show any increase in field resistance to *H. virescens* in Stoneville 213 cotton. Jenkins et al. (1987) reported slight changes in allelochemicals, but none seemingly great enough to affect natural resistance of Stoneville 213 cotton to *H. virescens*.

However, when we applied PIX to cotton and pecan (*Carya illinoensis* Koch), it caused internode shortening but it did not elicit an increase in resistance in cotton to the tobacco budworm or in pecan to pecan scab [*Cladosporium caryigenum* (Ell. et Lang) Gottwald]. Also, changes in content of four known allelochemicals (condensed tannins, gossypol, anthocyanins, flavonoids) for these pests were minimal. An unexpected finding was the increase in content of several nutritional factors that may be related to greater, rather than lesser, growth of tobacco budworm larvae feeding on cotton tissues (Hedin et al., 1984). A possible explanation for our different results from those of Zummo et al. (1983) is that there was much more rainfall during our tests, and we used a different cultivar of cotton.

The present report was part of a larger study to evaluate a number of plant growth regulators when applied to growing cotton for their effects on levels of nutrients and allelochemicals, on any induced plant resistance to the tobacco budworm, and on yield. Two applications of 16 bioregulators at three rates (zero, low, high) were sprayed on growing cotton in statistically designed field plots. Of the plots half were infested with tobacco budworm larvae five times. Plant tissue was gathered 4 weeks after the first application, freeze-dehydrated, and analyzed for allelochemicals. The cotton was machine-harvested once. Flavonoid content and yields were significantly altered by several of the bioregulators.

MATERIALS AND METHODS

1986 Bioregulator Field Test. The commercial cultivar Stoneville 213 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. Insects

Table II. Proximate Analyses and Allelochemicals of Leaves and Seed Cotton Yields of Bioregulator-Treated, Tobacco Budworm Infested ST-213 Plants[a]

		%					yield, kg/ha	
sample	rate	gossypol	tannin	anthocyanin	flavonoid		uninfested	infested
1-CCC	0	0.45 a	8.68 a	0.25 a	1.96 a		3532	3068
	L	0.49 a	8.50 a	0.24 a	2.02 a		3543	2987
	H	0.46 a	7.32 a	0.25 a	1.91 a		3252	2662
						LSD 0.05	NS	NS
2-BAS-105	0	0.45 a	7.46 a	0.23 a	1.75 a		4032	3281
	L	0.40 a	8.69 a	0.26 a	1.80 a		3386	2330
	H	0.44 a	8.97 a	0.25 a	1.78 a		3474	2531
						LSD 0.05	535	875
3-BAS-109	0	0.41 a	4.85 b	0.27 a	1.74 a		3766	3121
	L	0.40 a	6.16 a	0.28 a	1.91 a		4043	2251
	H	0.46 a	6.16 a	0.29 a	1.92 a		3549	3123
						LSD 0.05	NS	725
4-PIX	0	0.46 b	5.63 a	0.29 a	1.99 a		3727	3327
	L	0.54 a	5.02 a	0.30 a	1.88 a		3156	2598
	H	0.59 a	4.07 a	0.28 a	1.75 a		3472	2417
						LSD 0.05	1337	330
5-DINOSEB	0	0.36 a	6.10 a	0.23 a	1.71 a		3128	2326
	L	0.39 a	6.51 a	0.23 a	1.77 a		3497	2736
	H	0.40 a	7.72 a	0.24 a	1.72 a		3133	2780
						LSD 0.05	NS	NS
6-BURST	0	0.51 a	5.51 a	0.25 a	1.91 a		3666	2373
	L	0.56 a	6.31 a	0.28 a	1.96 a		3275	2767
	H	0.56 a	5.71 a	0.28 a	1.88 a		3725	2570
						LSD 0.05	NS	NS
7-XE-1019	0	0.55 a	5.59 a	0.27 a	1.85 a		3682	2862
	L	0.55 a	5.42 a	0.28 a	1.85 a		3213	2893
	H	0.64 a	5.68 a	0.30 a	1.89 a		3783	3173
						LSD 0.05	497	NS
8-V-2307	O	0.66 a	4.09 a	0.17 a	1.58 a		3625	3020
	L	0.54 b	4.92 a	0.14 b	1.60 a		3319	2982
	H	0.44 b	3.77 a	0.08 c	1.33 b		3068	2084
						LSD 0.05	NS	379
9-DCPTA	O	0.30 a	5.32 a	0.17 a	1.69 a		3529	3362
	L	0.25 a	6.31 a	0.18 a	1.76 a		3700	3055
	H	0.34 a	5.33 a	0.18 a	1.82 a		3618	2967
						LSD 0.05	NS	NS
10-GLYPH	O	0.35 a	6.09 a	0.27 a	2.07 a		3487	2836
	L	0.39 a	8.01 a	0.24 a	1.99 a		3310	3058
	H	0.36 a	4.59 a	0.25 a	1.98 a		1964	2678
						LSD 0.05	NS	617
11-V-3183	O	0.50 a	9.06 a	0.28 a	1.97 a		3055	2185
	L	0.46 a	9.67 a	0.32 a	2.09 a		2532	1285
	H	0.55 a	6.68 a	0.39 b	2.15 a		1964	898
						LSD 0.05	523	396
12-GA	O	0.35 a	5.43 a	0.25 a	1.89 a		2747	1872
	L	0.41 a	7.34 a	0.32 b	1.96 a		3250	1648
	H	0.44 a	5.56 a	0.31 b	1.97 a		2648	1742
						LSD 0.05	584	NS
13-IAA	O	0.35 a	7.46 a	0.26 a	1.91 a		2925	2165
	L	0.39 a	5.59 a	0.26 a	1.89 a		2692	2483
	H	0.41 a	6.71 a	0.27 a	1.96 a		3107	2387
						LSD 0.05	NS	NS
14-KIN	O	0.40 a	4.80 a	0.19 a	1.62 a		3434	2521
	L	0.39 a	5.61 a	0.22 a	1.96 a		3777	3241
	H	0.38 a	6.41 a	0.24 a	1.88 a		3625	2878
						LSD 0.05	NS	320
15-AG	O	0.46 a	5.85 a	0.21 a	1.87 a		3454	2783
	L	0.46 a	5.34 a	0.20 a	1.95 a		3785	2701
	H	0.45 a	5.53 a	0.21 a	1.88 a		3249	2502
						LSD 0.05	NS	NS
16-TREF	O	0.39 a	8.19 a	0.29 a	1.92 a		3990	2705
	L	0.44 a	6.46 a	0.28 a	1.91 a		3202	2610
	H	0.45 a	7.03 a	0.32 a	2.15 a		3078	2322
						LSD 0.05	NS	NS
av 16 controls		0.43 ± 0.05	6.26 ± 0.28	0.24 ± 0.04	1.84 ± 0.12		3486	2738

[a] Data from analysis of allelochemicals were subjected to analysis of variance, and means were separated with Duncan's new multiple-range test. Data obtained from the determination of yields were subjected to analysis of variance, and LSD values were calculated.

were controlled all season in environment one. Environment two (the other half of the plots) had an artificially induced infestation of tobacco budworms. Plots were infested weekly, beginning 15 July, for 5 weeks with 8–10 first instar larvae 30-cm^{-1} row (Jenkins et al., 1982).

Sixteen plant growth regulators (Table I) were applied at three rates (zero, low, high) on 7 July and 21 July. The dates were selected to coincide with onset of squaring and the buildup of normal insect infestations. Each compound was weighed and dissolved in 5–10 mL of specified solvent

Table III. Proximate Analyses and Allelochemicals of Squares and Seed Cotton Yields of Bioregulator-Treated, Tobacco Budworm Infested ST-213 Plants[a]

		%					yield, kg/ha	
sample	rate	gossypol	tannin	anthocyanin	flavonoid		uninfested	infested
1-CCC	O	0.23 b	3.01 a	0.05 a	1.13 a		3532	3068
	L	0.30 a	2.81 a	0.06 a	0.95 b		3543	2987
	H	0.30 a	3.00 a	0.06 a	0.99 ab		3252	2662
						LSD 0.05	NS	NS
2-BAS-105	O	0.21 a	5.72 a	0.05 a	1.05 a		4032	3281
	L	0.21 a	6.41 a	0.06 a	1.05 a		3386	2330
	H	0.18 a	6.09 a	0.05 a	1.13 a		3474	2531
						LSD 0.05	565	875
3-BAS-109	O	0.19 a	5.11 a	0.05 a	1.12 a		3766	3121
	L	0.20 a	5.86 a	0.05 a	1.09 a		4043	2251
	H	0.18 a	5.71 a	0.04 a	1.01 a		3549	3123
						LSD 0.05	NS	725
4-PIX	O	0.27 b	4.80 a	0.05 a	1.10 a		3727	3327
	L	0.39 a	4.35 a	0.04 a	1.07 a		3156	2598
	H	0.40 a	4.32 a	0.05 a	0.97 a		2472	2417
						LSD 0.05	1337	330
5-DINOSEB	O	0.21 a	2.52 a	0.05 a	1.04 a		3128	2326
	L	0.23 a	2.60 a	0.05 a	1.05 a		3497	2736
	H	0.18 a	2.40 a	0.04 a	1.05 a		3133	2780
						LSD 0.05	NS	NS
6-BURST	O	0.21 a	2.11 a	0.05 a	1.12 a		3666	2373
	L	0.23 a	2.46 a	0.05 a	1.15 a		3275	2767
	H	0.23 a	2.19 a	0.05 a	1.11 a		3725	2570
						LSD 0.05	NS	NS
7-XE-1019	O	0.22 a	2.86 a	0.06 a	1.26 a		3682	2862
	L	0.23 a	2.83 a	0.06 a	1.45 a		3213	2893
	H	0.23 a	2.77 a	0.05 a	1.29 a		3783	3173
						LSD 0.05	497	NS
8-V-2307	O	0.18 a	2.99 a	0.06 a	1.24 b		3625	3020
	L	0.18 a	2.63 a	0.05 a	1.35 ab		3319	2982
	H	0.19 a	2.91 a	0.05 a	1.43 a		3068	2084
						LSD 0.05	NS	379
9-DCPTA	O	0.21 a	3.26 a	0.07 a	1.23 a		3529	3362
	L	0.21 a	3.06 a	0.06 a	1.23 a		3700	3055
	H	0.21 a	2.92 a	0.06 a	1.20 a		3618	2967
						LSD 0.05	NS	NS
10-GLYPH	O	0.11 b	3.13 a	0.06 a	1.25 a		3487	2836
	L	0.14 a	3.27 a	0.07 a	1.27 a		3310	3058
	H	0.14 a	3.01 a	0.06 a	1.23 a		3469	2678
						LSD 0.05	NS	617
11-V-3183	O	0.22 a	4.28 a	0.07 a	1.40 a		3055	2185
	L	0.19 ab	4.11 a	0.06 a	1.33 a		2532	1285
	H	0.17 b	4.17 a	0.07 a	1.17 a		1964	898
						LSD 0.05	523	396
12-GA	O	0.16 a	5.57 ab	0.05 a	1.30 a		2747	1872
	L	0.16 a	6.32 a	0.06 a	1.37 a		3250	1648
	H	0.16 a	5.27 b	0.06 a	1.26 a		2648	1742
						LSD 0.05	584	NS
13-IAA	O	0.12 a	3.26 a	0.05 a	1.25 a		2925	2165
	L	0.14 a	3.52 a	0.05 a	1.34 a		2692	2483
	H	0.13 a	3.13 a	0.06 a	1.26 a		3107	2387
						LSD 0.05	NS	NS
14-KIN	O	0.13 a	3.21 a	0.05 a	1.02 a		3434	2521
	L	0.15 a	2.94 a	0.05 a	0.97 a		3777	3241
	H	0.14 a	3.08 a	0.06 a	1.01 a		3625	2878
						LSD 0.05	NS	320
15-AG	O	0.13 a	3.28 a	0.06 a	0.73 b		3454	2783
	L	0.13 a	3.27 a	0.05 a	1.13 a		3785	2701
	H	0.13 a	3.37 a	0.05 a	1.17 a		3249	2502
						LSD 0.05	NS	NS
16-TREF	O	0.14 a	5.12 a	0.05 a	1.05 a		3990	2705
	L	0.15 a	5.74 a	0.05 a	0.99 a		3202	2610
	H	0.15 a	5.70 a	0.05 a	0.99 a		3078	2322
						LSD 0.05	NS	NS
av controls		0.18 ± 0.03	3.76 ± 0.19	0.06 ± 0.01	1.14 ± 0.10		3486	2738

[a] Data from analysis of allelochemicals were subjected to analysis of variance, and means were separated with Duncan's new multiple-range test. Data obtained from the determination of yields were subjected to analysis of variance, and LSD values were calculated.

(Table I). One-milliliter portions of the emulsifiers Span 80 and Tween 80 were then added. The solution was made up to 3 L with water and stored at 4 °C until use. They were applied with a CO_2-pressurized back-pack sprayer delivering 203 L ha^{-1} at 207-kPa pressure. Each compound in each environment was handled as a separate randomized complete block experiment with six replications. The plots were machine-harvested one time to determine yield of seed cotton.

Analysis of Allelochemicals. Analyses for gossypol

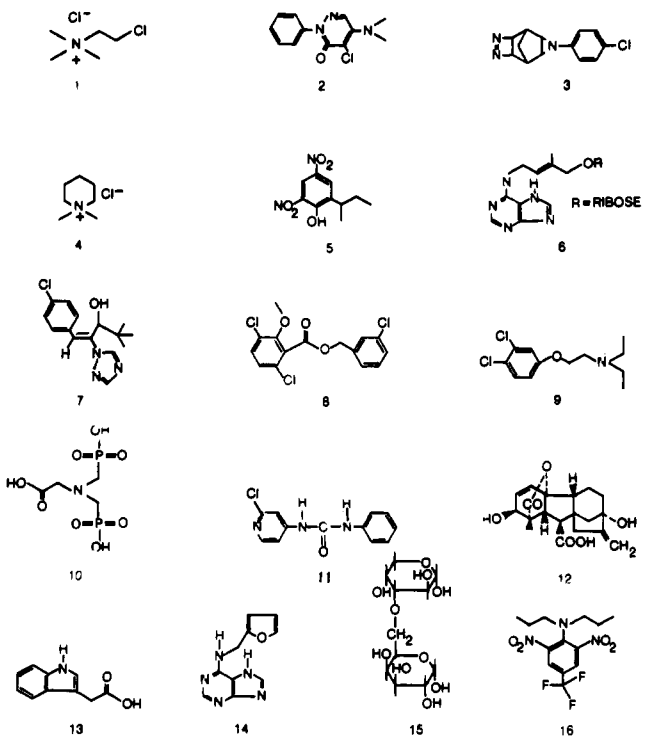


Figure 1. Structures of 16 naturally occurring and synthetic growth regulators applied to cotton plants. See Table I for nomenclature, trivial names, recommended treatments, and rates applied.

and related terpenoid aldehydes were performed on cyclohexane/ethyl acetate/acetic acid (500/500/1; CHEA) extracts of plant tissue by the phloroglucinol reaction (2% in absolute EtOH/concentrated HCl (1/1); stand 1 h) with subsequent spectrometric analysis at 550 nm. The concentration was determined by comparison with data obtained from authentic gossypol and is expressed as gossypol equivalents. Condensed tannin analyses were performed on 70% aqueous methanol (MW) extracts of tissue. The anthocyanidin chromophore was developed by boiling 1 h with 1-butanol/HCl (95/5) (Hedin et al., 1983a,b). The concentration was determined by comparison with the color obtained at 550 nm from a purified cotton condensed tannin sample, the structure of which was elucidated by Collum et al. (1981). The anthocyanin content was determined by measuring the absorbancy at 540 nm of an extract of freeze-dried tissue extracted with methanol/water/HCl (79/19/3), using the molar extinction coefficient (E) of cyanidin 3-β-glucoside (Hedin et al., 1967). Flavonoids were determined after extraction of freeze-dehydrated tissue with 70% aqueous acetone. Diphenylboric acid–ethanolamine complex (Natural Product Reagent A, Aldrich Chemical Co., 1%) in methanol was added, and the chromophore absorptivity at 440 nm was determined and compared to that obtained from a purified sample of isoquercitrin, the most prevalent flavonoid in cotton.

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

Statistical Treatments. Data obtained from the analyses of the allelochemicals were subjected to analysis of variance, and means were separated with Duncan's new multiple-range test. Data obtained from the determination of yields were subjected to analysis of variance, and LSD values were calculated.

Table IV. Abbreviated Summary of the Data in Tables II and III Identifying Bioregulators Having an Effect on Flavonoids and Yield[a]

compd	flavonoid	yield: uninfested	yield: infested	agronom/physiol act.
		(1) Inactive Compounds		
IAA	---	---	---	rooting and growth
DCPTA	---	---	---	inc photosynth
Treflan	---	---	---	preemerg control
Glyphosine	---	---	---	ripener
		(2) Compounds Decreasing Yield		
BAS-105	---	↓	↓	alters 18:2/18:3
PIX	---	↓	↓	internode shortener
V-3183	---	↓	↓	cytokinin
		(3) Compounds Increasing Yield		
Kinetin	---	↑?	↑	cytokinin
CA	↑LV	↑	---	shoot growth
		(4) Compounds Changing Flavonoids Only		
CCC	↓SQ	↓?	---	GA antagonist
AG	↑SQ	---	↓?	induces phytoalexins
BAS-109	↑LV	---	↓↑?	GA antagonist
V-2307	↓LV↑SQ	↓?	↓?	sugar inc
		(5) Compounds Showing Trends		
DINOSEB	↑LV?	↑	↑?	herticide, insecticide
BURST	↑LV?	---	↑?	cytokinin
XE-1019	↑LV?	↓↑?	↑?	GA antagonist

[a] Key: LV = leaves, SQ = squares (buds), ↓ = decreased, ↑ = increased, --- = no effect.

RESULTS AND DISCUSSION

Cotton leaves (terminals) and squares (buds) were analyzed for three classes of flavonoids: (1) flavonoids consisting primarily in cotton of flavones, flavonols, and their glycosides; (2) anthocyanins (cyanidin 3-β-glucoside is the only one in cotton); (3) condensed tannin (proanthocyanidins). The cotton tissues were also analyzed for gossypol. The data obtained from these analyses and yields of seed cotton are presented in Tables II (leaves) and III (squares). The yield of lint cotton from seed cotton is approximately 38–40%. An abbreviated summary of the data in Tables II and III is given in Table IV along with reported agronomic/physiological activities.

In uninfested cotton, BAS-105 and V-3183 significantly decreased yield of seed cotton at either one or both of the levels. With CCC, PIX, treflan, and V-2307, the trend was downward, but failed significance. With XE-1019, a compound stated by the supplier to reduce the rate of stem elongation, the yield was significantly decreased at the low level, but slightly above the control at the high level, therefore making the results difficult to interpret. The low rate of GA gave a significant increase in yield, whereas the high rate was not different from the control.

In infested cotton, PIX, V-3183, BAS-105, and BAS-109 (low level only) and V-2307 (high level only) significantly decreased yield. Only Kinetin at both levels significantly increased yield. Compounds showing trends toward protection against insects though not statistically significant were BAS-109 and XE-1019 at the high level and Dinoseb and Burst at both levels.

V-2307 decreased flavonoids in leaves but increased flavonoids in squares. CCC decreased flavonoids by 19% in squares, while arabinogalactan (AG) increased flavonoids by 60% in squares. GA significantly decreased tannins in the square by 6%, while BAS-109 significantly increased tannins in the leaf by 27%. V-2307 significantly decreased anthocyanins (53%) in the leaf while GA and V-3183 increased anthocyanins in the leaf by 28 and 39% respectively. Table IV suggests that flavonoids generally are increased where yields are (or appear to be) increased, while flavonoids generally are decreased where yields ap-

pear to be decreased. Obviously, further work would be required to confirm this possible relationship, which, tenuous as it may be, provides some basis for searching for bioregulators that could induce greater pest resistance in cotton.

Attempts to correlate reported agronomic/physiological activity with flavonoids and/or yield were generally not successful except for the cytokinins, which generally tended to increase yield, pest resistance, and flavonoids. V-3183, an exception, was evidently applied at too high a level, because the yield was severely decreased at the high level. There may also be a positive correlation of yield with shoot growth because GA improved yield, while PIX, an internode shortener, decreased yield.

In summary, this bioregulator study provided some limited encouragement for developing a crop strategy in which appropriate compounds are applied to cotton in order to increase yields and pest resistance. There appeared to be a small positive correlation between the flavonoid concentration, yield, and pest resistance, but it is not to be inferred that the increase in flavonoids was causal.

Registry No. 1, 999-81-5; 2, 3707-98-0; 3, 77788-93-3; 4, 24307-26-4; 5, 88-85-7; 6, 114718-87-5; 7, 83657-22-1; 8, 101191-06-4; 9, 65202-07-5; 10, 2439-99-8; 11, 68157-60-8; 12, 77-06-5; 13, 87-51-4; 14, 525-79-1; 15, 9036-66-2; 16, 1582-09-8; gossypol, 303-45-7.

LITERATURE CITED

Bader, R. F.; Niles, G. A. "Response of Short- and Full-Season Cotton Cultivars to Mepiquat Chloride. I. Morphological and Phenological Variables". Proceedings of the Beltwide Cotton Producers Research Conference, Las Vegas, NV, Jan 4–9, 1986; pp 513–517.

Bell, A. A.; Stipanovic, R. D. "The Chemical Composition, Biological Activity, and Genetics of Pigment Glands in Cotton". Proceedings of the Beltwide Cotton Producers Research Conference, 1977; p 244.

Campbell, B. C.; Chan, B. G.; Creasy, L. L.; Dreyer, D. G.; Rabin, L. B.; Waiss, A. C. "Bioregulation of Host Plant Resistance to Insects". In *Bioregulators*: Chemistry *and Uses*; Ory, R. L., Rittig, F. R., Eds.; ACS Symposium Series No. 257; American Chemical Society: Washington, DC, 1984; pp 194–203.

Chan, B. G.; Waiss, A. C.; Lukefahr, M. "Inhibition of Lepidopterous Larval Growth by Cotton Constituents". *Entomol. Exp. Appl.* **1978a**, *24*, 294–300.

Chan, B. G.; Waiss, A. C.; Lukefahr, M. "Condensed Tannin, An Antibiotic Chemical from *Gossypium hirsutum* (L.)". *J. Insect Physiol.* **1978b**, *24*, 113–118.

Coggins, C. W.; Scora, R. W.; Lewis, L. N.; Knapp, J. C. F. "Gibberellin-Delayed Senescence and Essential Oil Changes in the Navel Orange Rind". *J. Agric. Food Chem.* **1969**, *17*, 807–809.

Collum, D. H.; Hedin, P. A.; White, W. H.; Parrott, W. L.; Jenkins, J. N.; Grimley, E. B. "Studies on the Structural Properties of Cotton Tannin and Its Toxicity to the Tobacco Budworm". *Abstracts of Papers*, 182nd National Meeting of the American Chemical Society, New York, NY, 1981; American Chemical Society: Washington, DC, 1981; PEST 54.

Elliger, C. A.; Chan, B. G.; Waiss, A. C. "Flavonoids as Larval Growth Inhibitors: Structural Factors Governing Toxicity". *Naturwissenschaften* **1980**, *67*, 358–360.

Graham, C. T., Jr.; Jenkins, J. N.; McCarty, J. C.; Parrott, W. L. "Effects of Mepiquat Chloride on Natural Plant Resistance to Tobacco Budworm in Cotton". *Crop Sci.* **1987**, *27*, 360–361.

Greany, P. I. "Citrus Chemicals Knock Out Fruit Fly". *Agricultural Research*; U.S. Department of Agriculture: Washington, DC, 1978; Vol. 27, pp 3–4.

Hedin, P. A.; Waage, S. K. "Roles of Flavonoids in Plant Resistance to Insects". *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological, and Structure Activity Relationships*; Alan R. Liss: New York, 1986; pp 87–100.

Hedin, P. A.; Minyard, J. P.; Thompson, A. C.; Struck, R. F.; Frye, J. "Constituents of the cotton Bud. Identification of the Anthocyanin as Chysanthemin". *Phytochemistry* **1967**, *6*, 1165–1167.

Hedin, P. A.; Miles, L. R.; Thompson, A. C.; Minyard, J. P. "Constituents of the Cotton Bud. Formulation of a Boll Weevil Feeding Stimulant Mixture". *J. Agric. Food Chem.* **1968**, *16*, 505–513.

Hedin, P. A.; Collum, D. H.; White, W. H.; Parrott, W. L.; Lane, H. C.; Jenkins, J. N. "The Chemical Basis for Resistance in Cotton to *Heliothis* Insects". *Proceedings of the International Conference on Regulation of Insect Development and Behavior*; Wroclaw-Technical University: Wroclaw, Poland, 1981; pp 1071–1086.

Hedin, P. A.; Jenkins, J. N.; Collum, D. H.; White, W. H.; Parrott, W. L.; MacGown, M. W. "Cyanidin-3-β-Glucoside a Newly Recognized Basis for Resistance in Cotton to the Tobacco Budworm *Heliothis virescens* (F.)". *Experientia* **1983a**, *39*, 799–801.

Hedin, P. A.; Jenkins, J. N.; Collum, D. H.; White, W. H.; Parrott, W. L. "Multiple Factors in Cotton Contributing to Resistance to the Tobacco Budworm *Heliothis virescens* (F.)". In *Plant Resistance to Insects*; Hedin, P. A., Ed.; ACS Symposium Series No. 208; American Chemical Society: Washington, DC, 1983b; pp 347–365.

Hedin, P. A.; Jenkins, J. N.; McCarty, J. C.; Mulrooney, J. E.; Parrott, W. L.; Borazjani, A.; Graves, C. H.; Filer, T. H. "Effects of 1,1-Dimethylpiperidinium Chloride on the Pests and Allelochemicals of Cotton and Pecan". In *Bioregulators: Chemistry and Uses*; Ory, R. L., Rittig, F. R., Eds.; ACS Symposium Series No. 257; American Chemical Society: Washington, DC, 1984; pp 171–191.

Jenkins, J. N.; Parrott, W. L.; McCarty, J. C.; White, W. H. "Breeding Cotton for Resistance to the Tobacco Budworm: Techniques to Achieve Uniform Field Infestations". *Crop. Sci.* **1982**, *22*, 400–404.

Jenkins, J. N.; Hedin, P. A.; McCarty, J. C.; Parrott, W. L. "Effects of Mepiquat Chloride on the Allelochemicals of Cotton". *J. Miss. Acad. Sci.* **1987**, *32*, 73–78.

Kittock, D. L.; Arle, H. F.; Bariola, L. A. "Chemical Termination of Cotton Fruiting in Arizona in 1974". Proceedings of the Beltwide Cotton Producers Research Conference, New Orleans, LA, Jan 6–8, 1975; p 71.

Mulrooney, J. E., USDA, Mississippi State, MS, personal communication, 1985.

Nickell, L. G. "Sucrose Increases with Bioregulators". In *Bioregulators: Chemistry and Uses*; Ory, R. L., Rittig, F. R., Eds.; ACS Symposium Series No. 257; American Chemical Society: Washington, DC, 1974; pp 101–112.

Ory, R. L.; St. Angelo, A. J.; Conkerton, E. J.; Chapital, D. C.; Rittig, F. R. "Properties of Peanuts (*Arachis hypogaea* L.) from Bioregulator-Treated Plants". In *Bioregulators: Chemistry and Uses*; Ory, R. L., Rittig, F. R., Eds.; ACS Symposium Series No. 257; American Chemical Society: Washington, DC, 1984; pp 83–92.

Singleton, V. L.; Kratzer, F. H. "Toxicity and Related Physiological Activity of Phenolic Substances of Plant Origin". *J. Agric. Food Chem.* **1969**, *17*, 497–512.

Virk, J. A.; Sharma, S. R.; Tripathi, H. P. "Effect of Cycocel on Yield and Other Characters of Cotton Variety F 414". *J. Res. Punjab Agric. Univ.* **1984**, *21*, 483–488.

Waage, S. K.; Hedin, P. A.; Sikorowski, P. P. "Relationships Between Flavonoid Structure and *Heliothis zea* Gut Bacteria Growth". *J. Miss. Acad. Sci.* **1985**, *30*, 23–29.

Willard, J. I. "Behavior of PIX Plant Regulator on the Cotton Plant and the Environment". Proceedings of the Beltwide Cotton Producers Research Conference, Phoenix, AZ, Jan 7–11, 1979; p 52.

Williams, M. W. "Use of Bioregulators to Control Vegetative Growth of Fruit Trees and Improve Fruiting Efficiency". In *Bioregulators: Chemistry and Uses*; Ory, R. L., Rittig, F. R., Eds.; ACS Symposium Series No. 256; American Chemical Society: Washington, DC, 1984; pp 93–99.

Yokoyama, H. "Affecting Photosynthesis With Bioregulators". *Agricultural Research*; U.S. Department of Agriculture: Washington, DC, 1984; No. 14, p 4.
York, A. C. Response of Cotton to Mepiquat Chloride with Varying N Rates and Plant Populations". *Agron. J.* **1983**, *75*, 667–672.
Zummo, G. R.; Benedict, J. H.; Segers, J. C. "Effects of a Plant Growth Regulator (PIX) on Host-Plant Resistance to *Heliothis zea* in Cotton". Proceedings of the Beltwide Cotton Producers Research Conference, San Antonio, TX, Jan 2–6, 1983; pp 73–74.

Received for review September 17, 1987. Revised manuscript received February 24, 1988. Accepted March 30, 1988. Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

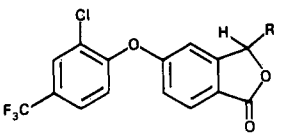
Some Novel Diphenyl Ether Herbicides with Peroxidizing Activity

Patrick Camilleri,* Karen Weaver, Michael T. Clark, John R. Bowyer, and Beverly J. Hallahan

5-[2-Chloro-4-(trifluoromethyl)phenoxy]phthalide and its 3-alkoxy derivatives are a new class of diphenyl ether herbicides with partitioning properties and symptoms of plant phytotoxicity similar to those shown by nitrodiphenyl ethers. At an applied concentration of 1 μM, they induce membrane lipid peroxidation on treated leaves at a rate similar to that seen with nitrodiphenyl ethers, with the 3-methoxyphthalide being the most active compound. Their redox properties preclude reduction by the photosynthetic electron-transport chain, nor do they significantly inhibit photosynthetic electron transport at herbicidally active concentrations. These compounds should prove useful in the identification of the primary mechanism of action of nitrodiphenyl ether and related herbicides.

It is now well established that nitrodiphenyl ether (NDPE) herbicides require both light and oxygen to elicit their activity on whole plants (Matsunaka, 1969; Orr and Hess, 1982; Kunert, 1984). However, the primary mode of interaction of these compounds, possibly at a receptor site within the chloroplast or the chloroplast envelope, is not understood. One of the hypotheses that has been proposed (Kunert and Böger, 1981; Lambert et al., 1984) is that the activity of NDPE's depends on the relative ease by which these compounds can be reduced by chloroplast photosystem I (PS I) in a way similar to that of paraquat, a well-known PS I electron acceptor. Such a mechanism would lead to the formation of a reactive anion radical that can transfer its electron to oxygen, leading to highly active oxygen species, such as H_2O_2 and $^{\bullet}OH$, which would peroxidize unsaturated lipid membranes. The occurrence of this mechanism has been questioned for a number of reasons. Thus, diuron treatment protects plants from paraquat toxicity but is much less effective in reversing or diminishing the phytotoxicity caused by NDPE's (Matsunaka, 1969; Orr and Hess, 1982; Ensminger and Hess, 1985). Moreover, recent electrochemical studies have shown that diphenyl ether compounds where the nitro group has been replaced by a chlorine atom cannot readily accept an electron to form an anion radical, as in the case of the nitro analogues (Ensminger et al., 1985). Despite these differences in electrochemical behavior, both the nitro and chloro compounds were found to be as effective on the green unicellular alga *Chlamydomonas eugametos* and in three weed species (*Xanthium pennsylvanicum*, *Abutilon theophrasti*, *Ipomoea*). Finally we have recently shown (Bowyer et al., 1987a) that a typical NDPE, namely 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitroacetophenone oxime *O*-(acetic acid methyl ester), which we called DPE I, causes rapid leaf wilting, membrane lipid peroxidation, and chloroplast destruction in barley mutants that are known to lack either PS I or PS II. As expected, these mutants were found to be resistant to paraquat action.

In this publication we introduce four novel diphenyl ether herbicides, which are derivatives of 5-[2-chloro-4-(trifluoromethyl)phenoxy]phthalide and show similar symptoms of phytotoxicity (rapid chlorosis and necrosis) on whole plants as NDPE's. The chemical structures of the four compounds we have studied are as follows:



I, R = H
II, R = OCH_3
III, R = OC_2H_5
IV, R = OC_3H_7

Like most conventional NDPE's these compounds contain a 2-chloro-4-(trifluoromethyl)phenoxy group. However, unlike NDPE's a phthalide ring replaces the nitro substituent. This structural property is of interest in the use of these compounds as important "tools" to help identify structure–activity features essential for the phytotoxicity of diphenyl ethers, in general.

MATERIALS AND METHODS

Chemicals. Phthalides I–IV (purity >95%) were synthesized (Clark and Gilmore, 1984) by the Organic Chemistry Department, Shell Research Centre, Sittingbourne.
Hill Inhibition: The procedure used for the preparation of the pea (*Pisum sativum*) thylakoid membranes and the measurement of photosynthetic electron transport have been outlined by us previously (Bowyer et al., 1987a).
Measurement of One-Electron Reduction Potential. The one-electron reduction potential of phthalide diphenyl ether II was measured from the equilibrium concentrations

Sittingbourne Research Centre, Shell Research Limited, Sittingbourne, Kent ME9 8AG, U.K. (P.C., K.W., M.T.C.), and Department of Biochemistry, Royal Holloway and Bedford New College, Egham Hill, Egham, Surrey TW20 0EX, U.K. (J.R.B., B.J.H.).

0021-8561/88/1436-1061$01.50/0