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EFFECTS OF FOLIAR APPLIED HARPIN PROTEIN ON COTTON LINT YIELD, FIBER QUALITY, AND CROP MATURITY*

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ABSTRACT

Yield-enhancing compounds are among the many inputs used in cotton (*Gossypium hirsutum* L.) production systems across the United States Cotton Belt. Some of these products, however, have not been adequately tested in field settings and their impact on cotton

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yield and quality is unknown. Messenger, marketed by the Eden Bioscience Corporation (Bothell, WA), is a new product containing a protein that may stimulate the hypersensitive response of higher plants, resulting in increased yields. The objective of our investigation was to determine if Messenger applications would result in enhanced cotton crop maturity, lint yield or fiber quality. Messenger studies were conducted in Colquitt, Grady, and Tift Counties in South Georgia and at the University of Georgia Coastal Plain Experiment Station (UGA-CPES) in Tifton in 2000. Plot size at each location ranged from 1.2 ha (Grady County) to 0.01 ha (UGA-CPES). Messenger was applied as a foliar treatment at several stages of crop development at each location with either a John Deere (Moline, IL) high clearance sprayer or a CO_2 backpack sprayer. Mid- and late-season plant maps at each location revealed no significant differences in crop maturity among the treatments. Lint yields in Colquitt, Grady, and Tift Counties and the UGA-CPES averaged 1159, 941, 1292, and 1654 kg ha^{-1}, respectively with no significant treatment differences within a location. Likewise, Messenger did not significantly affect fiber properties at any location.

INTRODUCTION

Yield-enhancing compounds are among the many inputs available for cotton production systems across the United States Cotton Belt. The usefulness of some of these products in producing high yielding, high quality cotton, however, remains to be resolved. Messenger, marketed by the Eden Bioscience Corporation (Bothell, WA), is a new product that was recently introduced in the Mid-south and Southeastern cotton states. In addition to the possibility of increased lint yield, Messenger may also aid in the suppression of insects, mites, nematodes and diseases as well as improve plant vigor, growth and stress tolerance (1). While somewhat novel in its mode of action, Messenger contains a protein that may bind to cellular receptors and stimulate the cotton plant's natural defense mechanism, known as the systemic acquired resistance (SAR) pathway.

The initial component of the natural defense mechanism of higher plants, the hypersensitive response (HR), is associated with plant defense against many bacteria, fungi, viruses and nematodes (2). The HR is characterized by the rapid, localized death of tissues affected by a pathogen (3). After the death of localized tissues, the SAR pathway is activated (4). Systemic acquired resistance activation results in the development of a broad-spectrum, systemic resistance (5,6). Ryals et al. (4) reported that an understanding of the biochemical pathway leading to this resistance could enable the development of plant protection compounds that act by stimulating the plant's inherent disease resistance mechanisms.

In 1992 researchers from Cornell University (Ithaca, NY) reported the discovery of a proteinaceous elicitor of the HR of tobacco (*Nicotiana tabacum* L.) that was isolated from *Erwinia amylovora*, the bacterium that causes fire blight of rosaceous plants (3). This elicitor, named harpin, is an acidic, heat-stable, cell-envelope-associated protein with a molecular weight of 44 kilodaltons. The Eden Bioscience Corporation further developed and marketed this harpin protein (trade name Messenger) for use on agricultural crops. The objective of this investigation was to evaluate the usefulness of Messenger in cotton production systems. Our specific objectives were to determine if Messenger would (1) enhance cotton crop development and maturity, (2) increase lint yield and/or (3) improve fiber quality.

MATERIALS AND METHODS

Messenger studies were conducted in 2000 on grower farms in Colquitt, Grady and Tift Counties in South Georgia and at the University of Georgia Coastal Plain Experiment Station (UGA-CPES) in Tifton. Soils at the Colquitt County, Tift County, and UGA-CPES locations are Tifton loamy sands (Fine-loamy, kaolinitic, thermic Plinthic Kandiudults). The soil at the Grady County location is a Varina Sandy Loam (Fine, kaolinitic, thermic Plinthic Paleudults). Water stress was minimized in all studies with overhead sprinkler irrigation systems. Fertility, weed, nematode and insect control techniques in all studies were consistent with the University of Georgia Cooperative Extension Service recommendations (7). The timing and rate of the various Messenger treatments at each location are presented in Table 1. Messenger was applied in each study at the two-leaf (TL), pin-head square (PHS), two weeks after PHS, first flower (FF) and/or three weeks after FF stages of crop development. Across all studies, the two-leaf stage of crop development occurred from 19 to 25 days after planting (DAP), the pin-head square stage occurred from 39 to 40 DAP, and the first flower stage occurred from 55 to 61 DAP. All Messenger treatments were applied with non-chlorinated water. Immediately after machine harvest ≈1000 g of seed cotton was collected from all the plots in each study. These samples were subsequently ginned on a 10-saw laboratory gin (Continental Eagle Corporation, Prattville, AL) for determination of lint percentage. Fiber quality was determined at the United States Department of Agriculture Cotton Classing Office in Macon, GA. More detailed methodology of each study is as follows.

Table 1. Timing and Rate of the Various Messenger Treatment Applications in Studies Conducted at Colquitt, Grady, and Tift Counties and the University of Georgia-Coastal Plain Experiment Station (UGA-CPES) in 2000 (All Applications Were Made as Broadcast Foliar Sprays)

Location	Treatment	2-Leaf[a] (g a.i. ha^{-1})	PHS[b] (g a.i. ha^{-1})	PHS + 2 wk[c] (g a.i. ha^{-1})	FF[d] (g a.i. ha^{-1})	FF + 3 wk[e] (g a.i. ha^{-1})
Colquitt Co.	Messenger 1	4.7	4.7	4.7		
	Messenger 2	4.7	4.7		4.7	
	Messenger 3	4.7	4.7	4.7	4.7	
	Untreated					
Grady Co.	Messenger		4.7	4.7	4.7	4.7
	Untreated					
Tift Co.	Messenger	4.7	9.4[f]		9.4[f]	
	Untreated					
UGA-CPES	Messenger 1	4.7	4.7	4.7	4.7	4.7
	Messenger 2	4.7	4.7	4.7		
	Messenger 3	4.7	4.7		4.7	
	Messenger 4	4.7	4.7	4.7	4.7	
	Messenger 5		4.7		4.7	4.7
	Messenger 6		4.7	4.7	4.7	4.7
	Messenger 7	4.7			4.7	4.7
	Untreated					

[a] Two-leaf stage of crop development.
[b] Pin-head square stage of crop development.
[c] Pin-head square plus two weeks stage of crop development.
[d] First flower stage of crop development.
[e] First flower plus three weeks stage of crop development.
[f] Carrier volume for these applications were 187 L ha^{-1}, all others were 93 L ha^{-1}.

Colquitt County

Cotton 'DeltaPine NuCOTN 35B' was planted on April 27, 2000 at a rate of one seed every 10 cm. Row widths were 97 cm and plot size was 16 rows by 560 m. Each plot was approximately 0.87 ha in size. All Messenger treatments were applied with a John Deere (Moline, IL) high clearance sprayer equipped with DG-8002-VS tips. Treatments were arranged in a randomized block design with 4 replications. At 112 and 125 DAP nodes above cracked boll (NACB) were recorded on 10 randomly selected plants in each plot. For this analysis, the number of main stem nodes from the uppermost first sympodial position cracked boll to the uppermost node containing a harvestable boll was counted. A harvestable boll was considered a boll that had reached full size. The study was machine harvested on September 27, 2000.

Grady County

Cotton 'SureGrow 747' was strip-till planted into wheat stubble on May 25, 2000. While planting, three seeds were hill dropped every 28 cm. Row widths were 91 cm and plot size was 20 rows by 672 m. Each plot was approximately 1.2 ha in size. All Messenger treatments were applied with a John Deere high clearance sprayer equipped with DG-8003 tips. Treatments were arranged in a randomized block design with 4 replications. At 76, 111, and 126 DAP growth analyses were conducted in which 5 plants from each plot were randomly selected and first and second sympodial position fruit type and number were recorded as well a plant height, main stem node number and the number of main stem nodes above the uppermost first sympodial position white flower (NAWF). The study was machine harvested on October 31, 2000.

Tift County

Cotton 'DeltaPine 458BRR' was planted on April 20, 2000 at a rate of one seed every 10 cm. Row widths were 91 cm and plot size was 6 rows by 30 m. Each plot was approximately 0.02 ha in size. All Messenger treatments were applied with a CO_2 backpack sprayer equipped with 8002-LP tips. Treatments were arranged in a randomized block design with 3 replications. Nodes above cracked boll were recorded on ten randomly selected plants in each plot at 126 and 130 DAP. The study was machine harvested on September 28, 2000.

UGA-CPES

Cotton 'Phytogen 355' was planted on May 3, 2000 at a rate of one seed every 10 cm. Row widths were 91 cm and plot size was 6 rows by 15 m. Each plot was approximately 0.01 ha in size. All Messenger treatments were applied with a CO_2 backpack sprayer equipped with 8002 tips. Treatments were arranged in a randomized block design with 5 replications. At 75 DAP a growth analysis was conducted in which 10 plants from each plot were randomly selected and first sympodial position fruit type and number were recorded as well a plant height, main stem node number and NAWF. Nodes above cracked boll were recorded on ten randomly selected plants in each plot at 106 and 111 DAP. The study was machine harvested on September 20, 2000.

Statistical Analyses

All data were analyzed using Proc MIXED (8) where replications, main plot error, and sub-samples were considered as random effects and treatments as fixed effects. Mean separation using LSD was taken from the least square mean results of Proc MIXED.

RESULTS AND DISCUSSION

Results from the growth analyses at 76 DAP in Grady County and 75 DAP at the UGA-CPES are presented in Table 2. Plant height in Grady County was slightly greater than 81 cm plant^{-1} for both treatments while plant height at the UGA-CPES was greater than 100 cm plant^{-1} across all eight treatments. Treatment differences were not statistically significant within a location. Wright et al. (9) conducted studies for three growing seasons with Messenger and also reported no significant differences in plant height. Main stem node number in our studies was slightly greater than 17 nodes plant^{-1} across all treatments and did not significantly differ (Table 2). The number of main stem nodes above the uppermost first sympodial position white flower (NAWF) also did not differ among treatments within a location, indicating there was no difference in crop maturity (Table 2).

Table 3 shows the percentage of fruiting sites with squares, bolls, and aborted positions at 76, 111, and 126 DAP in Grady County and 75 DAP at the UGA-CPES. Within a location and DAP, Messenger applications resulted in no significant difference in percent squares, bolls or aborted positions. These data also indicate crop maturity did not differ among the treatments. Crop maturity was also assessed in three of the four studies using the nodes above cracked boll

Table 2. Plant Height, Number of Main Stem Nodes, and Nodes Above White Flower (NAWF) at 76 Days After Planting (DAP) in Grady County and 75 DAP at the University of Georgia Coastal Plain Experiment Station (UGA-CPES) in Messenger Studies Conducted in 2000

Location	Treatment	Plant Height (cm plant^{-1})	Main Stem Nodes (plant^{-1})	NAWF (plant^{-1})
Grady Co.	Messenger	81.2 a[a]	17.4 a	5.9 a
	Untreated	81.1 a	17.3 a	6.0 a
	P > F	0.3725	0.7741	0.8493
UGA-CPES	Messenger 1	104 a	17.5 a	4.6 a
	Messenger 2	102 a	17.1 a	4.3 a
	Messenger 3	102 a	17.1 a	4.3 a
	Messenger 4	104 a	17.0 a	4.2 a
	Messenger 5	104 a	17.4 a	4.6 a
	Messenger 6	105 a	17.4 a	4.6 a
	Messenger 7	104 a	17.0 a	4.5 a
	Untreated	103 a	17.4 a	4.5 a
	P > F	0.8395	0.5059	0.6964

[a] Means followed by the same letter within a location and column are not significantly different ($P > 0.05$).

(NACB) technique (Table 4). At 111 DAP the average NACB across all treatments at the UGA-CPES was 6.3. In Colquitt County the average NACB at 112 DAP was 11.6 while in Tift County the average NACB at 126 DAP was 9.2. These data indicate crop maturity at the UGA-CPES occurred as much as two weeks earlier than the other locations. These maturity differences could be due to differences in cultivar selection between the three locations. Significant differences in NACB, however, were not observed within a location and DAP (Table 4). These data again indicate that Messenger did not result in enhanced crop maturity.

Wright et al. (9) observed increased lint yields with Messenger in two of three years. In one study, lint yield was increased 457 kg ha^{-1} while a more modest increase was reported in another (155 kg ha^{-1}). In our studies, average lint yields in Colquitt, Grady, and Tift Counties and the UGA-CPES were 1159, 941, 1292, and 1654 kg ha^{-1}, respectively with no significant treatment differences within a location (Table 5). Lint percentages ranged from 37.4 (Colquitt County) to 41.0% (UGA-CPES) and did not differ within a location (Table 5). In addition, Messenger did not significantly affect fiber properties at any location (Table 5).

Table 3. Percentage of Fruiting Sites with Squares, Bolls, and Aborted Fruit at 76, 111, and 126 Days After Planting (DAP) in Grady County and 75 DAP at the University of Georgia Coastal Plain Experiment Station (UGA-CPES) in Messenger Studies Conducted in 2000 (First and Second Sympodial Fruit Were Recorded in Grady County and First Sympodial Fruit Only Were Recorded at UGA-CPES)

			Total Fruiting Positions (%)		
Location	DAP	Treatment	Squares	Bolls	Aborted
Grady Co.	76	Messenger	47.4 a[a]	24.0 a	23.4 a
		Untreated	47.2 a	23.9 a	23.4 a
		P > F	0.9703	0.9788	0.9924
	111	Messenger	8.83 a	41.3 a	49.7 a
		Untreated	6.80 a	40.9 a	53.0 a
		P > F	0.6491	0.4922	0.4702
	126	Messenger	0.01 a	33.5 a	59.0 a
		Untreated	0.01 a	35.0 a	58.2 a
		P > F	0.3718	0.7310	0.8463
UGA-CPES	75	Messenger 1	36.2 a	41.2 a	12.5 a
		Messenger 2	37.4 a	44.2 a	8.2 a
		Messenger 3	36.6 a	43.6 a	10.1 a
		Messenger 4	35.4 a	41.8 a	12.5 a
		Messenger 5	38.1 a	41.0 a	11.4 a
		Messenger 6	37.5 a	42.5 a	11.6 a
		Messenger 7	38.3 a	41.9 a	11.7 a
		Untreated	37.0 a	41.8 a	12.4 a
		P > F	0.9999	0.9996	0.7699

[a] Means followed by the same letter within a location, DAP and column are not significantly different ($P > 0.05$).

CONCLUSIONS

Various types of SAR elicitor molecules have been discovered and include complex carbohydrates, salicylic acid, fatty acids, jasmonates, amino acids, ethylene, glycoproteins, yeast-derived elicitors and microbial metabolites. While many of these elicitors are effective at inducing plant resistance under laboratory conditions, most are unlikely to result in commercial products used for disease control in crops. Reasons for this include the level of disease control achieved by many elicitors is low compared to the levels which can be achieved with

Table 4. Nodes Above Cracked Boll (NACB) at Two Different Days After Planting (DAP) in Colquitt and Tift Counties and at the University of Georgia Coastal Plain Experiment Station (UGA-CPES) in Messenger Studies Conducted in 2000 (Data Were Recorded in Colquitt County on 112 and 125 DAP; Data Were Recorded in Tift County on 126 and 130 DAP; Data Were Recorded at the UGA-CPES on 106 and 111 DAP)

Location	Treatment	NACB First Recording	NACB Second Recording
Colquitt Co.	Messenger 1	11.2 a[a]	5.0 a
	Messenger 2	11.5 a	4.8 a
	Messenger 3	11.6 a	4.6 a
	Untreated	12.1 a	4.7 a
	P > F	0.5241	0.6957
Tift Co.	Messenger	10.2 a	9.2 a
	Untreated	08.2 a	8.0 a
	P > F	0.1064	0.3925
UGA-CPES	Messenger 1	8.3 a	6.6 a
	Messenger 2	7.8 a	5.9 a
	Messenger 3	9.1 a	6.0 a
	Messenger 4	8.8 a	5.9 a
	Messenger 5	8.9 a	6.5 a
	Messenger 6	9.0 a	6.3 a
	Messenger 7	9.2 a	6.4 a
	Untreated	8.8 a	6.4 a
	P > F	0.5661	0.5795

[a] Means followed by the same letter within a location, and column are not significantly different ($P > 0.05$).

fungicides and the elicitors may be highly specific to a certain crop or crop cultivar (10). These issues have not been addressed with harpin elicited SAR in cotton in the current scientific literature.

In the current studies, Messenger was not examined in an entomological or pathological context. Thus, if cotton SAR were induced with Messenger, the possible benefits from insect or pathogen control would not have been observed in our studies due to the objectives established and experimental design utilized. Messenger applications increased lint yield in another study with the same basic design and objectives as this study (9). Objectives for this study were to further investigate the possibility of Messenger as a yield-enhancing production input. Utilization of SAR elicitors as yield-enhancers is not a new concept. In field experiments, treatment of winter barley with an elicitor of SAR produced by a

Table 5. Lint Percentage, Lint Yield, Fiber Length, Percent Uniformity, Fiber Strength, Short Fiber Index (SFI), and Fiber Micronaire in Colquitt, Grady, and Tift Counties and at the University of Georgia Coastal Plain Experiment Station (UGA-CPES) in Messenger Studies Conducted in 2000

Location	Treatment	Lint (%)	Lint Yield ($kg\,ha^{-1}$)	Length (cm)	Uniformity (%)	Strength ($kN\,m\,kg^{-1}$)	SFI	Micronaire
Colquitt Co.	Messenger 1	37.4 a[a]	1156 a	2.76 a	81.8 a	277.5 a	3.1 a	4.2 a
	Messenger 2	37.9 a	1153 a	2.71 a	82.0 a	273.4 a	3.9 a	4.5 a
	Messenger 3	38.0 a	1166 a	2.76 a	82.1 a	279.3 a	3.2 a	4.3 a
	Untreated	37.8 a	1161 a	2.71 a	81.4 a	272.4 a	4.8 a	4.2 a
	$P > F$	0.6653	0.9344	0.3469	0.3310	0.5171	0.2661	0.1265
Grady Co.	Messenger	40.0 a	979 a	2.87 a	84.1 a	278.5 a	1.8 a	4.9 a
	Untreated	39.2 a	902 a	2.86 a	84.7 a	281.5 a	1.5 a	4.7 a
	$P > F$	0.3360	0.1750	0.7177	0.2210	0.6376	0.1265	0.3672

Tift Co.	Messenger	39.6 a	1310 a	2.78 a	82.6 a	270.0 a	3.7 a	4.6 a
	Untreated	39.2 a	1274 a	2.76 a	82.6 a	266.1 a	4.0 a	4.6 a
	P > F	0.5306	0.5833	0.6985	1.0000	0.3313	0.5352	0.8399
UGA-CPES	Messenger 1	40.0 a	1610 a	2.88 a	84.3 a	282.0 a	1.8 a	4.4 a
	Messenger 2	40.6 a	1676 a	2.83 a	83.9 a	289.5 a	1.6 a	4.6 a
	Messenger 3	38.3 a	1669 a	2.85 a	84.2 a	290.1 a	1.9 a	4.4 a
	Messenger 4	39.7 a	1624 a	2.89 a	84.6 a	293.2 a	1.5 a	4.5 a
	Messenger 5	41.0 a	1680 a	2.84 a	84.2 a	287.9 a	1.6 a	4.5 a
	Messenger 6	40.1 a	1651 a	2.87 a	84.3 a	289.3 a	1.5 a	4.6 a
	Messenger 7	40.5 a	1681 a	2.86 a	83.8 a	288.7 a	1.5 a	4.4 a
	Untreated	40.5 a	1639 a	2.83 a	84.4 a	288.5 a	1.7 a	4.6 a
	P > F	0.2093	0.9650	0.4796	0.4675	0.7360	0.3427	0.3778

[a]Means followed by the same letter within a location, column are not significantly different ($P > 0.05$).

Bacillus subtilis strain led to increased yield after infection with *Blumeria graminis* f.sp. *hordei*, which could not be accounted for by the reduction in disease severity alone (11). Thus, the possibility of cotton yield enhancement with harpin induced SAR appears to be scientifically based. In the current study however, Messenger treatments did not enhance cotton crop maturity, lint yield or fiber quality.

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