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Use of Glandless Breeding Stocks to Evaluate Unknown *Heliothis* Growth Inhibitors (X-Factors) in Cotton[1]

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ABSTRACT

A significant linear relationship (r = 0.86 to 0.90) was found between reduction in larval weight of tobacco budworm, *Heliothis virescens* (F.), and flowerbud gossypol content of 1) F_3 progenies of Upland stocks crossed with three Texas race stocks (Texas 216, Texas 490, and Texas 1134) of cotton, *Gossypium hirsutum* L., and 2) 221 entries from the race stock collection. Most of the suppression of growth of larvae which fed on diets containing extracts of progenies from the three race stocks could be attributed to gossypol or to other compounds that occur in pigment glands and can be measured by the colorimetric method for gossypol involving reaction with aniline. Subsequently, 317 glandless progenies from glandless strains crossed with derivatives of Texas 27, Texas 194 and Texas 254 were assayed for their effect on larval growth. None of the glandless selections tested caused a reduction in larval growth compared with insects feeding on a casein-wheat germ diet, which suggest that the X-factor effect results from one of the following: 1) gossypol; 2) synergist or additive effects of gossypol and other toxic compounds in flowerbuds; 3) gossypol-related compound that gives the same colorimetric test as gossypol but is more toxic than gossypol. Some of the glanded sister lines from the crosses suppressed larval weight by more than 50%, but generally this reduction was within the confidence limits of the reduction expected from gossypol content.

Additional index words: ***Gossypium hirsutum*** **L., Host plant resistance, Gossypol, Pigment glands, *Heliothis virescens* (F.).**

IT was recognized in the early 1960's that the tobacco budworm, *Heliothis virescens* (F.), was developing resistance to insecticides. A research propect was initiated in 1967 to discover new sources of resistance to these pests in *Gossypium*. Previously it had been demonstrated that cotton strains containing high levels of pigment glands (referred to as high gossypol strains) showed some resistance to the *Heliothis* complex compared to normally glanded cultivars (4, 5, 8, 9). Lines tested for new sources of resistance were taken from the regional *Gossypium hirsutum* L. primitive race collection (also referred to as the Texas race stock collection) maintained by USDA and the Texas Agric. Exp. Stn., College Station (16). These wild and feral cottons have been collected in Mexico, Central and South America, the Soviet Union, Africa, and numerous Atlantic, Caribbean, and Pacific islands (6).

Some of the lines tested appeared to exhibit more resistance than could be attributed to the gossypol content alone (10, 13), although gossypol concentrations in the flowerbud were greater than in standard lines used for comparison. Lines containing this unknown resistance factor have been referred to as X-factor lines by various authors (1, 2, 7, 11).

We crossed three of the X-factor lines (T-216, T-490, and T-1134) with a day-neutral glanded cotton and tested the correlation between gossypol content in the flowerbuds of F_3 progeny rows and suppression of larval growth. In addition, we initiated a study of three of the X-factor lines [Texas 27 (T-27), T-194, and T-254] in an attempt to: 1) transfer the unknown factor into a glandless background to eliminate any interference from gossypol in chemical and genetic studies of the material, 2) determine the relationship between X-factor and gossypol content in glanded sister lines; and 3) identify glandless lines that suppress larval development should the X-factor prove to be truly independent of gossypol content.

MATERIALS AND METHODS

Bud Gossypol vs. Larval Growth in F_3 Progeny of T-216 X 542, T-490 X 542, and T-1134 X 542. Three of the Texas race stocks that appeared to possess resistance in initial screening for new sources of resistance were crossed with 542 (not Texas 542), a day-neutral strain of *G. hirsutum* that had been used as one of the standards in earlier evaluation tests. The race stocks used were T-216, T-490, and T-1134. Because they are photoperiodic and require short days and cool nights to initiate fruiting, F_2 progenies of these crosses segregated for this character, and the individual F_2 populations were rogued for their ability to produce flowerbuds. Plants that had not produced flowerbuds approximately 100 days after planting were discarded. Seeds from day-neutral F_2 plants were harvested and planted in progeny rows. Flowerbuds from these F_3 progenies were collected on at least four separate dates (weekly intervals) and bioassayed similar to the method outlined by Shaver and Lukefahr (12). Flowerbud powders were extracted with diethyl ether in a Soxhlet extractor, and the extracts were coated on alphacel and incorporated into a casein-wheat germ diet (3) and the extract obtained from 5 g of lyophilized flowerbuds was blended into 50 ml of diet. All test diets were dispersed into 2-ml capacity polyethylene rearing cups (32 cups/test diet), and each cup was infested with either a newly hatched or a 3-day-old larva that had previously been fed on the casein-wheat germ diet containing no extract. All insects used in this study were taken from a laboratory colony of tobacco budworms maintained on a soybean-wheat germ diet (14) at Brownsville, TX. Controls used in this test were M-8 glanded and 542. Gossypol content of flowerbuds was determined by the method of Smith (15).

Entries from each of the three F_3 series were grouped in gossypol concentration ranges of 0.1% (from 0.40 to 0.49% to 1.00 to 1.09%) and larval weights and percentage gossypol were averaged for each group. Also, 221 entries of Texas race stocks that were assayed in a search for X-factor lines were handled in the same manner and bioassayed as before. A regression coefficient was calculated from a comparison of percent gossypol vs. larval weight. Larval weight and percent gossypol in each series were analyzed and the means were separated by Duncan's Multiple Range Test.

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Table 1. Tobacco budworm development on diets containing ether extracts of flowerbuds with various levels of gossypol.

Diet	Texas race stocks		Texas 490 F_3's		Texas 1134 F_3's		Texas 216 F_3's	
Texas lines (Gossypol)	Larvae weight	Gossypol	Larval weight	Gossypol	Larval weight	Gossypol	Larval weight	Gossypol
%	mg	%	mg	%	mg	%	mg	%
0.40–0.49	250 abc*	0.46	–	–	250 abc	0.46	–	–
0.50–0.59	238 bc	0.55	238 ab	0.56	238 bc	0.55	233 bc	0.57 e
0.60–0.69	212 cd	0.64	205 bc	0.65	208 cd	0.64	231 bc	0.65 de
0.70–0.79	174 de	0.75	162 ce	0.76	172 de	0.73	186 cd	0.75 cd
0.80–0.89	150 ef	0.84	140 de	0.85	136 ef	0.84	158 de	0.84 bc
0.90–0.99	117 f	0.94	108 e	0.94	114 f	0.90	118 ef	0.95 ab
1.00–1.09	101 f	1.05	114 e	1.02	–	–	94 f	1.07 a
	r = −0.90		r = −0.87		r = −0.86		r = −0.87	
Check								
M-8	276 ab	0.49	263 a	0.48	281 ab	0.48	281 ab	0.50 e
542	269 ab	0.55	268 a	0.53	271 ab	0.55	267 ab	0.56 e
Artificial diet	290 a		279 a		298 a		289 a	

* Numbers not followed by the same letter in a column are significantly different at the 0.05 level of probability by Duncan's Multiple Range Test.

Table 2. Comparison of effect of ether extracts of glanded and glandless cotton on larval weights of 3-day-old or neonate tobacco budworm larvae, 1973 test.

	Texas 27			Texas 254		
		Larval weight			Larval weight	
Diet	Gossypol	3 day	Neonate	Gossypol	3 day	Neonate
	%	mg		%	mg	
Glandless	0.05	228 a*	152 a	0.05	246 a	113 a
X-factor parent	0.69	102 b	14 c	0.77	36 c	5 c
M-8 glanded (control)	0.52	189 a	89 b	0.55	120 b	31 b
Regular diet	0.00	218 a	134 ab	0.00	253 a	110 a

* Numbers followed by the same letter in a column are not significantly different at the 0.05 probability level according to Duncan's Multiple Range Test.

Transfer of X-factor into a Glandless Background. T-27, T-194, and T-254 were crossed to glandless stocks. These three race stocks appear to possess an X-factor or an unknown chemical(s) in the flowerbud that retards development in *H. virescens* larvae. In March 1973, F_3 progeny rows of the M-8 glandless X T-27 crosses were planted at Brownsville, Tex. A total of 120 glandless and 140 glanded F_3 progeny rows were planted; however, sufficient flowerbuds were obtained from only 113 and 123 of the glandless and glanded progeny rows, respectively. Also, in August 1973, 100 glandless and 100 glanded F_3 progeny rows of a cross between T-254 and Watson OB-1 glandless were planted near Tuxpan, Veracruz, Mexico.

Flowerbuds collected from individual progeny rows were frozen on dry ice, debracted, and freeze-dried before being ground through a 50-mesh screen and stored in a laboratory freezer for subsequent analysis. Gossypol content was determined for each flowerbud sample by the method of Smith (15). At least four collections were made of all the lines (weekly intervals), and bioassays were made as previously described. The 3-day-old larvae were weighed after feeding on the test diets for 5 days, and the newly hatched (neonate) larvae were weighed after feeding for 7 days. Glanded controls used in this test were M-8 glanded and 'Stoneville 213'.

In March 1975, glanded and glandless progeny rows of upland stocks crossed with T-27, T-194, and T-254 were planted near Brownsville, Tex. T-194 and T-254 were initially hybridized with 542, and T-27 was hybridized with M-8 glanded by M. J. Lukefahr to eliminate the photoperiodic response of the three race stocks. Progenies from these crosses that possessed X-factor biological activity (10) were obtained from Lukefahr and hybridized with M-8 glandless. Flowerbuds from plants in the glanded and glandless progeny rows (F_3) of each race stock were harvested three times during the summer of 1975 and evaluated as described in the preceding tests.

Table 3. Comparison of effect of ether extracts of flower buds containing varying levels of gossypol on larval weight of tobacco budworm, 1973 test.

Diet	Texas 27			Texas 254		
	Larval weight			Larval weight		
Texas lines (Gossypol)	3 day	Neonate	Gossypol	3 day	Neonate	Gossypol
%	mg		%	mg		%
0.10–0.19	304 a*	236 a	0.16	–	–	–
0.20–0.29	307 a	252 a	0.24	223 a	96 a	0.21
0.30–0.39	295 ab	180 b	0.36	190 ab	68 b	0.34
0.40–0.49	250 bc	132 c	0.44	150 bc	44 c	0.45
0.50–0.59	216 c	75 d	0.54	130 c	32 d	0.54
0.60–0.69	158 d	34 e	0.64	93 d	13 e	0.65
0.70–0.79	166 d	49 de	0.72	80 de	8 ef	0.74
0.80–0.89	–	–	–	64 e	4 f	0.88
Checks						
X-factor parent	139 d	39 e	0.69	69 de	9 ef	0.77
St. 213	183 cd	41 e	0.69	82 de	8 ef	0.74
M-8 glanded	249 bc	115 c	0.55	151 bc	31 d	0.56
Artificial diet	316 a	259 a	–	228 a	103 a	–

* Numbers followed by the same letter in a column are not significantly different at the 0.05 probability level according to Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Glanded Day-neutral F_3 Progenies between 542 and T-216, T-490, and T-1134. The significant negative correlations between reduction in larval weight of tobacco budworm and flowerbud gossypol content of F_3 progenies of 542 crossed with the three Texas race stocks and the 221 entries from the race stock collection indicate that most of the larval growth suppression can be attributed to gossypol or other compounds measured by the colorimetric method (Table 1). In the T-216 F_3 series, 28.4% of the progeny produced larvae that weighed no more than 50% of those fed on control diets. The gossypol concentrations of these progenies were 0.77 to 1.09% (0.94% average). Also, 26.2 and 10.5% of the progenies of T-490 and T-1134, respectively, produced larvae that were at least 50% lighter than the controls. The gossypol concentration of T-490 F_3 progeny which produced 50% or greater reduction ranged from 0.77 to 1.04% (0.88% average); the range for T-1134 was 0.67 to 0.90% (0.79%

Table 4. Comparison of effect of ether extract of flowerbuds obtained from selections of crosses of 3 Texas race stocks × glandless on tobacco budworm larval weight, Brownsville, 1975.

Diet	Texas 27			Texas 194			Texas 254		
	Larval weight			Larval weight			Larval weight		
Texas lines (Gossypol)	3 day	1 day	Gossypol	3 day	1 day	Gossypol	3 day	1 day	Gossypol
%	mg		%	mg		%	mg		%
0.20–0.29	247	146	0.23	217	143	0.23	162	136	0.27
0.30–0.39	229	109	0.35	220	113	0.34	169	68	0.33
0.40–0.49	177	50	0.45	208	102	0.46	132	45	0.46
0.50–0.59	144	29	0.55	179	68	0.56	115	25	0.53
0.60–0.69	112	23	0.63	152	48	0.63	86	10	0.65
0.70–0.79	78	10	0.77	135	32	0.75	77	7	0.76
0.80–0.89	77	6	0.85	108	18	0.87	60	4	0.86
0.90–0.99	–	–	–	–	–	–	63	4	0.94
1.00–1.09	–	–	–	–	–	–	59	4	1.04
Glandless	247	129	–	234	153	–	202	131	–
X-factor parent	110	18	0.79	106	20	0.77	61	11	1.03

average). A 50% or greater reduction in larval weight compared with control lines and flowerbud gossypol content less than 1.0% are two criteria used for selecting lines with resistance or X-factor activity (12).

Glanded and Glandless Selections from Crosses between Derivatives of T-27, T-194, and T-254 X Glandless Strains. None of the 113 glandless selections of a cross between a T-27 derivative and Watson OB-1 glandless grown near Brownsville, Tex. in 1973 inhibited the growth of tobacco budworm larvae compared to larvae fed on a regular laboratory diet. In fact, larvae grown on diets containing extracts of these glandless progenies were slightly larger than those reared on regular diet, although this difference was not significant (Table 2). The X-factor parent used in the test caused a 46% reduction in larval weight compared with the glanded control. In tests comparing larval weights and gossypol content of the 123 glanded entries from the above cross, negative correlation coefficients of 0.75 and 0.86 were obtained using 3-day-old and neonate larvae, respectively (Table 3). Five of the glanded entries met the resistance criteria of having less than 1.0% gossypol and causing a 50% or more reduction in larval weight compared to a glanded check. Only one entry (0.61% gossypol) fell outside the 95% confidence limit established for this test by analysis of variance. However, three other entries (with 0.47, 0.55, and 0.59% gossypol) fell outside the confidence limit but reduced larval weight only 39, 43, and 47%, respectively.

None of the 96 glandless selections of the cross between a T-254 derivative and Watson OB-1 glandless grown near Tuxpan, Mexico in 1973 inhibited larval growth (Table 2), although the X-factor parent caused a 70% reduction in larval weight compared with the glanded check and an 86% reduction compared with the regular diet. Twenty-two of the glanded selections met the criteria of 1.0% gossypol and 50% or greater reduction in larval weight. Four of these selections fell outside the confidence limits calculated by comparing larval weight and gossypol content.

Results were essentially the same in 1975 (Table 4) as in 1973. A total of 40, 43, and 25 glandless selections were tested from T-27, T-194, and T-254, respectively. None produced larvae that were smaller than larvae fed on regular diet. Glanded selections of these crosses caused reductions in larval weight that were strongly correlated with gossypol content of flowerbuds (correlation coefficients of 0.76, 0.79, and 0.60 for T-27, T-254, and T-194 derivatives, respectively). Although 10 of the glanded T-27 progeny reduced larval weight by 50% or more, none fell outside the confidence limits set by a two-way analysis of variance comparing gossypol content with larval weight. Five of the T-194 glanded selections caused a 50% or more reduction in larval weight, but none of the five fell outside the confidence limits; however, three additional entries (with 0.15, 0.25, and 0.69% gossypol) did cause a greater reduction in larval weight than could be attributed to gossypol content alone, but the reduction in larval weight was less than 50%. Nineteen of the glanded selections of T-254 progeny reduced the larval weight more than 50%, but none reduced larval weight more than that attributed to gossypol content alone.

The fact that X-factor activity was not detected in any glandless progeny from T-27, T-194, and T-254 derivative strongly suggests that the X-factor effect in these lines results from one of the following: 1) gossypol; 2) synergistic or additive effects of gossypol and other toxic compounds found in cotton flowerbuds; 3) gossypol-related or possibly unrelated compounds found in cotton flowerbuds that either are not detected by the aniline colorimetric method for gossypol or give a significantly reduced analysis; 4) a gossypol-related compound that gives the same colorimetric test as gossypol but is significantly more toxic than gossypol.

Several gossypol-related compounds isolated from various cotton flowerbud samples have been implicated as having a role in X-factor activity (1). Results of the tests described herein tend to support that conclusion. However, none of these compounds are as toxic to *Heliothis* spp. as gossypol. Further work is needed to correlate their activity with molar response to the aniline test for gossypol and to determine possible additive and synergistic effects of combinations of these compounds with gossypol.

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