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Interference of Gossypol in Bioassay for Resistance to Tobacco Budworm in Cotton[1]

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ABSTRACT

The effect of gossypol on suppressing larval growth of tobacco budworms, *Heliothis virescens* (F.), was enhanced by diethyl ether extracts of flowerbuds of cotton (*Gossypium hirsutum* L.). This effect was greater with extracts of glanded than with glandless flowerbuds. The greater increase in larval growth suppression caused by these extracts is sufficient to interfere with the laboratory bioassay for detecting sources of antibiosis other than gossypol in cotton flowerbuds. Also, results of these laboratory tests suggest that a significant and useful larval growth suppression can be obtained with less than 1.2% gossypol in the flowerbud.

***Additional index words:* *Heliothis virescens* (F.), *Gossypium hirsutum* L.**

A research program was initiated in the early 1960's to search for sources of resistance in cotton (*Gossypium hirsutum* L.) to the bollworm, *Heliothis zea* (Boddie), and the tobacco budworm, *H. virescens* (F.). Glabrousness, nectarilessness, and increased flowerbud (square) gossypol content were among the first plant characters studied. The value of these individual characters and their combinations have been reported from laboratory, field-cage, and field tests (3, 4, 6). However, additional sources of resistance with different mechanisms of action would be desirable to supplement the action of known plant characters.

The bioassay technique (7) used to detect sources of resistance due to antibiosis in tobacco budworm larvae is based on comparing weights of larvae grown on diets containing extracts of lyophilized flowerbuds from experimental lines to weights of larvae grown on diets containing similar extracts from the check material, usually a commercial-type cotton. Gossypol is known to have a detrimental effect on larval growth and development in tobacco budworms (5); thus, the level of gossypol in the test material must always be considered in this type of bioassay.

In the bioassay technique described by Shaver and Lukefahr (7), lines causing at least a 50% reduction in larval weight compared to the check and containing less than 1.0% gossypol in the flowerbuds are subsequently bioassayed for larval response on fresh, detached squares. The upper limit of 1.0 gossypol in flowerbuds was chosen on the basis of laboratory tests using purified gossypol isolated from cottonseed glands. Also on the basis of laboratory tests with purified gossypol, Lukefahr and Houghtaling (2) stated that "it appeared that the amount of total gossypol in the cotton squares would have to exceed 1.2% (dry weight basis) to effectively inhibit the larval development of *Heliothis* spp."

The tests reported herein were undertaken to study the relationship between gossypol and larval weight when gossypol is added to the insect diet as purified gossypol or as extracts from flowerbuds containing this substance.

MATERIALS AND METHODS

All insects used in this study were taken from a laboratory colony of tobacco budworms maintained at Brownsville on a soybean-wheat germ diet developed by Shaver and Raulston (9).

Flowerbuds were collected from individual F_3 progeny rows that originated from a cross between an experimental high gossypol line ('Soccoro Island' background) and 'Stoneville 213'. The flowerbuds were frozen on dry ice, debracted, and freeze-dried before analysis for gossypol content by the colorimetric method of Smith (10). Selections were made from the F_3 progeny rows so that entries with gossypol concentrations of 0.6 to 1.7% in increments of 0.1% gossypol would be represented in the test. The entries with these varying levels of gossypol (hereafter referred to as VLG lines) were extracted with diethyl ether in a soxhlet extractor. The extracts were coated on Alphacel[3] and incorporated into a casein-wheat germ diet as described by Berger (1). The extract obtained from 5 g of lyophilized flowerbuds was blended into 50 ml (42.5 g) of diet.

Gossypol was added to flowerbuds of M-8 glanded (0.6% gossypol) and M-8 glandless (0.05% gossypol) to provide gossypol concentrations of 0.6 to 1.7% in 0.1% increments. Extracts were prepared and incorporated into the insect diet in the same manner as for the VLG lines. A fourth series of test diets was also prepared by incorporating purified gossypol directly into the diet in amounts equivalent to those contained in the 5-g flowerbud samples containing 0.6 to 1.7%. All four series of test diets were formulated to contain a final gossypol concentration ranging from 0.07 to 0.20% (weight/weight).

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 which had fed on the casein-wheat germ diet. The 3-day-old larvae that were placed on the test diet were weighed after having fed for 5 days. Newly hatched larvae that were placed on the test diet were weighed after 7 days. Each test (newly hatched and 3-day-old larvae) was replicated six times. The larval weights of the four treatments were statistically compared at each gossypol concentration by two-way analysis of variance.

RESULTS

Data from tests using 3-day-old larvae are presented in Table 1. Larval weights were depressed at all corresponding gossypol concentrations when ether extracts of VLG lines and M-8 glanded + gossypol were added to the diet at gossypol concentrations greater than 0.6%. These larvae weighed significantly less than those feeding on diets containing equivalent concentrations of gossypol added as gossypol: acetic acid except 0.9 and 1.5% for VLG lines and 0.9 and 1.7% for M-8 glanded + gossypol. Diets containing gland-

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[3] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.

Table 1. Comparative effect of gossypol on larval weight when 3-day-old tobacco budworm larvae fed for 5 days on diets containing purified gossypol or gossypol in extracts of flowerbuds (extract of 10 g flowerbed/100 ml diet).

Gossypol in flowerbud	Larval weight when gossypol added as: Gossypol: acetic acid†	M-8 glandless + gossypol‡	M-8 glanded + gossypol‡	Sib selections§ of HG × ST 213
%	mg			
0.6	297 a*	345 a	284 a	308 a
0.7	299 a	295 a	252 b	195 c
0.8	290 a	284 ab	239 bc	223 c
0.9	238 a	243 a	200 a	159 a
1.0	250 a	253 a	192 b	167 b
1.1	224 a	191 ab	173 b	157 b
1.3	198 a	155 b	137 bc	111 c
1.5	173 a	147 ab	106 b	145 ab
1.7	129 a	110 ab	103 ab	89 b
Mean	233 a	225 a	187 b	173 b

* Numbers followed by the same letter in horizontal line are not significantly different at the 0.05 probability level according to Duncan's new multiple range test. † The amount of gossypol added was equivalent to gossypol contained in flowerbud with the indicated concentration. ‡ Gossypol was added to M-8 glanded and M-8 glandless flowerbuds prior to extraction with diethyl ether. § Sib selections were made from F_3 progeny rows originating from a cross between an experimental high gossypol line (HG) and 'Stoneville 213'.

Table 2. Comparative effect of gossypol on larval weight when newly hatched tobacco budworm larvae fed for 7 days on diets containing purified gossypol or gossypol in extracts of flowerbuds (extract of 10 g flowerbud/100 ml diet).

Gossypol in flowerbud	Larval weight when gossypol added as: Gossypol: acetic acid†	M-8 glandless + gossypol‡	M-8 glanded + gossypol‡	Sib selections§ of HG × ST 213
%	mg			
0.6	215 a*	183 a	51 b	39 b
0.7	187 a	122 b	27 c	19 c
0.8	145 a	34 b	10 b	10 b
0.9	148 a	5 b	5 b	3 b
1.0	131 a	6 b	5 b	3 b
1.1	112 a	4 b	3 b	2 b
1.3	60 a	2 b	2 b	3 b
1.5	63 a	1 b	2 b	2 b
1.7	37 a	2 b	1 b	1 b

* Numbers followed by the same letter in the horizontal lines are not significantly different at the 0.05 probability level according to Duncan's new multiple range test. † The amount of gossypol added is equivalent to gossypol contained in flowerbud with the indicated concentration. ‡ Gossypol was added to M-8 glanded and M-8 glandless flowerbuds prior to extraction with diethyl ether. § Sib selections were made from F_3 progeny rows originating from a cross between an experimental high gossypol line (HG) and 'Stoneville 213'.

less + gossypol extracts produced smaller larvae than gossypol alone at all concentrations above 1.0%, but the difference was significant only with 1.3% gossypol. Mortality was less than 5% on all diets containing gossypol (data not shown), and was approximately the same as the mortality in larvae reared on the casein-wheat germ diet containing no gossypol.

A two-day analysis of variance was calculated for the combined data from the nine gossypol concentrations of the four treatments using 3-day-old larvae, and the means were ranked for significance. The mean larval weights were equal in larvae feeding on diets containing extracts of M-8 glanded (187 mg) and VLG lines (173 mg), but both were significantly less than those feeding on diets containing purified gossypol (233 mg) and M-8 glandless + gossypol (225 mg).

Larval growth was reduced significantly in the newly hatched larvae reared on diets containing extracts from VLG lines and M-8 glanded + gossypol, even at concentrations of 0.6% gossypol (Table 2). Larvae that on these two diets at all gossypol concentrations were ca. one-quarter or less the weight of larvae that fed on diets containing gossypol alone. Also, larvae reared on diets containing extracts of VLG lines and M-8 glanded + gossypol were ca. one-quarter the weights of larvae reared on diets containing extracts of M-8 glandless + gossypol at 0.6, 0.7, and 0.8% gossypol. However, from 0.9 to 1.7% gossypol, the weights of larvae reared on these three diets were equal. The maximum effect of these three diets on larval weight, when infested with newly hatched larvae, was obtained at 0.9 to 1.0% gossypol. Essentially no growth was obtained over the 7-day feeding period at these and higher gossypol concentrations.

There was no difference in mortality rate (data not shown) of newly hatched larvae in any of the test diets and in the casein-wheat germ diet containing no gossypol.

DISCUSSION

These results demonstrate that there are compounds in ether extracts of cotton flowerbuds that enhance or supplement the action of gossypol in inhibiting larval growth in tobacco budworms. This effect is greater in extracts of glanded flowerbuds but significant in extracts of glandless lines to which equivalent quantities of gossypol have been added. Apparently, the ether soluble factors are present in the flowerbuds in insufficient quantities to affect larval weight in the absence of gossypol. An ether extract of the glandless material with no gossypol added had no effect on neonate or 3-day-old larvae, and an extract of the M-8 glanded alone had no effect on 3-day-old larvae.

Therefore, much of the weight differences in larvae fed on diets from the commercial-type line (check) and on those from the test lines could be due to increased amounts of gossypol in the latter, rather than to other compounds. For example, present cultivars contain ca. 0.6% gossypol in the flowerbuds. The response of 3-day-old larvae to gossypol incorporated in an extract of flowerbuds into the insect diet as shown in Table 1 suggests that if the flowerbud gossypol content could be increased to 1.0%, the larval weight as obtained in the bioassay technique would be decreased by 50%, thereby giving a significant and useful larval growth suppression with less than 1.2% gossypol concentration. The data in Table 2 suggest that a 50% reduction in larval weight can be obtained by increasing gossypol content from 0.6 to 0.8% when newly hatched larvae are tested.

The increased sensitivity of the neonate larvae to gossypol is similar to that reported by Shaver and Parrott (8) for toxicity of the gossypol to bollworms, tobacco budworms, and pink bollworms, *Pectinophora gossypiella* (Saunders), but the effect is much more pronounced with extracts of flowerbuds containing gossypol and other pigment gland constituents.

REFERENCES

1. Berger, R. S. 1963. Laboratory techniques for rearing *Heliothis* species on artificial medium. USDA, ARS-33-84. 4 p.
2. Lukefahr, M. J., and J. E. Houghtaling. 1969. Resistance of cotton strains with high gossypol content to *Heliothis* spp. J. Econ. Entomol. 62:588-591.
3. ———, ———, and D. G. Cruhm. 1975. Suppression of *Heliothis* spp. with cottons containing combinations of resistant characters. J. Econ. Entomol. 68:743-746.
4. ———, ———, and H. M. Graham. 1971. Suppression of *Heliothis* populations with glabrous cotton strains. J. Econ. Entomol. 64:486-488.
5. ———, and D. F. Martin. 1966. Cotton-plant pigments as a source of resistance to the bollworm and tobacco budworm. J. Econ. Entomol. 59:176-179.
6. ———, ———, and J. R. Meyer. 1965. Plant resistance to five Lepidoptera attacking cotton. J. Econ. Entomol. 58: 516-518.
7. Shaver, T. N., and M. J. Lukefahr. 1971. A bioassay technique for detecting resistance of cotton strains to tobacco budworms. J. Econ. Entomol. 64:1274-1277.
8. ———, and W. L. Parrott. 1970. Relationship of larval age to toxicity of gossypol to bollworms, tobacco budworms, and pink bollworms. J. Econ. Entomol. 63:1802-1804.
9. ———, and J. R. Raulston. 1971. A soybean-wheat germ diet for rearing the tobacco budworm. Ann. Entomol. Soc. Am. 64:1077-1079.
10. Smith, F. H. 1967. Determination of gossypol in leaves and flowerbuds of gossypium. J. Am. Oil Chem. Soc. 44:267-269.