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Genetic Relationship Between Tobacco Budworm Feeding Response and Gland Number in Cotton Seedlings[1]

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ABSTRACT

Tobacco budworms, *Heliothis virescens* (F.), discriminated among seedlings of Upland cotton (*Gossypium hirsutum* L.) possessing the nine possible genotypes involving the gland-determining alleles Gl_2, gl_2, Gl_3^{rai}, and gl_3. The insect larvae preferred glandless seedlings ($gl_2gl_2gl_3gl_3$), then showed a decreasing amount of preference as the number of Gl_2 and Gl_3^{rai} alleles increased. Seedling damage, number of larvae left on the seedlings, number of pigment glands on the cotyledonary petiole, and percent seed-gossypol were correlated *inter se*. A diallel analysis showed that additive effects accounted for 84 to 97% of the total genetic variances for seedling damage, number of larvae per seedling, and gland number. Dominance and epistatic effects were generally small, but sometimes statistically significant. The analysis showed also that Gl_2 usually contributed more to the total additive genetic variance than did Gl_3^{rai}, an expected result because the former allele has a more profound effect on gland density and thus on gossypol content.

***Additional index words:* *Gossypium hirsutum* L., *Heliothis virescens* (F.), Seed-gossypol content, Diallel analysis, Larval non-preference.**

THIS paper discusses action of the gland-determining alleles Gl_2 and Gl_3^{rai} in seedlings of cotton, *Gossypium hirsutum* L., in relation to the feeding response of the tobacco budworm, *Heliothis virescens* (F.).

Cotton possesses minute pigment glands throughout the plant body. A major constituent of these glands, at least in the parts of the plant that have been studied, is gossypol (8,8′-dicarboxaldehyde-1,1′, 6,6′,7,7′-hexahydroxy-5,5′-diisopropyl-3,3′-dimethyl-2, 2′-binapthalene). Gossypol is toxic to the tobacco budworm when added to an artificial diet (Shaver and Lukefahr, 1969). Cotton lines possessing high levels of gossypol in the flower buds significantly reduce larval growth (Lukefahr and Houghtaling, 1969).

Plant breeders are presently investigating the possibility of transferring high levels of gossypol into agronomically acceptable cottons to protect the plants from tobacco budworm and the closely related species, cotton bollworm, *Heliothis zea* (Boddie).

Although tobacco budworms do not normally attack cotton seedlings, Wilson (1971) showed that they discriminated among the four true-breeding phenotypes of cotton seedlings carrying Gl_2 and Gl_3 ($Gl_2Gl_2Gl_3Gl_3$: fully glanded; $Gl_2Gl_2gl_3gl_3$ and $gl_2gl_2Gl_3Gl_3$: reduced glandedness; $gl_2gl_2gl_3gl_3$: glandless). The larval reaction was correlated with the number of pigment glands in the cotyledonary petioles of the seedlings and with gossypol content of the seed.

The discriminating power of the larvae suggested the possibility of a more precise analysis of the interrelationships of gland density and gossypol content in the plant with the feeding response of the insect.

MATERIALS AND METHODS

Lee (1962) and Lee, Cockerham, and Smith (1968) investigated the inheritance of glandulosity and of gossypol level in cotton. Lee made available seeds of the four parents and of all F_1 hybrid combinations from 4 × 4 diallel crosses made in the field in North Carolina, 1969. He also analyzed seed meats for gossypol content according to the methods of Smith (1958). These stocks carried the native Gl_2 allele, located in the A subgenome, and Gl_3^{rai}, transferred from *Gossypium raimondii* Ulbr., and located in the D subgenome of the amphidiploid *G. hirsutum*. Since an earlier study (Lee et al., 1968) had shown that reciprocal and maternal effects were not significant, we decided to test only the four parents (coded A, B, C, D; Table 1) and the five possible F_1 genotypes (coded AB, AC, etc., in Table 1; the AD double-heterozygote was included and BC was excluded).

Wilson planted seeds of the nine genotypes in paper cups in a greenhouse at College Station, Texas on February 20, 1970. When seedlings were two weeks old (March 6, 1970), he counted the number of pigment glands in the cotyledonary petioles in 10 seedlings of each genotype. The cups, each containing a single seedling, were then plunged in agricultural perlite in 25.4-cm plastic pots. Each of the 10 replications included nine pots; each pot contained four seedlings of a single genotype.

M. J. Lukefahr, Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture, Brownsville, Texas, supplied tobacco budworm eggs and media cups containing a standard wheatgerm diet. The eggs hatched March 19, 1970 and the larvae were transferred to the diet. Four days later, March 23, 1970, Wilson infested each seedling with two larvae. On March 24, 25, and 30, he counted the number of larvae left on the seedlings, and on the two latter dates made estimates of insect damage to the first and second true leaves of the seedlings.

Damage-estimate values were analyzed directly, but larval and gland counts were transformed ($\sqrt{\times + 0.5}$) because zero values appeared in both instances. We analyzed the data from each reading separately and then compared means using Duncan's New Multiple Range Test.

We also used the modification of Hayman's (1954) diallel analysis proposed by Lee et al. (1968). In Hayman's model, the n^2-1 degrees of freedom for entries (genotypes) in the complete diallel table are subdivided into general, specific, maternal and reciprocal effects. Lee et al. applied this analysis to a two-gene model and further subdivided the general and specific effects into various additive, dominance, and epistatic components. Methods of estimation are reported in detail in the two papers cited and will not be repeated here.

Since the program available to us called for a complete diallel set, we employed it by (a) entering our data (genotype within

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replication means) in both halves of the 4 × 4 table, and (b) considering the effects of the double heterozygote comparable whether it resulted from A × D or B × C. To estimate additivity, dominance, and epistasis, we subdivided the 89 total degrees of freedom as follows:

Source	*D. F.*
Replications	9
Genotypes	8
Additive Gl_2	1
Additive Gl_3^{rai}	1
Additive × Additive	1
Dominance Gl_2	1
Dominance Gl_3^{rai}	1
Epistasis	3
Error	72

RESULTS

Table 1 shows mean pigment gland number in the cotyledonary petiole and percent seed gossypol in the nine genotypes studied. The high correlation (r = .975) between gland number and gossypol content is interpreted as the result of the dependence of the latter upon the former (Wilson, 1971). Both gland number and percent gossypol were distributed into five groups which corresponded with the number of Gl_2 and Gl_3^{rai} alleles present (4, 3, 2, 1, or 0). However, results suggest that Gl_2 affected gland number and gossypol content more profoundly than did Gl_3^{rai}. Results also showed that the addition of only one Gl_2 or Gl_3^{rai} allele to the glandless base raised gland number and gossypol level minimally.

Table 2 presents means for seedling damage caused by tobacco budworm to the first and second true leaves, and for number of larvae per seedling on March 25, 1970 (48 hours after infestation) and March 30, 1970 (168 hours after infestation). Larval counts made 24 hours after infestation are not shown in Table 2 because differences between genotypes were small and not statistically significant.

Significant differences between genotypes were indicated for every characteristic except first leaf damage at 48 hours. In general, plant damage increased, and more larvae remained on the seedlings as the number of gl_2 and gl_3 alleles increased. Results suggested that (a) larvae preferred the second leaf over the first leaf; (b) a better evaluation of larval preference was obtained at the second reading than at the first.

Mean squares from the diallel analysis are presented in Table 3 for first and second-leaf damage, for number of larvae per seedling, and the number of glands on the cotyledonary petiole. Additive effects are much larger than dominance or epistatic effects, although the latter two sources of variation sometimes show

Table 1. Pigment gland numbers and percent seed gossypol in four true-breeding lines (A, B, C, D) and their F_1 hybrids.

Genotype	Code	Mean number of glands in cotyledonary petiole, 10 seedlings	% seed gossypol
$Gl_2\ Gl_2\ Gl_3^{rai}Gl_3^{rai}$	A	117.9 a*	1.475
$Gl_2\ Gl_2\ Gl_3^{rai}gl_3$	AB	93.8 b	1.351
$Gl_2\ gl_2\ Gl_3^{rai}Gl_3^{rai}$	AC	83.6 c	1.268
$Gl_2\ Gl_2\ gl_3gl_3$	B	56.8 d	.885
$Gl_2\ gl_2\ Gl_3^{rai}gl_3$	AD	48.6 e	.864
$gl_2\ gl_2\ Gl_3^{rai}Gl_3^{rai}$	C	33.9 f	.783
$Gl_2\ gl_2\ gl_3gl_3$	BD	2.8 g	.087
$gl_2\ gl_2\ Gl_3^{rai}gl_3$	CD	1.5 g	.083
$gl_2\ gl_2\ gl_3gl_3$	D	0.0 h	.015

* Means with letters in common are not significantly different at the 5% level, according to Duncan's New Multiple Range Test.

Table 2. Leaf damage ratings, and number of tobacco budworm larvae on cotton seedlings 48 hours and 168 hours after infestation (two larvae put on each seedling March 23, 1970).

		Leaf damage rating, 1=none; 4=severe				Number of larvae/seedling	
		First leaf		Second leaf			
Genotype	Code	Mar. 25	Mar. 30	Mar. 25	Mar. 30	Mar. 25	Mar. 30
$Gl_2\ Gl_2\ Gl_3^{rai}Gl_3^{rai}$	A	1.75 a*	2.32 a	1.77 a	2.22 a	.60 a	.27 a
$Gl_2\ Gl_2\ Gl_3^{rai}gl_3$	AB	1.65 a	2.35 a	1.87 a	2.42 a	.87 abc	.32 a
$Gl_2\ gl_2\ Gl_3^{rai}Gl_3^{rai}$	AC	1.63 a	2.32 a	1.95 ab	2.70 b	1.05 bcd	.52 ab
$Gl_2\ Gl_2\ gl_3gl_3$	B	1.65 a	2.85 b	2.25 c	3.12 c	.80 ab	.75 bc
$Gl_2\ gl_2\ Gl_3^{rai}gl_3$	AD	1.73 a	2.70 ab	2.20 bc	3.12 c	1.02 bcd	.76 bcd
$gl_2\ gl_2\ Gl_3^{rai}Gl_3^{rai}$	C	1.73 a	2.50 ab	2.32 c	3.22 c	1.10 bcd	.80 bcd
$Gl_2\ gl_2\ gl_3gl_3$	BD	1.65 a	3.35 c	2.37 cd	3.82 d	1.24 cd	.92 cd
$gl_2\ gl_2\ Gl_3^{rai}gl_3$	CD	1.75 a	3.30 c	2.65 d.	3.80 d	1.35 d	.97 cd
$gl_2\ gl_2\ gl_3gl_3$	D	1.78 a	3.60 c	2.32 c	3.92 d	1.18 bcd	1.07 d

* Means with letters in common not significantly different at the 5% level, according to Duncan's New Multiple Range Test.

Table 3. Mean squares from diallel analyses of leaf damage ratings, number of larvae per seedling, and gland number.

		Leaf damage			No. of larvae per seedling		
		First leaf	Second leaf				
Source	df	3/30	3/25	3/30	3/25	3/30	Number†
Genotypes	8	3.699**	1.271**	5.848**	.126**	.253**	237.43**
Add. Gl_2	1	9.975**	5.913**	25.880**	.666**	1.220**	1,059.35**
Add. Gl_3^{rai}	1	16.882**	2.194**	18.528**	.087*	.640**	688.55**
Add. × Add.	1	.413	.282	.080	.006	.011	6.02**
Dom. Gl_2	1	.112	.047	.225*	.039	.056	3.82*
Dom. Gl_3^{rai}	1	.172	.172	.225*	.020	.006	8.01**
Epistasis	3	.609	.540*	.604**	.058	.024	42.54**
Error	72	.283	.181	.055	.040	.031	.56

* P < .05. ** P < .01. † Number glands, cotyledonary petiole.

statistical significance. Gl_2 contributed more than Gl_3^{rai} to the additive genetic variance, except in first-leaf damage.

Table 4 shows a subdivision of the genetic variances for plant damage, number of larvae, and gland number into additive, dominance and epistatic components. The additive variance is consistently high; in fact, it was above 95% at the second reading for plant damage and number of larvae. The dominance variance is less than 2% of the total in every instance. While the epistatic variance is usually higher than the dominance variance, it is always a minor source of variation when compared with the additive effects.

DISCUSSION

Gland number, gossypol content, number of larvae remaining on the seedling, and leaf damage ratings were correlated *inter se*. Examination of gland number and percent seed gossypol reveals an interesting type of gene action for both Gl_2 and Gl_3^{rai}. For example, addition of a single Gl_2 to the $gl_2gl_2gl_3gl_3$ (glandless) base raises mean gland number from 0.0 to only 2.8. However, addition of a second Gl_2 raises mean gland number to 56.8. On the other hand, addition of one or two Gl_2 or Gl_3^{rai} alleles to a genotype already possessing at least one of these alleles increases gland number and gossypol level by regular increments. The observed phenomenon is interpreted as a threshold effect, where a gland-determining allele occurring alone cannot fully express its potential, but when another allele is added the two interact to enhance both gland number and gossypol materially. This same type of gene action is also indicated in the seed-gossypol data presented by Lee et al. (1968) for the native alleles Gl_2 and Gl_3 in 'Coker' and 'Empire' varietal backgrounds.

Table 4. Subdivision of the genetic variances into additive, dominance, and epistatic components; first- and second-leaf damage, number of larvae per plant, and number of glands on two dates in March 1970.

	First leaf damage		Second leaf damage				Number of larvae					
	March 30		March 25		March 30		March 25		March 30		Number of glands	
Source	Var.	%	Var.	%	Var.	%	Var.	%	Var.	%	Var.	%
Additive	.1648	96.0	.0486	88.5	.2761	95.7	.0044	84.6	.0113	97.3	10.914	92.7
Dominance	-.0001	0.0	.0001	1.8	.0024	.8	.0001	1.9	.0002	1.8	.068	.6
Epistatic	.0070	4.0	.0062	9.7	.0100	3.5	.0007	13.5	.0001	.9	.793	6.7
Total	.1717	100.0	.0549	100.0	.2885	100.0	.0052	100.0	.0116	100.0	11.775	100.0

The larvae showed the greatest preference for glandless ($gl_2gl_2gl_3gl_3$) seedlings, but a decreasing amount of preference as the number of gland-determining alleles increased. Because of the known sensitivity of tobacco budworms to gossypol (Shaver and Lukefahr, 1969), it is tempting to attribute the progressive decrease in preference of the larvae to a progressive increase in gossypol level in the seedling tissues. We cannot demonstrate that the larvae responded directly to gossypol content of the seedlings, but we can say that they responded indirectly to the gossypol level of the seeds having the same genotypes as those seedlings.

Number of larvae remaining on the plants and damage inflicted by the larvae show largely additive effects (dominance and epistasis never accounted for more than 15.4% of the total variance), and are negatively correlated with gland density in the seedlings. The high heritability of gossypol level suggests that breeders can proceed with confidence in their efforts to select high gossypol lines. However, we should emphasize that (a) no one as yet has studied the inheritance of gossypol level in the flower parts; (b) the levels of gossypol found in the lines that we studied were below the range believed to be effective for practical resistance to the bollworm (Lukefahr and Houghtaling, 1969).

Information is needed in the following areas: (a) the reactions of insect larvae to seedlings carrying higher levels of gossypol than heretofore studied; (b) the nature of the inheritance of gossypol at those higher levels; (c) the relationship between seed gossypol and flower-bud gossypol; (d) the relationship between gland size, gland number, and gossypol level in various parts of the plant; and (e) the relationship of insect damage to seedling leaves to that of flowering parts. Such information may enable us to decide whether seedling screening will be of value in selecting for insect resistance. But perhaps more important, it may help us to decide whether visual selection will be effective in isolating plants with high gossypol levels, thus reducing the need for extensive insect bioassays or chemical tests.

The relationship between seed and floral gossypol content is particularly important. Plant breeders would like to be able to manipulate levels of seed and floral gossypol separately to produce combinations of value both to seed processors (low gossypol level in cottonseed) and to those interested in protecting the plant against insect attack (high gossypol level in floral parts). Since the major gland-determining genes Gl_2 and Gl_3 affect gossypol content in both parts of the plant, there seems to be a practical limit to the amount of divergence that one might expect. However, we must investigate thoroughly the possibility of the presence of independent factors and their effects before making a decision about the utility of this breeding objective.

Our method of counting glands on the cotyledonary petiole is unfortunately not precise enough to separate seedling phenotypes completely. However, it should be precise enough to allow one to make a preliminary separation in a segregating population by counting glands and relating these counts to gland numbers in the parental lines. We have not investigated the effects of other genetic backgrounds on gland number; modifiers (Lee, 1962) could materially alter results. Seedling development also affects gland counts. We have observed that gland number increases until seedlings are 7 to 10 days old, after which it remains constant.

Gland numbers in the cotyledonary petioles may be helpful in discriminating completely glandless plants. McMichael (1969) emphasized that the genotypes $Gl_2gl_2gl_3gl_3$ and $gl_2gl_2Gl_3gl_3$ are hard to detect because their hypocotyls are glandless and they have only a few light glands at the base or apex of the cotyledons. All of the seedlings of the genotypes that we examined had at least one gland in at least one of the two cotyledonary petioles, and most seedlings had several glands per petiole. Seedlings thus marked can be discarded in the search for glandless cotton.

LITERATURE CITED

Hayman, B. I. 1954. The analysis of variance of diallel tables. Biometrics 10:235-244.

Lee, J. A. 1962. Genetical studies concerning the distribution of pigment glands in the cotyledons and leaves of Upland Cotton. Genetics 47:131-142.

————, C. C. Cockerham, and F. H. Smith. 1968. The inheritance of gossypol level in *Gossypium*. I. Additive, dominance, epistatic, and maternal effects associated with seed gossypol in two varieties of *Gossypium hirsutum* L. Genetics 59:285-298.

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.

McMichael, S. C. 1969. Selection for glandless seeded cotton plants. Crop Sci. 9:518-520.

Shaver, T. N. and M.J. Lukefahr. 1969. Effect of flavonoid pigments and gossypol on growth and development of the bollworm, tobacco budworm, and pink bollworm. J. Econ. Entomol. 62:643-646.

Smith, F. H. 1958. Spectrophotometric determination of total gossypol in cottonseed meal and cottonseed meats. J. Am. Oil Chem. Soc. 35:261-265.

Wilson, F. D. 1971. Reactions of tobacco budworm larvae to cotton seedlings carrying various combinations of gland-determining alleles. Crop Sci. (in press)