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# Response of Tobacco Budworm Larvae to Cotton Seedlings Carrying Various Combinations of Gland-determining Alleles[1]

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## ABSTRACT

**Five- and 6-day-old larvae of the tobacco budworm, *Heliothis virescens* (F.), discriminated among seedlings of cotton, *Gossypium hirsutum* L., carrying the 4 true-breeding combinations of the gland-determining alleles $Gl_2$ and $Gl_3$. Seedling damage was least, and larval numbers were lowest on dimeric glanded, $Gl_2Gl_2Gl_3Gl_3$, intermediate on the 2 monomerics, $Gl_2Gl_2gl_3gl_3$ and $gl_2gl_2Gl_3Gl_3$, and highest on glandless, $gl_2gl_2gl_3gl_3$, when readings were made 24 and 48 hours after infestation. However, seedling damage was less at the second reading and numbers of larvae left on the seedlings were less at both readings in $Gl_2Gl_2gl_3gl_3$ than in the other monomeric line. Larval response was correlated both with the number of pigment glands in the cotyledonary petioles of the seedling, and with percent gossypol in seed meats of the genotypes studied. Results suggest that this bioassay technique may be useful in selecting seedlings with varying levels of gossypol. In fact, it may be possible to select seedlings by counting glands, without resorting to a bioassay. However, the effects of varied genetic backgrounds on gland number have not been studied. Also, the relationship of seedling resistance to mature-plant resistance is not known. Several applications of this bioassay technique are discussed in relation to a number of other breeding, entomological, and genetic problems.**


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

THIS paper reports the differential reactions of larvae of the tobacco budworm, *Heliothis virescens* (F), to seedlings of cotton, *Gossypium hirsutum* L., carrying the four true-breeding combinations of the gland-determining genes $Gl_2$ and $Gl_3$. It also discusses infestation techniques and the potential applications and importance of this type of bioassay in genetic and plant breeding investigations.

The genetic mechanism in the New World cultivated cottons that determines the inheritance patterns of the pigment, or gossypol, glands is one of several duplicate-factor combinations that have been identified in these amphidiploid species. Cotton plants have glands (glands appear as small, black dots to the unaided eye) if one or more dominant alleles are present at the $Gl_2$ and $Gl_3$ loci. Plants are completely glandless if both of these loci bear only recessive alleles (McMichael, 1960, 1969). Most presently grown commercial varieties are conspicuously glanded and are of the genotype $Gl_2Gl_2Gl_3Gl_3$.

The major constituent of the pigment glands is gossypol (8, 8′ - dicarboxaldehyde - 1, 1′, 6, 6′, 7, 7′ - hexahydroxy - 5, 5′ - diisopropyl - 3, 3′ dimethyl - 2, 2′ - binapthalene). This substance is toxic to certain Lepidopterous insects (Bottger et al., 1964; Lukefahr and Margin, 1966; Shaver and Lukefahr, 1969), and to poultry and some mammals (Eagle, 1960). Bell (1967) suggested that gossypol is also involved in resistance of cotton to diseases caused by fungi.

Cotton workers are attempting at present to combine the high-gossypol level from a wild cotton (Fryxell and Moran, 1963; Lukefahr and Houghtaling, 1969) with the acceptable agronomic properties of varieties of Upland cotton, *G. hirsutum*. A large part of this effort involves the identification of lines carrying levels of gossypol high enough to be effective against the target insects. The two approaches to this problem have been *(1)* visual inspection of gland density on the plant and *(2)* chemical analyses of flower bud tissue (the part of the plant attacked by early instars of *Heliothis* spp.) to determine gossypol levels. The first technique, though useful, has proved to be relatively inefficient, while the second technique is precise but expensive. A seedling bioassay technique, if effective, could circumvent the disadvantages of both of these techniques. However, a seedling technique will be effective only *(1)* if the larvae discriminate between plants varying in gossypol level and *(2)* if seedling resistance is highly correlated with mature-plant resistance. The present paper explores the first facet of this problem.

Along with practical plant-breeding considerations is the intrinsic value of insect-cotton seedling interrelationships in genetic studies. Some possible genetic applications of this technique are discussed in this paper.

## MATERIALS AND METHODS

J. A. Lee, USDA, North Carolina State University, Raleigh, provided seeds of the four true-breeding, gland-determining genotypes (Lee, 1962) in the background of the variety 'Empire,' as follows: $Gl_2Gl_2Gl_3Gl_3$ (dimeric), $Gl_2Gl_2gl_3gl_3$ (monomeric-2), $gl_2gl_2Gl_3Gl_3$ (monomeric-3), and $gl_2gl_2gl_3gl_3$ (glandless). Empire cotton was of particular value because its seed gossypol levels had been calculated previously (Lee et al., 1968). Seedlings of the 4 entries were grown to the 2-leaf stage in 170-g (6-ounce) paper cups. The cups were then arranged randomly in nine flats (two seedlings per genotype per flat) and placed in a small, portable greenhouse at College Station, Texas. M. J. Lukefahr, USDA, Entomology Research Division, Brownsville, Texas, supplied tobacco budworm eggs and media cups containing a wheatgerm diet. The eggs hatched on June 19, 1969, and the larvae were transferred to the diet. On June 23, 1969, two larvae were transferred from the artificial diet to each seedling. The larvae were not confined or restricted in any way after they were placed on the seedlings.

Twenty-four hours and 48 hours after the plants were infested, the seedlings were rated visually for damage to cotyledons, first true leaf, and second true leaf, and the number of larvae left on each plant was counted. One week later, the seedlings were rated for damage to the growing point.

The pigment glands were counted in the cotyledonary petioles of each seedling. These counts were compared with the insect response and with previously published data on seed-gossypol percentages (Lee et al., 1968).

Damage estimates were analyzed directly, but larval counts were transformed ($\sqrt{x + 0.5}$) before analysis. Single-degree-of-freedom comparisons were made to determine individual gene effects and interactions, as follows:

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**Table 1. Cotyledon, leaf and growing point damage to cotton seedlings infested with tobacco budworm larvae, and numbers of larvae left on the seedlings 24 and 48 hours after infestation (damage rating, cotyledons and leaves: 1 = undamaged, to 4 = severely damaged; growing point: 1 = undamaged, 2 = damaged, 3 = dead).**

| Genotype | | Cotyledon damage | | First leaf damage | | Second leaf damage | | No. larvae per plant | | Growing point damage, one week |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 24 hrs | 48 hrs | 24 hrs | 48 hrs | 24 hrs | 48 hrs | 24 hrs | 48 hrs | |
| $Gl_2\ Gl_2\ Gl_3\ Gl_3$ | | 1.44 | 2.00 | 2.05 | 2.59 | 2.38 | 2.66 | .72 | .11 | 1.72 |
| $Gl_2\ Gl_2\ gl_3\ gl_3$ | | 1.94 | 2.33 | 2.50 | 2.72 | 2.66 | 2.94 | .83 | .33 | 2.05 |
| $gl_2\ gl_2\ Gl_3\ Gl_3$ | | 1.83 | 2.88 | 2.50 | 2.77 | 2.72 | 3.47 | 1.16 | .44 | 2.00 |
| $gl_2\ gl_2\ gl_3\ gl_3$ | | 2.05 | 2.94 | 2.94 | 3.22 | 3.61 | 3.88 | 2.05 | 1.00 | 2.11 |
| Analysis of variance: mean squares | | | | | | | | | | |
| Source | DF | | | | | | | | | |
| Replicates | 8 | 1.07 | 1.18 | 1.18 | .73 | 1.67 | 1.42 | 1.44 | 1.23 | 1.42 |
| Genotypes | 3 | 2.54* | 7.43** | 4.74** | 2.32** | 10.10** | 11.21** | 13.14** | 5.14* | 1.07 |
| $Gl_2$ vs. $gl_2$ | 1 | 2.25 | 20.25** | 7.11** | 3.36** | 14.40** | 26.69** | 25.00** | 9.00* | 1.00 |
| $Gl_3$ vs. $gl_3$ | 1 | 4.69* | 1.36 | 7.11** | 2.25* | 12.00** | 4.69* | 9.00 | 5.44 | 1.77 |
| $(Gl_{2,3} + gl_{2,3})$ vs. $(Gl_2gl_3 + gl_2Gl_3)$ | 1 | .69 | .69 | 0.00 | 1.36 | 3.29 | 2.25 | 5.44 | 1.00 | .44 |
| Error | 24 | .67 | 1.53 | .51 | .36 | 1.02 | 1.02 | 2.31 | 1.18 | .88 |
| Percentage of total genotypic variance attributable to: | | | | | | | | | | |
| $Gl_2$ vs. $gl_2$ | | 29.5 | 90.8 | 50.0 | 48.2 | 48.4 | 79.4 | 63.4 | 58.3 | |
| $Gl_3$ vs. $gl_3$ | | 61.5 | 6.1 | 50.0 | 32.3 | 40.4 | 13.6 | 22.8 | 35.2 | |

* $P < .05$. ** $P < .01$.

(a) $\sigma^2_{(Gl_2 \text{ vs. } gl_2)} =$

$$\left[\frac{\Sigma(Gl_2\ Gl_2\ Gl_3\ Gl_3 + Gl_2\ Gl_2\ gl_3\ gl_3) - (gl_2\ gl_2\ Gl_3\ Gl_3 + gl_2\ gl_2\ gl_3\ gl_3)}{36}\right]^2$$

(b) $\sigma^2_{(Gl_3 \text{ vs. } gl_3)} =$

$$\left[\frac{\Sigma(Gl_2\ Gl_2\ Gl_3\ Gl_3 + gl_2\ gl_2\ Gl_3\ Gl_3) - (Gl_2\ Gl_2\ gl_3\ gl_3 + gl_2\ gl_2\ gl_3\ gl_3)}{36}\right]^2$$

(c) $\sigma^2_I =$

$$\left[\frac{\Sigma(Gl_2\ Gl_2\ Gl_3\ Gl_3 + gl_2\ gl_2\ gl_3\ gl_3) - (Gl_2\ Gl_2\ gl_3\ gl_3 + gl_2\ gl_2\ Gl_3\ Gl_3)}{36}\right]^2$$

## RESULTS

Means and variances are presented in Table 1 for seedling damage ratings and for number of larvae per seedling. The results are summarized as follows: *(1)* damage ratings were higher, and numbers of larvae per seedling were lower on all four entries after 48 hr than they were after 24 hr of feeding time; *(2)* order of larval preference among plant parts was (a) second leaf; (b) first leaf; (c) cotyledons; *(3)* the larvae preferred glandless over glanded seedlings; *(4)* larvae discriminated between the dimeric glanded and the monomeric types, and in most instances, also between the 2 monomerics; *(5)* differences among genotypes were statistically significant in every case, except growing point damage; *(6)* the comparison $Gl_2$ vs. $gl_2$ was significant in every instance except in cotyledon damage at 24 hr and in growing point damage; *(7)* the comparison $Gl_3$ vs. $gl_3$ was usually significant, but in only one instance did it contribute more to the total genotypic variance than $Gl_2$ vs. $gl_2$; *(8)* the third single-degree-of-freedom comparison was in every case nonsignificant, which suggests that seedling damage and larval numbers increased linearly as gland number decreased.

Table 2 presents the relationships between genotype, number of pigment glands in the cotyledonary petiole, and percent gossypol in dried seed meat (gossypol data from Lee et al., 1968). Results show that the dimeric strain had the most glands and the highest gossypol level, that monomeric-2 ranked second, monomeric-3 ranked third, and that the glandless strain ranked fourth,but was not completely devoid of gossypol.

## DISCUSSION

*H. virescens* larvae were able to discriminate successfully among the four genotypes studied. Our data suggest that larval counts were more reliable after 24 than after 48 hr, because many larvae had left the plants before the second reading. However, genotypic differences in plant damage were expressed better 48 hr after infestation. The trend of more damage and more larvae on plants with lower gland number and gossypol level was consistent at both 24 and 48 hr and in all plant parts that were scored. However, it appears that a reading of the second leaf, coupled with a larval count, would have adequately characterized the degree of nonpreference exhibited by the insects.

The intimate relationship between gland density and gossypol level in seeds found in this study has also been noted by other workers (c.f. Rhyne et al., 1959; Lee et al., 1968). Presumably, $Gl_2$ and $Gl_3$ primarily affect gland number (perhaps gland size, also) and thus provide gossypol storage sites; apparently they have no control over the synthesis and transport of gossypol. For example, glandless cotton has not lost the ability to synthesize gossypol (Table 2). In fact, it will produce normal levels of this substance when induced to do so by the invasion of fungal spores, certain heavy metal ions, or various metabolic inhibitors (Bell, 1967).

From the data presented in this paper, it is not known whether the larvae responded to the amount of gossypol present in the plant tissues or to the gland density. However, it is safe to say that the larvae responded directly (negatively) to the *gossypol potential* of the seedlings.

The larvae left the dimeric seedlings in great numbers; 64% had moved away after 24 hr, and 95% after 48 hr. On the other hand, the number of larvae had increased slightly on the glandless seedlings after 24 hr. However, half of them had left after 48 hr, which suggested that even glandless seedlings were not particularly attractive to budworm larvae.

$Gl_2$ generally had a greater effect than $Gl_3$ on larval behavior, as expected, on the basis of gossypol levels

**Table 2. Relationship between number of pigment glands in the cotyledonary petioles of cotton seedlings at the 2-leaf stage, and percent gossypol in the seed meat of the Empire variety of cotton.**

| Genotype | Number of pigment glands mean ± S. E. | % gossypol in seed meat* |
|---|---|---|
| $Gl_2\ Gl_2\ Gl_3\ Gl_3$ | 78.9 ± 3.6 | 1.252 |
| $Gl_2\ Gl_2\ gl_3\ gl_3$ | 52.4 ± 3.0 | .848 |
| $gl_2\ gl_2\ Gl_3\ Gl_3$ | 14.4 ± 3.1 | .332 |
| $gl_2\ gl_2\ gl_3\ gl_3$ | 0.0 | .012 |

* from Lee et al. (1968)

present. For example, the proportion of the total genotypic variance attributed to $Gl_2$ vs. $gl_2$ was 79.4% for second leaf damage at 48 hr and 63.4% for number of larvae per plant at 24 hr. Comparable figures for $Gl_3$ vs. $gl_3$ were 13.6% and 22.8%, respectively. However, this effect was not evident in plant damage ratings 24 hr after infestation, and it was not as pronounced in numbers of larvae 48 hr after infestation.

Results from this test, and from subsequent tests not reported in this paper, suggest that tobacco budworm larvae exert their nonpreference for glanded seedlings by *(1)* staying on them for a shorter period and *(2)* by eating less plant material while they are present (cf. Oliver, Maxwell, and Jenkins, 1970). They will exhibit this nonpreference both under a free-choice situation, or where they are confined to plants of a single genotype in a cage, or in some other way.

The discriminating power by tobacco budworm larvae suggested several breeding, genetic, and entomological applications, as follows: *(1)* screening for high or low gossypol levels in seedlings (see Ferguson et al., 1970, as an example of the use of the Angoumois grain moth in breeding corn for high amylose content); *(2)* studying the effects of individual genes and gene-combinations on the insect response; *(3)* studying gene action in relation to insect response; *(4)* studying insect reactions under free-choice, limited-choice, and no-choice situations; *(5)* studying various aspects of insect/plant interactions, such as the amount and degree of movement of larvae from plant to plant; *(6)* studying the effects of various caging and other containment practices on larval behavior.

We include here a discussion of techniques, to help the reader benefit from our experience. We have used larvae of several ages, from newly-hatched ones to those a week old. We have placed larvae directly on the plants from the media and have starved them for several hours before placing them on the plants. We have placed them directly on the plants and have released them in the vicinity of the plants. We have caged them and left them uncaged. Our findings may be summarized as follows: *(1)* newly-hatched larvae were unsatisfactory because they grew slowly on seedlings and did not provide good damage-rating estimates; *(2)* larvae older than 3 or 4 days, having been fed on artificial media, sometimes moved around excessively, unless they were starved for several hours before they were put on the plants; *(3)* 3 to 4-day-old larvae, on the other hand, performed as well when they were moved directly from the artificial media to the plants as when they were starved first; *(4)* results were more satisfactory when the larvae were placed directly on the plants; *(5)* cages were unnecessary and were, in fact, a hindrance to watering and to data-gathering; *(6)* larvae expressed nonpreference best under a no-choice situation, when they were confined to a single genotype, either in or out of a cage. As shown in the test reported in this paper, however, the larvae did discriminate in a free-choice situation. Apparently the key to successful assessment of nonpreference where the larvae have a free-choice among plants in a segregating progeny is to gather data at the appropriate time. In practice, this can probably be accomplished by counting larvae and reading damage more than once, and by including check plants of known genotype with the segregating plants.

Cotton workers are searching for biochemical resistance in the cotton plant to *Heliothis* spp. Many stocks have been screened in attempts to reveal those that show a degree of resistance (Lukefahr et al., 1969). In fact, considerable emphasis is being placed on non-gossypol sources of resistance, because of the unfavorable effects of high gossypol levels in cottonseed oil and meal. However, the route that appears to show the most promise at present is to protect the plant parts that are attacked by *Heliothis* with high levels of gossypol. The seedling technique described in this paper is not offered as a practical preliminary screening tool to achieve this end, because the relationship of seedling resistance to mature-plant resistance remains to be determined.

A potentially important finding is that the larval response was highly correlated with the number of glands on the cotyledonary petiole and with percent gossypol in the seed meats of the genotypes studied. This finding suggests that the breeder may select seedlings by counting glands, without resorting to a bioassay. However, gland number varied considerably between plants of any given genotype in the Empire material. Also, the effects of other genetic backgrounds on gland number are largely unknown at present.

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