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Field Evaluation of Glanded and Glandless Cotton (*Gossypium hirsutum* L.) Lines for Boll Weevil (*Anthonomus grandis* Boh.) Susceptibility[1]

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ABSTRACT

The boll weevil is the major cotton insect in the rain-grown cotton belt. We investigated glandless cottons to determine if they were more susceptible to the boll weevil than the currently grown glanded varieties. Two years' results from large field plots with two pairs of glanded-glandless cottons indicated that the glandless genes gl_2 and gl_3 did not cause greater susceptibility in the Acala 4-42-77 and Rex Smoothleaf lines. Thirteen pairs of glanded-glandless lines were compared in small field plots. In each of the 13 comparisons no significant differences were found. Antibiosis tests showed slightly larger weevils on some glandless lines. These data suggest that the glandless character should not create any increased boll weevil susceptibility, especially with careful selection of genetic background.

Additional index words: **glandless cotton, cotton, boll weevil, *Anthonomus grandis*, *Gossypium hirsutum*, resistance, susceptiblility.**

SMALL lysigenous glands occur in all cotton plant parts except the roots in all species of Gossypium. Because gossypol is the major plant pigment in these glands, they are called gossypol glands. Lewton (4) and Fulton (2) reported that Hopi Cotton from central Arizona had a variable number of the pigment glands in the boll. McMichael (8,9) was the first to demonstrate that cottonseed without glands could be produced and the gossypol content of seed meats reduced to negligible amounts. He reported that two recessive genes, gl_2 and gl_3, were responsible for the glandless condition in the cotton plant and seed.

The detrimental effects of gossypol in cottonseed meal and oil have long been recognized by the cottonseed crushing industry. The removal of gossypol from cottonseed could result in an increased use of meal in animal rations and an increased use of cottonseed products in human nutrition. For example, a completely new source of protein from the cottonseed flour would be potentially available for human consumption.

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Since the original development of experimental lines of glandless cottons by McMichael (9) in 1959, entomologists and plant breeders have noticed that the glandless cottons were quite susceptible to certain phytophagous insects, notably the leaf feeders. These potential entomological problems have been adequately reviewed by Bottger et al. (1), Murray et al. (10), Jenkins et al. (3), and Maxwell et al. (6). These researchers, in general, reported an increase in susceptibility of the glandless cottons to the leaf feeding insects. Maxwell et al. (7) reported on a series of laboratory experiments involving several glandless lines and their glanded counterparts. The presence of the glandless genes gl_2 and gl_3 in the various genetic backgrounds investigated did not appear to increase significantly the susceptibility of the lines to the boll weevil.

The boll weevil is a major insect in the rain-grown cotton belt and is not a leaf feeder. From our best evidence it also seems that the boll weevil has evolved on gossypol containing plants throughout its history and development. We thus had reason to suspect that the boll weevil might respond differently than other insects to glandless cottons. Its economic importance as a pest also contributed to the need to evaluate its damage potential to glandless cotton.

We conducted four field experiments in 1964 and 1965 and these are reported herein. The purpose of these experiments was to determine if the glandless genes gl_2 and gl_3 in a number of genetic backgrounds increased boll weevil susceptibility over the glanded counterpart lines.

MATERIALS AND METHODS

The glanded and glandless pairs of cotton lines utilized are described under each of the various experiments. All the glandless lines except 'Pima S-2,' 'Rex Smoothleaf,' 'Acala 4-42-77,' and 'Empire' had been backcrossed four times to the glanded parental line followed by several generations of selfing. The four excepted lines were supplied by the respective breeders and were advanced lines in their programs.

Experiment I, 1964 Season. Three replications of 'Rex Smoothleaf, Rex Smoothleaf glandless, 'Acala 4-42-77,' Acala 4-42-77 glandless, and 'Deltapine Smooth Leaf' were planted in a completely randomized design. The plots were 24.38 m square with a 3.05-m alley on all sides of each. These covered a 1.2-hectare field. The seed were planted on May 15 and the data collection began on July 1. No insecticide was used at any time in these plots. We were interested in measuring the boll weevil infestation buildup during a season. The percentage of squares (buds) damaged by boll weevil oviposition was recorded at each record date. Twenty-one inspections were made of the plots from July 1 through August 31. The plots were infested naturally with weevils from favorable overwintering sites adjoining the field. At each inspection 100 squares were examined for egg punctures. All plots were infested with adult weevils by July 1, the date the first records were taken. Records were terminated in the Rex Smoothleaf glanded and glandless plots and the Deltapine plots when the squares reached 75% punctures, which occurred on August 17. The Acala glanded and glandless plots were terminated at 74.0 and 72.6%, respectively, on August 31.

Experiment II, 1965 Season. The same five cotton lines as in Experiment I were used in Experiment II. Several modifications of the 1964 test were made. Four replications were used in plots 30.48 m square, with 3.05-m alleys on all sides of each plot. The cotton lines were planted on May 4 and the first record was taken on June 24 and at approximately regular intervals for a total of 13 records. Two insecticide applications of 281 g methyl parathion per ha were applied on July 2 and 6 to reduce a high initial percentage infestation resulting from overwintering weevils. No further applications of insecticide were made during the season. The fruiting rate of the lines was measured by using a modification of the Point Sample Method of determining boll weevil infestation. From two randomly selected points in each plot, we picked 50 unflared squares at least 0.64 cm in diameter, the preferred size for oviposition. The row distance to obtain the 50 squares was measured accurately with a steel tape. The squares from each plot were examined in the laboratory for egg and feeding punctures and the percentage of squares infested was determined. In addition, the number of squares per .4047 ha (1 acre) and the number of infested squares per .4047 ha were calculated.

The square root transformation was used on the data prior to the analysis of variance and the analysis of covariance. The data were analyzed as a split plot in time with record date as the split plot and cotton lines as the main plot. In the covariance the number of squares per .4047 ha was the dependent variable. There was no gain in precision in these large plots with these lines from using the covariance analysis.

Experiment III, 1965 Season. Fourteen pairs of glanded-glandless cotton lines were tested in small field plots. Dr. James Meyer supplied the glanded and glandless versions of the following lines: 'Atlas,' 'Stardel,' 'Coker 100A,' 'Stoneville 7A,' M8, M11, 'Wescot,' 'Dixie King,' and 'Deltapine Smooth Leaf.' The Acala 4-42-77 pair was supplied by Dr. Angus Hyer. The Rex Smoothleaf pair was supplied by Mr. Carl Moosburg. The Empire glandless was supplied by Dr. William Manning and the 'Empire' glanded was commercial seed. The 'Pima S-2' pair was supplied by Dr. Ed Turcotte.

The cotton lines were planted on May 4 in plots 8 rows by 9.14 m long and the first records were taken on June 29. A heavy infestation of weevils at that time necessitated an application of 281 g of methyl parathion per hectare to reduce the weevil population to a low level in all plots. Records were made at regular intervals from July 6 through August 18 for 11 records. Data were collected as in Experiment II with the exception that only one sample per plot was picked because of the small size of the plots. The analysis was the same as in Experiment II. There was gain in precision from the covariance analysis in these small plots and with these lines.

Experiment IV, 1965 Season. An antibiosis test was conducted on the 14 pairs of lines grown in Experiment III. Approximately 400 squares containing egg punctures were picked from each of the glanded-glandless lines using all four replications to obtain the total of 400. These infested squares were brought into the laboratory and sorted to obtain approximately 200 of the same size of each line. They were then placed into emergence cages made from 13-liter cardboard containers. A hole was cut in the top of each container and a small glass jar inserted to provide light. Weevils moved into the jar upon emergence and were removed daily and weighed on a Mettler semi-micro balance[3]. The weights were recorded in milligrams. The data were analyzed via individual t tests to separate the means of each pair.

RESULTS

Experiment I, 1964 Season: Fig. 1 shows the percentage of egg punctured squares on each cotton line. Rex Smoothleaf glanded and glandless and Deltapine Smooth Leaf had higher initial infestations than the two Acala lines. Squares were available in sufficient quantities by July 1 for an infestation to develop in all plots. An infestation did not develop early in the Acala glanded and glandless plots. A lower initial preference for the Acalas may have contributed to this.

The first field generation of the season emerged around July 22; after July 26, the infestation increased rapidly on the two Rex Smoothleaf lines and Deltapine Smooth Leaf, exceeding 75% oviposition punctured squares by August 17. The two Rex Smoothleaf lines and Deltapine Smooth Leaf were not significantly

[3]The use of trade or proprietary names does not necessarily imply the endorsement of these products by the U. S. Department of Agriculture.

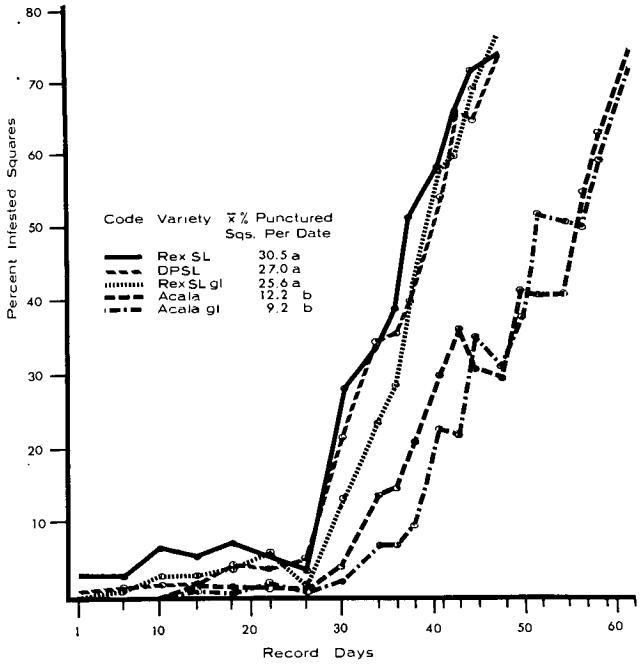

Fig. 1. Percentage egg punctured squares on five cotton lines during the 1964 season. Those means followed by the same letter are not significantly different at the 0.05 level (Tukey's Test).

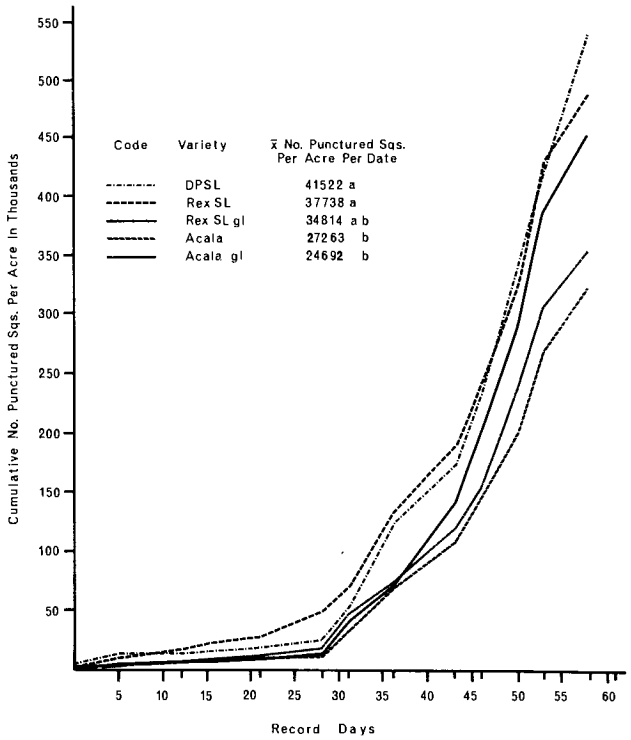

Fig. 2. Cumulative number of puncutred squares per .4047 ha (1 acre) on five cotton lines during the 1965 season. Those means followed by the same letter are not significantly different at the 0.05 level (Duncan's New Multiple Range Test).

different (Tukey's Test) in mean percentage of squares infested with boll weevil eggs per record time.

Figure 1 shows that the two Acala lines increased more slowly in percentage of squares damaged by egg punctures than did the other three lines. These two lines were not significantly different (Tukey's Test) from each other but were from the two Rex Smoothleafs and Deltapine Smooth Leaf. Fourteen days after the Rex Smoothleaf and Deltapine Smooth Leaf lines reached 75% infestation the two Acala lines were in the low seventies and the experiment was terminated.

Experiment II, 1965 Season: The analysis of covariance did not show a gain in precision; thus, the Analysis of Variance for split plots was used with the date of record being the split plot. This was not entirely unexpected with these five lines which are near commercial type and in large plots. Figure 2 shows the total number of egg and feeding punctured squares per .4047 ha for each line for the season. The data is plotted as cumulative number of punctured squares per .4047 ha by dates. Duncan's New Multiple Range Test was used to separate the five means. Again, as in Experiment I, the glandless lines were not different from their respective glanded counterpart. Rex Smoothleaf glanded and Deltapine Smooth Leaf were significantly different from the two Acala's. Rex Smoothleaf glandless was not significantly different from any line in the test.

Experiment III, 1965 Season: The data in this experiment were analyzed as a split plot analysis of covariance. The gain in precision by taking into account the varying numbers of squares on the lines was 50% for the main plots (cotton lines) and 25% for the split plot (record date). There was a significant effect due to cotton lines and record date (Table 1). They were divided into five overlapping groups. In each of the 14 comparisons of glanded vs. glandless lines, no significant difference was found. The lowest infestation group included Pima S-2 glanded and glandless, Acala glanded and glandless, D_2 glandless, M8 glandless, and M11 glanded. The last column in Table 1 shows the mean percentage of egg and feeding punctured squares for each line averaged over record

Table 1. Adjusted mean number of feeding and egg punctured squares per acre for the cotton lines in Experiment III, 1965. Mean of three replications.

Cotton line	Adjusted mean number* of punctured squares per .4047 hectare	x̄% infested squares per date
Atlas	37332 a†	34.0
Stardel glandless	36598 ab	30.0
Empire W R	36095 ab	34.0
Dixie King glandless	34979 ab	35.0
D_2	34911 ab	30.0
Coker 100A	34855 ah	31.0
Stardel	34509 abc	36.0
Coker 100A glandless	33608 abc	30.0
Stoneville 7A	33502 abc	31.0
M8	32712 abcd	32.0
Rex Smoothleaf glandless	32352 abcd	32.0
Wescot	31686 abcd	31.0
Wescot glandless	31409 abcd	29.0
Deltapine Smooth Leaf glandless	30431 abcd	28.0
Rex Smoothleaf	30011 abcd	30.0
M11 glandless	29876 abcd	30.0
Stoneville 7A glandless	28502 abcd	29.0
Atlas glandless	28270 abcd	30.0
Empire glandless	27207 abcd	28.0
Dixie King	26255 abcd	30.0
Pima S-2	24372 abcde	25.0
D_2 glandless	24105 abcde	26.0
M8 glandless	23767 abcde	25.0
Pima glandless	22652 bcde	22.0
Acala glandless	20908 cde	23.0
M11	19795 de	28.0
Acala	14596 e	20.0
All glanded	29574	30.0
All glandless	28904	28.3

* Means reported are the adjusted mean from the Analysis of Covariance based on the same regression coefficient for A and B. † Those means followed by the same letter are not significantly different at the 0.05 level using the Duncan New Multiple Range Test.

Table 2. Antibiosis data for weevil emergence weight on the 14 pairs of glanded-glandless lines in Experiment IV, 1965.

Cotton line	No. weevils emerged†		$\bar{x}$ wt. of emerged adults in mg	
	Glanded	Glandless	Glanded	Glandless
Pima S-2	145	104	9.14	10.00
Wescot	146	149	9.54	9.56
Deltapine Smooth Leaf	---	140	-----	9.70
Stoneville 7A	114	149	9.72	10.71*
Stardel	149	148	9.75	10.61*
Empire	103	123	9.78	10.53
M8	63	89	9.88	9.54
Atlas	149	145	9.97	10.29
Rex Smoothleaf	118	150	10.08	10.89*
M11	87	54	10.15	10.86*
Dixie King	156	103	10.16	11.09*
Acala 4-42-77	72	149	10.20	10.68
Coker 100A	86	153	10.24	9.83
$D_1$723	149	152	10.40	10.25
$\bar{x}$ of all lines	118	129	9.92	10.37

* Indicates significance at the 0.05 level using individual t test comparison between each glanded-glandless pair. † Unequal numbers of infested squares were installed in all emergence cages.

dates. These agree with the data on punctured squares per .4047 ha.

Experiment IV, 1965 Season: Results are recorded in Table 2. The mean emergence weight of adult weevils did not differ significantly between the glanded and glandless lines in nine pairs. In five pairs, Stardel, Stoneville 7A, Rex Smoothleaf, Dixie King, and M11, the weevils produced on the glandless lines were significantly larger than those produced on the respective glanded line. When all 14 pairs were averaged, there was no significant difference between the weights of adults produced on glanded and on glandless lines.

DISCUSSION

Our field plot results throughout the seasons of 1964 and 1965 indicate that the presence of the glandless genes gl_2 and gl_3 in 14 different genetic backgrounds neither increased nor decreased significantly their susceptibility in the field to boll weevil attack over that of their glanded counterparts. The significant increase in the size of weevils emerged from 5 of the 14 glandless lines suggests an increased problem if larger weevils live longer and puncture more squares than smaller ones. In previously conducted studies in the laboratory we have noticed a general trend for larger weevils to lay more eggs and live longer than smaller ones (11). We do not know whether statistical differences in size, noted in these instances, would be biologically significant in the field. We did not notice any increased damage in our glandless field plots over the 2-year period; thus, it does not appear that the size difference noted here would be of any significance in total population buildup during the season on these lines. However, it is obvious that the glandless genes are reacting somewhat differently to the weevil in the various genetic backgrounds, probably due to gene interactions. As each new glandless variety is developed and carried through the normal testing program toward ultimate release, we recommend that the variety be evaluated carefully for boll weevil reaction, to avoid any potential interactions between the glandless genes and the cotton line background which might result in increased susceptibility to this insect. Normal testing procedures during development, with special care to test specifically for boll weevil susceptibility, should prevent any pitfalls which may exist.

These data strongly support the conclusions drawn by Maxwell et al. (7) which were based on laboratory feeding, oviposition, and rearing tests on various selected glanded and glandless pairs of lines. It was interesting to notice that the Acala 4-42-77 glanded and glandless lines were in the lowest damage group in three different experiments over a 2-year period. The Acala 4-42-77 line apparently carries a degree of resistance to the boll weevil. This resistance seems to be associated with the preference mechanism of resistance. We are studying this variety further to determine if this low level of resistance can be successfully utilized in a breeding program for boll weevil resistance.

On the basis of the laboratory data reported by Maxwell et al. (7) and the data reported herein, it is concluded that the glandless genes in cotton should not increase the boll weevil problem if proper care is exercised in initially selecting the genetic background and subsequently testing the developing variety. These conclusions and recommendations are made only for the boll weevil and do not apply to other cotton insects.

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