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A Genetic and Breeding Study of Pink Bollworm Resistance and Agronomic Properties in Cotton[1]

F. D. Wilson and B. W. George[2]

ABSTRACT

A breeding stock of upland cotton (*Gossypium hirsutum* L.) designated as AET-5, consistently sustains less seed damage by pink bollworm (PBW) [*Pectinophora gossypiella* (Saunders)] than do cultivars. This stock is deficient in most agronomic and fiber properties, with the exception of lint percentage. We crossed AET-5 to DES 24-8ne, an advanced breeding line which lacks extrafloral nectaries [nectariless (2 ne_1, ne_2)], a morphological trait which imparts resistance to PBW in large field plantings. Parents and hybrid generations were compared for 2 years in insecticide-free environments. The objectives of these studies were to determine the inheritance of the AET-5 level of seed damage and of several agronomic properties and to predict the ease of transfer of the low seed damage and high lint percentage into a desirable agronomic background by backcrossing. The seed damage expected in a stock combining the AET-5 type of resistance with the nectariless character should be lower than in stocks carrying only one of those two resistance characters. Analyses of tests grown for 2 years indicated that gene action was primarily additive for both seed damage and lint percentage, that narrow-sense heritability estimates were significant, and that few genes conditioned those properties. A combined analysis of variance revealed that genotype × environment interactions were not significant for those traits. Therefore, it should not be difficult to transfer the AET-5 level of resistance and its associated high lint percentage into desirable backgrounds. Additive effects were also shown in two of the three tests for the other agronomic properties measured. Dominance, epistasis, or both were also shown. Heritability estimates were not consistently significant. Those non-additive effects should not be regarded as obstacles to breeding progress because our ultimate objective is to transfer by backcrossing only low seed damage and high lint percentage, but otherwise to reconstitute the phenotype of the nectariless parent.

Additional index words: ***Gossypium hirsutum*** **L.,** ***Pectinophora gossypiella*** **(Saunders), Host-plant resistance, Additive gene action, Dominance, Epistasis, Heritability, Genotype × environment interaction.**

A BREEDING stock of upland cotton (*Gossypium hirsutum* L.) designated as AET-5, consistently has sustained less seed damage from pink bollworm (PBW) [*Pectinophora gossypiella* (Saunders)] in Arizona than do current cultivars [35% less than 'Deltapine 16' or 'Deltapine 61' in 10 tests over 5 years (11)]. The mechanism of resistance has not been elucidated. We demonstrated that AET-5 has a low level of boll-content antibiosis, as reflected in reduced pupation percentage of PBW larvae in the boll (9), but this mechanism only partially accounts for the level of field resistance observed. We also observed that field-grown plants of AET-5 had fewer PBW entrance holes/boll and fewer PBW eggs than those of Deltapine 61 (2).

Irrespective of the mechanism of resistance, relative percentages of seed damage in parents and hybrids may be used as a tool to study the inheritance and ease of transmission of the AET-5 source of resistance. Results from a diallel experiment which included AET-5, two cultivars, plus other entries suggested that AET-5 would be a good combiner for low seed damage (6).

An aim of our long-term breeding program is to combine independent sources of resistance into superior breeding stocks. One potentially useful combination is the AET-5 level of resistance with a breeding stock or cultivar lacking extrafloral nectaries [nectariless (2 ne_1, ne_2)] because such a combination would be expected to show a higher level of resistance to PBW than would either character alone (10). We therefore crossed AET-5 to DES 24-8ne, a nectariless advanced breeding stock and produced parental, F_1, F_2, F_3, and backcross progenies to study the inheritance of the AET-5 level of seed damage and of several agronomic properties and to predict the ease of transfer of that level of seed damage and high lint percentage into a desirable agronomic background by backcrossing.

MATERIALS AND METHODS

The pedigree of AET-5, obtained from G.A. Niles, Texas Agriculture Experiment Station, is presented in Wilson et al. (11). The other parent, DES 24-8ne, a nectariless version of Deltapine 16 (4), was obtained from W.R. Meredith, Jr., USDA-ARS, Stoneville, Miss. In our tests, the nectariless trait did not show resistance to PBW because our plots were so small that the PBW moths could move freely between nectaried and nectariless cotton. Therefore, DES 24-8ne was expected to respond to PBW attack similarly to its susceptible recurrent parent, Deltapine 16.

We crossed AET-5 as the pistillate parent with DES 24-8ne at the University of Arizona Cotton Research Center, Phoenix. F_1 plants were self-pollinated and backcrossed to both parents at the National Cotton Council winter nursery at Iguala, Mexico. Randomly selected F_2 plants were selfed and advanced to the F_3 generation at Phoenix.

We planted seed of the two parents (DES 24-8ne = P_1; AET-5 = P_2) and four hybrid generations [F_1, F_2, B_1 (backcross to P_1), and B_2 (backcross to P_2)] in the greenhouse. Two-week-old

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[2] Research geneticist and research entomologist, respectively, Western Cotton Res. Lab., USDA-ARS, 4135 E. Broadway Rd., Phoenix, AZ 85040.

Table 1. Means, genetic effects, and heritabilities estimated from generation-mean analyses over years for two parents and four hybrid generations of cotton (Tests 1979-1 and 1980-1).

Parent or hybrid generation	Seed damage	Seedcotton yield/plant	Lint yield/plant	Boll size	Lint	Bolls/ plant
	%	g			%	no.
DES 24-8ne (P_1)	19.6 a†	95.6 a	35.9 ab	4.11 bc	37.73 d	23.8 a
B_1	16.9 ab	97.7 a	38.0 a	4.51 a	38.70 c	22.6 ab
F_1	11.5 c	86.4 b	34.7 b	4.37 ab	39.85 a	19.9 c
F_2	15.6 b	89.3 b	35.0 ab	3.86 cd	38.95 bc	23.4 a
B_2	11.0 cd	76.7 c	30.1 c	3.79 cd	39.55 ab	20.3 bc
AET-5 (P_2)	7.0 d	75.8 c	31.0 c	3.68 d	40.25 a	20.4 bc
Genetic effects						
Additive (A)	6.3 ± 0.6*	9.9 ± 1.8*	2.4 ± 0.7*	0.22 ± 0.03*	−1.26 ± 0.18*	1.7 ± 0.5*
Dominance (D)	−9.0 ± 8.0	−11.3 ± 26.3	−6.1 ± 10.5	2.86 ± 0.67*	1.03 ± 2.03	−15.9 ± 7.2*
Epistasis:						
A × A	−6.7 ± 3.1*	−8.4 ± 10.3	−3.7 ± 4.1	1.16 ± 0.26*	0.68 ± 0.78	−7.6 ± 2.8*
A × D	−0.9 ± 2.3	22.2 ± 7.7*	10.9 ± 3.1*	1.01 ± 0.19*	0.85 ± 0.63	1.1 ± 2.1
D × D	0.5 ± 5.1	3.7 ± 17.2	3.7 ± 6.9	−1.23 ± 0.44*	0.51 ± 0.30	5.8 ± 4.6
Heritabilities	0.48*	0.51*	0.53*	0.05	0.47*	0.55*

* Genetic effects or heritability estimates were significantly different from zero at the 0.05 level of probability.
† Means within a column followed by letters in common were not significantly different at the 0.05 level of probability, according to an LSD test (means adjusted after analysis).

seedlings were transplanted with a mechanical transplanter to the field at the Arizona State University Farm Laboratory, Tempe. Soil type was Contine clay loam, a member of the fine, mixed, hyperthermic Typic Haplargids. Planting and transplanting dates were 18 Apr. and 3 May 1979 (Test 1979-1) and 9 and 23 Apr. 1980 (Test 1980-1), respectively.

The experimental design was a randomized, complete block with five replications. Each plot contained 20 plants spaced 46 cm apart; row width was 1 m. Test plots were bordered by guard rows of Deltapine 61. The F_2 was represented by 200 plants/year; whereas, the parents and the other hybrid generations were represented by half that number.

Standard cultural practices for the area were followed except that plots received no insecticide. PBW populations were sufficient both years to infest the plants. Seed cotton was harvested from individual plants on 29 Oct. 1979 and 21 Oct. 1980, respectively. Seed cotton was weighed and ginned, and lint and seed samples were weighed. A random sample of seed (100 to 300) from each plant was x-rayed to provide radiographs for estimating seed damage by PBW (12). In addition to percent seed damage, other traits studied included seed cotton and lint yield/plant, lint percentage, bolls/plant, and boll size (g seed cotton/boll).

Generation-mean analyses (3, 5) were used to estimate genetic parameters.[3] Combined analyses of variance of the 1979 and 1980 data revealed no significant generation × season interactions; therefore, the data were pooled over both years for the generation-mean analyses. Narrow-sense heritabilities were estimated by the variance component method of Mather and Jinks (3).

From the pooled data, we estimated the number of genes controlling differences in seed damage and lint percentage using the formula given by Cavalli-Sforza and Bodmer (1) as follows: $[1/2(\overline{P}_1 - \overline{P}_2)]/2V_A$, where $\overline{P}_1$ and $\overline{P}_2$ are the means of the two parents and V_A is the additive genetic variance. This formula is appropriate if the two parents are inbred, i.e., homozygous for their respective alleles, and if there are no gene × gene or genotype × environment interactions.

Also in 1979, we conducted Test 1979-2 which included P_1, P_2, 14 F_1 families (coming from crosses of 14 paired combinations of staminate and pistillate parents), 14 F_2 families (from one selfed plant of each F_1 family), and 70 F_3 families (five from a randomly selected F_2 plant from each of the 14 F_2 families). Seedlings were transplanted into the same field on the same day as those in Test 1979-1. Each entry was represented in each replication by 20 plants spaced 46 cm apart in a single row 1 m wide (bordered by guard rows of Deltapine 61). Experimental design was a 10 × 10 simple lattice with two replications. Seed cotton was harvested from 10 plants/plot biweekly from early August to mid-September for four harvests with a fifth harvest in late October. Data on seed damage and agronomic properties were subjected to a combined analysis and are presented as seasonal means. Narrow-sense heritability was estimated by the F_3/F_2 regression method. That is, the means of each five F_3 families were regressed on the mean of their respective F_2 parents.

We also conducted Test 1980-2 which included P_1, P_2, and 34 hybrid families reared from nectariless F_2, F_3, or B_1 plants obtained from both 1979 tests. The 1979 nectariless F_2 and B_1 plants had also been selected for low or high seed damage. Planting dates and plot size were the same as in Test 1980-1. Experimental design was a 6 × 6 triple lattice with three replications. Seed cotton was harvested from 10 plants/plot biweekly from early August to mid-September for four harvests with a fifth harvest in late October. The same data were obtained as in the other tests described.

RESULTS

In all four tests, seed damage percentages were consistently and significantly lower in AET-5 than in DES 24-8ne (Tables 1, 2, and 4). F_1, F_2, and F_3 means were intermediate between those of the parents and significantly different from both, except that the F_1 mean was not significantly higher than AET-5 in Test 1979-2 (Table 2). Backcross means were intermediate between the F_1 and the respective recurrent parent. Neither was significantly different from the recurrent parent.

Generation-mean analyses from individual plant data, pooled over Tests 1979-1 and 1980-1, and from plot data in Test 1979-2, revealed significant additive genetic effects for seed damage (Tables 1 and 2). Also, additive × additive epistasis was significant in the pooled data. Narrow-sense heritability estimates were significantly different from zero when calculated by the variance component method (Table 1) or by the regression method (Table 2). The estimated number of genes for seed damage (pooled data) was approximately two.

In the six-generation comparisons, Tests 1979-1 and 1980-1, the two parents also differed significantly in the five agronomic properties measured (Table 1). DES 24-8ne produced more seedcotton yield/plant, lint yield/plant, and bolls/plant; and it had heavier bolls as well. AET-5

[3] A copy of the computer program used is available upon request.

Table 2. Means and genetic effects estimated from generation-mean analyses for two parents and three hybrid generations of cotton; heritabilities estimated by regression of F_3 on F_2 (Test 1979-2).

Parent or hybrid generation	Seed damage	Seedcotton yield/plant	Lint yield/plant	Boll size	Lint	Bolls/ plant
	%	g			%	no.
Means						
DES 24-8ne (P_1)	34.4 a†	99.4 a	36.4 a	3.75 ab	34.28 c	28.4 a
F_1	24.6 bc	113.7 a	44.7 a	3.94 a	37.27 ab	28.1 a
F_2	25.7 b	105.6 a	41.0 a	3.73 ab	37.00 ab	27.6 a
F_3	26.2 b	101.6 a	39.2 a	3.63 ab	36.86 b	27.3 a
AET-5 (P_2)	19.0 c	95.8 a	38.3 a	3.30 b	39.18 a	25.8 a
Genetic effects						
Additive	7.7 ± 2.2*	3.6 ± 11.5	−1.9 ± 2.7	0.22 ± 0.15	−2.45 ± 0.62*	2.6 ± 3.2
Dominance	−2.1 ± 1.3	32.3 ± 6.3*	14.7 ± 1.7*	0.41 ± 0.10*	0.54 ± 0.41	2.0 ± 1.7
Chi-square	0.6	0.1	0.1	1.01	0.97	0.2
Heritabilities	0.25*	−0.05	−0.04	−0.04	0.60*	−0.09

* Genetic effects, chi-squares (to test adequacy of additive-dominance model), or heritability estimates were significantly different from zero at the 0.05 level of probability.
† Means within a column followed by letters in common were not significantly different at the 0.05 level of probability, according to an LSD test (means adjusted after analysis).

Table 3. Performance of homozygous nectariless (AET-5 × DES 24-8ne)F_3 families selected from nectariless F_2 cotton plants (Test 1979-2).

Parent or F_3 family	Seed damage	Seedcotton yield/plant	Lint yield/plant	Boll size	Lint	Bolls/ plant
	%	g			%	no.
DES 24-8ne (P_1)	38.9 a*	108.6 a	38.1 a	3.45 ab	33.77 b	30.9 a
F_3(24)	18.6 c	87.9 a	34.4 a	3.34 ab	37.67 a	24.7 ab
F_3(32)	24.4 b	93.9 a	36.0 a	3.41 ab	36.38 a	25.9 ab
F_3(40)	24.2 bc	107.9 a	41.9 a	3.53 ab	36.93 a	30.3 a
F_3(61)	20.4 bc	88.2 a	34.6 a	3.83 a	37.82 a	21.5 b
AET-5 (P_2)	20.9 bc	101.6 a	40.2 a	3.20 b	37.86 a	27.5 ab

* Means within a column followed by letters in common were not significantly different at the 0.05 level of probability, according to an LSD test (unadjusted means).

Table 4. Seed damage percentages and agronomic traits for two parents and 34 nectariless families of cotton (Test 1980-2).

Parent or hybrid generation	Families	Seed damage	Seedcotton yield/plant	Lint yield/plant	Boll size	Lint	Bolls/ plant
	no.	%	g			%	no.
DES 24-8ne (P_1)	1	23.2 a*	107.9 a	38.9 a	3.85 a	35.3 b	28.7 a
F_4 from unselected F_3†	4	11.0 b	78.9 b	31.2 ab	3.40 b–d	38.2 a	21.2 b
F_3 from low damage F_2	5	9.8 b	82.7 b	33.1 ab	3.34 cd	38.8 a	22.4 b
F_3 from high damage F_2	2	8.8 b	72.0 b	29.2 b	3.54 a–c	38.0 a	18.3 b
B_1F_2 from low damage B_1	12	13.1 b	78.9 b	31.1 ab	3.75 ab	37.8 a	19.7 b
B_1F_2 from high damage B_1	11	13.6 b	90.4 ab	35.2 ab	3.84 a	37.5 a	21.9 b
AET-5 (P_2)	1	9.0 b	67.5 b	26.8 b	3.07 d	38.1 a	20.1 b

* Means within a column followed by letters in common were not significantly different at the 0.05 level of probability, according to an LSD test.
† Selected for lack of extrafloral nectaries, but not for low or high seed damage.

had a higher lint percentage. Significant additive genetic effects were shown for all five properties. All but lint percentage exhibited significant epistasis, and boll size and bolls/plant showed significant dominance. Heritability estimates were significantly different from zero for seed cotton yield/plant, lint yield/plant, lint percentage, and bolls/plot.

In the five-generation comparisons, Test 1979–2, the parents differed significantly among the agronomic properties measured only for lint percentage (Table 2). Chi-square values from the generation-mean analyses indicated that the additive-dominance model was adequate for all five traits. These results contradicted those for the four agronomic properties which had shown significant epistasis in Table 1. Additive effects were again shown for lint percentage, but not for the other traits. Dominance effects were indicated for boll size and for seed cotton and lint yield/plant. The heritability estimates for the agronomic traits were significant only for lint percentage. The estimated number of genes for lint percentage (pooled data) was about one.

Four of the 70 F_3 families in Test 1979–2 bred true for the nectariless character. All four had significantly lower seed damage and significantly higher lint percentage than DES 24-8ne; but they did not differ from either parent in the other agronomic properties measured, except family 61 had a heavier boll than did AET-5 and fewer bolls/plant than did DES 24-8ne (Table 3). The four F_4 families compared in Test 1980–2 (from the four 1979 F_3 families) also had significantly lower seed damage than DES 24-8ne, but damage not significantly different than AET-5 (Table 4). Also, in Test 1980–2, five F_3 families (reared from nectariless F_2 plants selected in 1979 for low seed damage) displayed low seed damage. Inexplicably, two F_3 families selected from F_2 plants with high seed damage had no higher seed damage than the AET-5 parent. Twelve B_1F_2 families from B_1 nectariless plants selected for low damage and eleven B_1F_2 families from B_1 nectariless plants selected for high damage in 1979 had about the same mean seed damage in 1980 (Table 4).

Mean lint percentages in the F_3, F_4, and B_1F_2 families were significantly higher than in DES 24-8ne, but were not significantly lower than in AET-5. With some exceptions, the other agronomic properties were significantly lower in the hybrid families than in DES 24-8ne (Table 4). The highest yielding B_1F_2 family (41.5 g lint/plant) had been selected in the B_1 for low seed damage. It also had low seed damage in 1980 (12.6%). This same family had a large boll (4.23 g seed cotton/boll) and a high lint percentage (39.36%), but had relatively few bolls/plant (21.4).

DISCUSSION

An efficient use of AET-5 in breeding is probably as a source of PBW resistance having high lint percentage to transfer into superior agronomic backgrounds by backcrossing because of AET-5's numerous agronomic and fiber-property deficiencies (7, 8). For the following reasons, we believe that transferring the AET-5 type of resistance may not be too difficult: i) relative resistance may be determined by estimating seed damage percentages from radiographs; ii) analyses indicated that a significant amount of the genetic variability for seed damage was additive, narrow-sense heritability estimates were significantly different from zero, the genotype × environment interaction for the trait was not significant, and resistance was conditioned by as few as two gene pairs. On the negative side, the additive × additive epistasis for seed damage would be expected to impair selection efficiency.

Our subsequent experience indicates that the main breeding problem will be in separating resistant plants from escapes. For example, F_3 and B_1F_2 families in 1980 from F_2 and B_1 plants selected for low seed damage in 1979 performed about as expected. That is, F_2 and B_1 plants selected for low seed damage had essentially the same seed damage in the next generation as AET-5. On the other hand, F_3 and B_1F_2 means from F_2 and B_1 plants selected for high seed damage were not significantly higher than that of AET-5. This result suggests that the light PBW infestation in 1980 failed to render a rigorous test and that some susceptible plants escaped severe damage.

This supposition is buttressed by 1981 data, in which only one of nine DES 24-8ne × AET-5 F_3 progenies, selected as F_2 plants in 1980 for low seed damage, had significantly lower seed damage than the DES 24-8ne parent (15.8 vs. 22.8% seed damage, respectively; LSD = 6.9% at the 0.05 level of probability). Also, only three of 13 B_1F_2 progenies in 1981, selected as B_1 plants in 1980 for low seed damage, had significantly lower seed damage than DES 24-8ne (14.7, 15.0, and 15.8% seed damage, respectively).

Another approach that we used in 1980 and 1981 was to count PBW entrance holes/boll in parents and various of their progenies to accelerate the breeding program. Theoretically, plants or progenies having fewer entrance holes/boll could be selected and used as parents the same season, or at least brought into the greenhouse for winter crosses, thus reducing the generation time. Unfortunately, this method proved not to be practical on the scale necessary for a breeding program. Its main deficiency was a high experimental error caused by variation in counts among observers.

Because of the problems discussed above, we have settled on a backcross breeding program that will require 2 years/cycle. After selection of F_3 families with low seed damage in the field in Arizona in year 1, plants from those families will be moved to the greenhouse and backcrossed to the recurrent parent in year 1-2. B_1 will be increased in Arizona in year 2 and B_1F_2 will be increased in Mexico in year 2-3. B_1F_3 families will be screened in the field in Arizona in year 3 and selected for low seed damage. The crossing-increase-testing cycle will then be repeated as often as judged necessary.

Transfer of high lint percentage from AET-5 into DES 24-8ne should also be relatively easy via backcrossing because the trait was inherited in an additive fashion, was apparently conditioned by only one gene pair, and was highly heritable. Some selection pressure for that trait would merely have to be exerted after each backcross.

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