

## BREEDING & GENETICS

### Evaluation of Injury by Tarnished Plant Bugs (*Lygus lineolaris* Palisot de Beauvois) to Blended Cotton Genotypes

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#### INTERPRETIVE SUMMARY

Yield loss estimates attributed to tarnished plant bugs are typically low, compared with losses associated with other cotton insect pests. However, this pest is pivotal because early-season spraying for plant bugs reduces beneficial insects and exacerbates other insect pest problems. Studies reported in the literature indicate the sensitivity of cotton genotypes is, to some degree, dependent on their morphological characteristics. Genotypes expressing the Frego bract characteristic are highly sensitive to plant bugs, while nectariless genotypes have some resistance. The effects of other characteristics are not well established.

Earlier work done in Texas suggested that in a mixed planting of two genotypes possessing contrasting morphological traits, damage by fleahoppers was reduced, compared with either genotype planted alone. In that report, the authors suggested that the additional time that fleahoppers spent moving among plants (searching) may have increased the effectiveness of the more resistant genotype.

Mixing of seed from different genotypes prior to planting (forming blends) is not a new concept. In other crops, blends composed of resistant and susceptible genotypes have been shown to effectively slow the development of new races of certain disease pathogens. Thus, the objective of this study was to determine whether blends of cotton genotypes that possess contrasting morphological traits might reduce damage from plant bugs.

Tests were conducted at the Cotton Branch Station near Marianna, AR, in 1991, and at the

Delta Branch Experiment Station near Clarkedale, AR, in 1991 and 1992. Within each test, five cotton genotypes (each of which can be distinguished by morphological traits) were planted as pure lines and as component lines in blends. Prior to planting, equal numbers of seed of the genotypes were blended to form all possible two-component blends.

The plots did not receive any insecticide treatment or irrigation. Large squares that did not have any boll weevil punctures were examined for plant bug injury; and the percentage of squares with discolored anthers was determined.

In all three tests, plant bug damage to each blend was intermediate to damage to its two component lines. Therefore, in contrast to that reported for fleahopper, mixing of genotypes did not reduce plant bug damage, compared with the more resistant component line.

In this study we demonstrate a relatively novel use of a genetic analysis. Using pure lines to represent parents, and the blends to represent the F<sub>1</sub> generation, the data were evaluated using diallel analysis. Estimates of general blending ability was assumed to reflect the additive effects of the genotypes, and specific blending ability was assumed to reflect the nonadditive effects of the genotypes. These allowed the relative influence of each line in blends to be estimated. The diallel analysis confirmed that variation among blends was additive, which means there is no advantage in reducing plant bug damage by blending these genotypes.

Components of blends that express significant additive and/or nonadditive effects (in the direction of less injury) might be good candidates for parents in a breeding program to improve resistance to plant bugs.

#### ABSTRACT

**Plant bugs, *Lygus lineolaris* (Palisot de Beauvois) and *Neurocolpus nubilius* (Say), can delay**

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early fruiting of cotton (*Gossypium hirsutum* L.), and thus increase risks associated with other cotton insect pests. Injury from plant bugs might be lessened by seeding mixtures of cotton genotypes (blends) rather than pure lines. Our objectives were to evaluate plant bug injury in pure lines and all possible two-component blends of five cotton genotypes and to illustrate the use of diallel analysis for evaluating these blends for plant breeding purposes. Five cotton genotypes, which had distinguishing morphological traits, and 10 blends (all possible two-part combinations of the five genotypes) were evaluated in tests at Marianna, AR, in 1991 and at Clarkedale, AR, in 1991 and 1992. Following plant bug infestation, squares were randomly collected from each plot and evaluated for plant bug injury to anthers. Blending ability for plant bug injury was evaluated using a diallel analysis with the pure lines representing parents and the blends representing the  $F_1$  generation. Variation among the genotypes evaluated as pure lines was consistent with previous reports. The diallel analysis indicated significant general blending ability (additive effects) and nonsignificant specific blending ability (nonadditive effects) for plant bug injury in each test. Plant bug injury to blends was intermediate to injury to their respective component genotypes. Blends had no advantage over pure lines of the genotypes with respect to plant bug injury. No synergism was found in the blend of the two most resistant lines. This approach may be an effective method for choosing potential parents in a breeding program that emphasizes host plant resistance.

From 1991 through 1996, estimated cotton yield losses associated with plant bugs averaged less than 0.8% in Arkansas with a high of 2.2% in 1996 (Hardee and Herzog, 1997). These estimates do not reflect the seriousness of these pests to many cotton growing regions. By damaging small squares, plant bugs may have little direct effect on yield unless damaging population levels persist for an extended time.

However, loss of small squares delays critical fruiting and maturation and disturbs the vegetative-to-fruiting balance. Increased vegetative growth then enhances internal shading and physiological shedding. Any delay in fruiting will increase the plant's vulnerability to other major cotton pests. Thus, control of plant bugs is often pivotal to avoiding damage by other pests, maintenance of optimum plant growth and development, and precise crop management.

Pack and Tugwell (1976) described damage by plant bugs to include injury to anthers in flower buds and punctures of carpel walls in young bolls. Their research indicated that the insect preferred "pinhead" (less than 3 mm) squares to older fruit. Tugwell (1983) indicated that damage by plant bugs to small squares could be detected by slicing squares and evaluating anther damage. Using this approach, Maredia et al. (1994) developed a field technique for evaluating cotton genotypes for injury from plant bugs. Independently, Jones et al. (1984) developed and used damaged androecia as a means of evaluating response of cotton genotypes to plant bugs.

Blending seed (prior to seeding) of genotypes that vary in resistance to a specific pest can sometimes assist pest control. Van der Plank (1968) indicated that multilines (blends of near-isogenic lines) can be an effective means to avoid epiphytotic by stabilizing disease pathogens and preserving vertical-resistant genes. Similarly, refugia planting of conventional cotton genotypes with transgenic genotypes has been suggested as a means to preserve the effectiveness of the Bt gene in cotton (Benedict, 1996).

In addition to preserving genes conferring resistance to insect pests, a mixture of contrasting genotypes may reduce insect activity by causing them to spend more time moving between plants. Cantelo and Sanford (1984) found different pests varied in their responses to mixed and uniform populations of resistant and susceptible cultivars. Benedict et al. (1986) showed that mixed populations of glabrous and hirsute cotton genotypes incurred less damage by fleahoppers, *Pseudatomoscelis seriatus* (Reuter), and yielded more at first and total harvest than uniform seeding of either component line. Control of tarnished plant bugs in cotton might be enhanced if a similar response could be found to mixed populations.

To be effective in a blend, component genotypes must vary in response to the pest. Variation in response to tarnished plant bugs in cotton has been associated with several morphological traits including pubescent, compared with glabrous leaves and stems (Meredith and Schuster, 1979); nectariless compared with nectaried leaves and flowers (Laster and Meredith, 1974; Meredith et al., 1973; Schuster et al., 1976); and normal bracts compared with flego/rolled bracts (Jones et al., 1969; Lincoln et al.,

1971; Maredia et al., 1993). Most of these morphological traits have specific positive and negative effects with regard to response to insect pests or to agronomic performance (Jenkins, 1982).

The objectives of this study were to determine the effect of blended populations of cotton genotypes (blends) on degree of tarnished plant bug injury and to demonstrate the use of diallel analysis for evaluating blends made of two equal components.

## MATERIALS AND METHODS

Five cotton genotypes were evaluated when seeded alone (pure lines) and as component lines in blends. The genotypes included three cultivars (Deltapine 50, Stoneville 132, and Stoneville 825), a released germplasm line (Arkot 8110, Bourland et al., 1997), and a breeding line, Frego 25 (developed at the University of Arkansas by B.A. Waddle). Distinguishing morphological traits of the genotypes are listed in Table 1.

Prior to seeding, equal numbers of seed of the genotypes were blended to form all possible two-component blends. The five genotypes and 10 blends were seeded at the Cotton Branch Station near Marianna, AR, on 10 May 1991, and at the Delta Branch Experiment Station near Clarkedale, AR, on 21 May 1991 and 6 May 1992. Soil type at Marianna was Calloway silt loam (fine-silty, mixed, active, thermic Glossaquic Fragiudalfs) and at Clarkedale was Dubbs (fine-silty, mixed, active, thermic Typic Hapludalfs)–Dundee (fine-silty, mixed, active thermic Typic Endoaqualfs) complex. Plots were two rows, 13.5m long,  $\approx$  1 m apart, and were arranged in a randomized complete block design with four replications. Except for no insecticide treatment and no irrigation, standard production practices were followed.

A large square (prior to corolla extension) was taken from near the plant apex of 25 random plants within each plot. Squares were sampled just prior to flowering from the test at Marianna and on 1 Aug. 1991, and from the tests at Clarkedale on 7 Aug. 1992. Squares punctured by boll weevil, *Anthrenus grandis* Boheman, were discarded from the samples. The number discarded in any one sample never exceeded three. Each remaining square bud was cut transversely at the point of its largest diameter. The pointed end of the bud was rolled to

**Table 1. Contrasting morphological traits of the five cotton genotypes used in this Arkansas study.**

Genotype	Bract shape	Leaf/floral nectaries	Pubescence	
			Leaf	Stem
Arkot 8110	Normal	Present	Glabrous	Glabrous
Frego 25	Rolled	Present	Dense	Dense
Deltapine 50	Normal	Present	Glabrous	Moderate
Stoneville 132	Normal	Present	Moderate	Moderate
Stoneville 825	Normal	Absent	Dense	Dense

expose the anthers and examined for plant bug injury, as described by Maredia et al. (1994).

The percentage of squares with discolored anthers was determined for each plot and evaluated by analysis of variance. The percentage data were subjected to arc sine transformation prior to analysis. Because the test by entry interaction was significant, the data were analyzed for each test site separately. Using pure lines to represent parents and the blends to represent the  $F_1$  generation, the data were then evaluated by Griffing's (1956) diallel analysis, method 2, model 1 (fixed). Combining ability was not estimated from offspring of genetic combinations, so the terms *general blending ability* and *specific blending ability* are used instead of *general combining ability* and *specific combining ability*. Estimates of general blending ability and specific blending ability were assumed to reflect the additive and nonadditive effects of the genotypes, respectively. The relative influences of each line in blends could then be estimated. This use of diallel analysis and terminology is similar to Gizlice et al. (1989), who studied blends of soybean.

## RESULTS AND DISCUSSION

Among the genotypes evaluated as pure lines, Frego 25 had the greatest injury while Stoneville 825 and Arkot 8110 had the least injury within each test (Table 2). The high injury level for the rolled bract genotype and low injury for the nectariless genotype were expected. The relative resistance of Arkot 8110 to plant bugs was consistent with other tests (Bourland et al., 1996) and suggests that its resistance level is greater than expected for genotypes possessing glabrous leaves and stems (Laster and Meredith, 1974; Jones et al., 1977).

In each test, relative damage to the pure lines of Stoneville 132 and Deltapine 50 varied, but was always intermediate to the other genotypes (Table 2)

**Table 2. Levels of anther damage to pure lines and blends of five cotton genotypes found in an Arkansas study.**

Genotype by planting method	Percentage of squares with damaged anthers <sup>†</sup> by test		
	1991 Clarkedale	1991 Marianna	1992 Clarkedale
<b>Pure lines</b>			
Frego 25	69	49	85
Stoneville 132	16	23	44
Deltapine 50	33	29	21
Arkot 8110	16	14	25
Stoneville 825	8	12	19
<b>Blends</b>			
Frego 25 + Stoneville 132	44 (42) <sup>‡</sup>	20 (36)	72 (65)
Frego 25 + Deltapine 50	52 (53)	28 (39)	38 (53)
Frego 25 + Arkot 8110	43 (42)	29 (31)	52 (55)
Frego 25 + Stoneville 825	28 (38)	18 (31)	48 (52)
Stoneville 132 + Deltapine 50	18 (27)	24 (26)	29 (33)
Stoneville 132 + Arkot 8110	24 (16)	33 (18)	21 (35)
Stoneville 132 + Stoneville 825	16 (12)	19 (16)	24 (32)
Deltapine 50 + Arkot 8110	24 (27)	24 (21)	24 (23)
Deltapine 50 + Stoneville 825	20 (23)	11 (21)	21 (20)
Arkot 8110 + Stoneville 825	9 (12)	11 (13)	22 (22)
LSD 0.05	18	17	17

<sup>†</sup> Anther damage determined for 25 squares/plot using techniques of Maredia et al. (1994).

<sup>‡</sup> Mean of component lines is in parenthesis.

Stoneville 132 exhibited relatively high resistance in the 1991 Clarkedale test, but was relatively susceptible in the two other tests. Its early maturity and more determinate growth habit might have contributed to this variation. Deltapine 50 was the only smooth leaf, hirsute (moderate) stem genotype in this test. The effect of the smooth leaf trait on plant bugs is not well established.

Anther damage associated with each blend was generally intermediate to the mean damage associated with its respective component lines (Table 2). This suggests that the combination of genotypes having contrasting response to plant bugs did not provide any advantage for reducing injury.

Across the tests, responses of blends to plant bugs would be expected to vary more than the responses of pure lines because relative growth and competitiveness of plants from the component lines may vary in the different environments. Thus, environment might affect which plants become more dominant within a blend. Three of the blends (Frego 25 + Stoneville 132, Stoneville 132 + Deltapine 50, and Stoneville 132 + Arkot 8110) that were the most variable in the tests all had Stoneville 132 as one component.

Diallel analysis of the data confirmed that the influence of the component lines was additive (Table

**Table 3. Diallel analysis of anther damage for cotton genotypes evaluated as pure lines and blends<sup>†</sup> in a 2-yr study in Arkansas.**

Source of variation	Mean squares by test year and location		
	1991 Clarkedale	1991 Marianna	1992 Clarkedale
<b>Blend</b>	463*	1161**	1628**
Additive effects (general blending ability)	1051**	3857**	5240**
Nonadditive effects (specific blending ability)	228	82	183
Error	148	166	132

\* Significantly different from zero at  $P = 0.05$ .

\*\* Significantly different from zero at  $P = 0.01$ .

<sup>†</sup> Parental and  $F_1$  generations represented by pure lines and blends, respectively.

3). Additive effects (general blending ability) were significant in each test, and were the major source of variation. The intermediate performance of blends was confirmed by the nonsignificant estimates of nonadditive effects (specific blending ability). Thus, performance of blends could be predicted by performance of their component lines.

Therefore, in contrast to that found for fleahoppers (Benedict et al., 1986), mixed populations of resistant and susceptible lines did not provide an advantage over the resistant lines. Apparently, tarnished plant bugs were not confused by the mixed populations of plants with varying morphological traits. The relative attractiveness of the genotypes was apparently constant in pure lines and blends.

The relative influence of each genotype on anther damage of the blends was determined by comparing estimates of their general blending ability. When included in a blend, Frego 25 was the only genotype that was consistently associated with an increase in anther damage (Table 4). As found when evaluated as pure lines, the influence of Stoneville 132 and Deltapine 50 varied greatly among the tests. Both Stoneville 825 and Arkot 8110 negatively influenced damage when they were a component line of a blend. The performance of these two genotypes, which have contrasting morphological traits and contrasting genetic backgrounds, suggests the presence of different sources and/or mechanisms of resistance to plant bugs. However, the Arkot 8110 + Stoneville 825 blend was not superior to the pure line of Stoneville 825. Thus, no synergism between the two mechanisms for resistance was exhibited.

**Table 4. Additive effects (general blending ability) associated with anther damage for cotton genotypes evaluated as pure lines and blends in a 2-yr study in Arkansas.**

Genotype	Additive effects by test year and location		
	1991 Clarkedale	1991 Marianna	1992 Clarkedale
Frego 25	7.3	19.3	22.9
Deltapine 50	0.4	1.7	-9.1
Stoneville 132	3.6	-4.7	2.3
Arkot 8110	-2.6	-5.0	-6.9
Stoneville 825	-8.8	-11.8	-9.2
Standard error	2.1	2.2	2.0

Diallel blend analysis might be a useful method of determining potential good parental combinations for improving resistance to tarnished plant bugs. Genotypes suspected to have some resistance could be evaluated in various blends. Blends having significant additive and/or nonadditive effects in the direction of less injury would likely be good parental combinations. If this strategy for selecting parents were effective, superior lines could be developed more efficiently.

## CONCLUSIONS

Resistance to tarnished plant bugs was not improved by blending seed of cotton genotypes that had different levels of resistance. Responses of the blends to plant bugs was generally intermediate to their respective component lines. Evaluation of two-component blends using diallel analysis may be an effective means of assisting breeders to choose the best parental combinations for improving resistance to plant bugs.

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