

Development of Cotton Germplasm for Reduced Insecticide Use Production Systems

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ABSTRACT. Management of the bollworm (*Helicoverpa zea* [Boddie]) and tobacco budworm (*Heliothis virescens* [F.]) complex in cotton (*Gossypium hirsutum* L.) is a significant production cost and a large contributor of insecticides to the environment. Genetic resistance or tolerance could help alleviate these problems. We conducted a trial to identify and exploit sources of bollworm/tobacco budworm resistance in the Pee Dee (PD) cotton germplasm. The experiment consisted of selecting phenotypically desirable cotton plants in segregating populations when produced with limited control of bollworm/tobacco budworm. Although this methodology had previously been successful in identifying resistant types, we were unable to demonstrate a response to selection. The study did find that PD 0762, a germplasm released as possessing resistance to bollworm/tobacco budworm based on lint yield in limited control experiments, likely is tolerant of square loss as its reaction to this insect complex does not suggest resistance. We also found that a new germplasm PD 0786 has a low level of resistance to bollworm/tobacco budworm and therefore could be a component of sustainable cotton production

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INTRODUCTION

Sustainable agricultural systems rely on fewer chemical inputs including pesticides. Since the elimination of the boll weevil (*Anthonomus grandis grandis* Boheman) as an economic pest in much of the southeastern USA, cotton production requires less insecticide. However, the cotton bollworm (*Helicoverpa zea* [Boddie]) and tobacco budworm (*Heliothis virescens* [F.]) remain destructive cotton insect pests, annually requiring 4-7 insecticide applications for their control (M.E. Roof, personal communication). Resistant cultivars would be a valuable component of sustainable cotton production systems for the USA and developing countries. Recently developed transgenic cultivars that produce the delta-endotoxin from the bacterium *Bacillus thuringiensis* subsp. *kurstaki* (Berliner) (Bt cotton) for control of bollworm and tobacco budworm provide growers with a new tool to manage this insect complex (Benedict, 1996). Despite the impressive control of bollworm/budworm achieved with the Bt technology, we feel that host resistance breeding should not simply be abandoned. Technology fees levied by biotechnology companies to utilize Bt cultivars could be cost prohibitive for developing countries and some concern exists about the sustainability of the Bt resistance with repeated exposure to large insect populations (Benedict, 1996).

The study of various plant characteristics as host resistance traits for the bollworm/tobacco budworm complex has produced conflicting results. Reduced trichome density on cotton leaves has been associated with less oviposition (Jenkins et al. 1979, Lukefahr et al. 1971, Lukefahr et al. 1975) and with reduced oviposition and fewer larvae in several studies (Lukefahr et al. 1965, Jones et al. 1977, Robinson et al. 1980). However, Culp et al. (1979) were unable to demonstrate reduced oviposition on glabrous versus hirsute cottons. High square gossypol content can lower larval survival and growth (Lukefahr et al. 1966, Oliver et al. 1971, Lukefahr et al. 1975). In contrast, Lee (1976) reported that high gossypol did not provide sufficient protection against square damage but was associated with reduced boll damage. Frego bract has also been associated with nonpreference and/or lower larval survival (Lincoln et al. 1971). Subsequent studies by Culp et al. (1979) questioned the utility of characteristics such as glabrousness and high gossypol content as resistance traits. Resistant germplasm developed by Culp et al. (1979) was compared with gla-

brous, glabrous-nectariless, and high gossypol lines under a reduced and no-insecticide regime. Genotypes not receiving insecticide produced no measurable yield of cotton and the study concluded that at least some insecticide would be necessary to supplement resistance traits.

Culp et al. (1979) reported an uncharacterized mode of resistance to the bollworm/budworm complex in the Pee Dee (PD) cotton germplasm collection. Resistant PD genotypes generally had one-half or less the density of live larvae compared with susceptible cultivars when evaluated under a regimen of one-half the recommended rate of insecticide applied at 3-7 or 5-7 day intervals. These tests relied on natural bollworm/tobacco budworm infestations. The authors eliminated oviposition preference and antibiosis resulting from plant terpenoids as possible sources of resistance to the complex. Subsequently, Culp (1979) released PD 695 and PD 875 as germplasm lines with resistance to bollworm/tobacco budworm. Jenkins et al. (1984) confirmed the resistance of PD 875 to *H. virescens* in artificially inoculated tests. These lines were then utilized to breed additional resistant germplasm including PD 0762 (Culp et al. 1990). Resistance of the germplasm released in 1990 was measured as lint yield compared with that of germplasm with a known level of resistance to bollworm/tobacco budworm when minimal control measures were employed. Reaction of this germplasm to bollworm/tobacco budworm when produced without insecticide has not been reported.

We conducted the following study to identify and exploit sources of bollworm/tobacco budworm resistance (Culp et al. 1990) and to measure reaction of the germplasm to this insect complex when produced without insecticide.

MATERIALS AND METHODS

Population Formation and Mass Selection

A released germplasm line PD 0762 (Culp et al. 1990), an unreleased germplasm PD 0786, and Stoneville 69132 (ST 69132) were chosen as parents of new populations based on their top yielding status in the cotton Regional Bollworm-Tobacco Budworm Test at Florence, SC in 1988 (USDA 1989). The purpose of the Bollworm/Tobacco Budworm Test was to measure host resistance at locations across the cotton belt of cotton germplasm to this insect complex. Based on these data implying possible resistance, we crossed PD 0762 and PD 0786 with Stoneville 69132 to create two new breeding populations for the further exploitation of boll-

worm/tobacco budworm resistance. The F1 populations were created in 1989 and each was self-pollinated at a winter nursery in Mexico in 1989-1990 to produce the F2 generation. The F2 generation of the two populations was evaluated in 1990 at the Pee Dee Research and Education Center (PDREC) near Florence, SC. Plot size was 2 rows, 10.6 m long with 96 cm row spacing. Four plots of each F2 population were thinned to a stand of one plant per 0.3 m at the 2-leaf stage to facilitate selection of individual plants. Mass selection was conducted whereby the phenotypically most desirable plants were kept and the remainder discarded when produced with limited insect control. Assessment of the response of individual plants to this insect complex on multiple occasions during the growing season is not feasible, hence the use of an indirect selection criteria. Thiodicarb (Larvin 3.2 AF [aqueous flowable], 0.90 kg [AI]/ha, Rhone-Poulenc Ag. Co., Research Triangle Park, NC) insecticide was applied only when densities of bollworm/tobacco budworm larvae or square damage exceeded twice the established threshold for economic loss based on Clemson University recommendations (Roof et al. 1990). A random sample of F2 plants was scouted twice weekly for the presence of bollworm/tobacco budworm utilizing recommended practices (Roof et al. 1988). This procedure resulted in a single insecticide application for control of bollworm/tobacco budworm in 1990. Only non-frego bract plants were selected, as the intent was to incorporate the uncharacterized source of resistance to the bollworm/tobacco budworm complex in the PD germplasm (Culp et al. 1979) into a normal-bract genetic background. The selection intensity was between 10 and 20% in both populations. At maturity, all open bolls on selected plants were harvested and the seedcotton was ginned. This seed represented the first cycle (Cycle 1) of selection. The second cycle of selection was completed in 1992. An equal amount of seed from each Cycle 1 selected plant was combined to form a composite sample within populations. A second cycle of mass selection was then accomplished using the same methods as in Cycle 1. Higher bollworm/tobacco budworm densities in 1992 required the application of cypermethrin (Cymbush 3EC, ICI Corp., Wilmington, DE) twice at 0.09 kg (AI)/ha and once at 0.12 kg (AI)/ha. Thiodicarb (0.22 kg [AI]/ha) was applied once as an ovicide in combination with cypermethrin.

Evaluation of Reaction to Bollworm/Tobacco Budworm

Response to bollworm/tobacco budworm was evaluated in 1993 and 1994 at the PDREC near Florence, SC. The Cycle 0 (remnant F2 seed from the cross of either PD 0762/Stoneville 69132 or PD 0786/Stoneville 69132), Cycle 1, and Cycle 2 of each population along with the two

parents PD 0762 or PD 0786, plus Stoneville 69132, and a non-resistant check, 'Coker 315,' were planted in separate randomized complete block designs with four replicates. Each test in 1993 and 1994 consisted of the three selection cycles (Cycles 0, 1, and 2), the two parents (ST 69132 and either PD 0762 or PD 0786), and Coker 315. Plot size was two rows, 10.6 m long with 96 cm row spacing. Other than not applying insecticides for control of bollworm/tobacco budworm, we followed cotton cultural practices recommended by the Clemson University Cooperative Extension Service.

Bollworm/tobacco budworm eggs, larvae, and damaged squares were counted on 25 plants per plot (12 consecutive plants in row 1 and 13 in row 2 of each plot), 100 plants per entry, twice weekly from late June through early August. Larvae were not identified as to species. Eggs collected by the junior author at the PDREC and reared to larval stage in addition to larval collections in seasons during and after completion of this experiment (1992-1996) indicated a range in ratio of 62:38% to 100:0% of bollworm:tobacco budworm, respectively. Bollworms comprised at least 80% of the complex on 16 of 18 collections. Squares examined for damage by bollworm/tobacco budworm were selected at random and represented various developmental stages. Damaged squares were defined as those exhibiting feeding symptoms from bollworm/tobacco budworm (Roof et al. 1988). Data from each replicate on each sample date were taken by a single individual and scouts were rotated among replicates between sample dates to avoid having the same individual take data from the same replicate over the season. A common insect scouting method is to randomly select plants (Roof et al. 1988) within the field from which insect and insect-damaged square counts are made. Because of the size of this experiment and the time necessary to examine 25 plants per plot, we chose to delineate the plants *a priori* within each plot from which bollworm/tobacco budworm data were taken. Nonrandom sampling methods were previously shown to detect economic infestations of bollworm and tobacco budworm (Hopkins et al. 1980). These data were enumerated on 16 dates in 1993 and 11 dates in 1994. After plants began to actively set squares, 2 squares per plant were examined for bollworm/tobacco budworm damage.

Data transformation procedures outlined by Gomez and Gomez (1984) were applied to the percent damaged square and density of bollworm/tobacco budworm larvae and eggs to satisfy statistical assumptions for analysis of variance (ANOVA). Sample dates were not cross-classified with years and thus, for combined ANOVA across yr, plot seasonal totals were utilized for percent damaged squares and densities of bollworm/tobacco

budworm larvae and eggs. In the combined ANOVA across yr, entry differences were tested against the entry \times yr mean square; yr were considered random effects. ANOVAs were also conducted within yr by sample date. All ANOVAs were conducted by SAS procedures (SAS Institute 1990). Herbicide damage caused loss of five plots in 1994 in the PD 0762/Stoneville 69132 population. For these data and combined analysis over yr, PROC GLM (SAS Institute, 1990) was used for ANOVA. Least-squares means from the GLM analysis were separated with the PDIFF option which utilizes a t-test for mean comparison (SAS Institute 1990). For the PD 0786/Stoneville 69132 data and the balanced 1993 data of the PD 0762/Stoneville 69132 population, genotype means were separated with LSD when the F test for genotypic differences was significant ($P < 0.05$).

RESULTS AND DISCUSSION

Selection based on a visual yield estimate at maturity with limited control of bollworm/tobacco budworm was not associated with lower percent damaged squares in the PD 0762/Stoneville 69132 population (Table 1; $F = 2.55$, $df = 5,5$; $P = 0.164$). Variation among entry means for percent damaged squares was small and not significant. Similar results were obtained when ANOVAs were conducted by sample date within yr (data not shown). Without insecticide for bollworm/tobacco budworm control, PD 0762 did not exhibit significantly reduced square damage compared with susceptible Coker 315, although it did harbor fewer bollworm/tobacco budworm larvae (Table 1). Based on lint yield, Culp et al. (1990) confirmed that PD 0762 was more resistant than its parents, PD 875 and PD 695, when bollworm/tobacco budworm were controlled if damaged squares reached 10% or two larvae per 100 plant terminals were found on PD 695. Apparently, the resistance of PD 0762 is overwhelmed when high densities of bollworm/tobacco budworm occur. Alternatively, PD 0762 is tolerant of insect induced square loss and not resistant per se, its yield in experiments by Culp et al. (1990) reflecting cottons ability to compensate for square loss (Mann et al. 1997).

Differences in percent damaged squares were significant ($F = 6.14$, $df = 5,5$; $P < 0.05$) among entries in the PD 0786/Stoneville 69132 population (Table 1). However, most of the significant comparisons were with PD 0786, which suffered less square damage than all the other entries except Cycle 0. As in the PD 0762/Stoneville 69132 population, selection was not associated with reduced square damage among Cycles 0, 1, and 2. ANOVAs within yr for the PD 0786/ST 69132 population revealed significant ($P < 0.05$) differences in percent damaged squares on two sample dates in

TABLE 1. Reaction of cotton germplasm to the bollworm/tobacco budworm complex produced without control for 2 years at Florence, SC.

Entry ^a	PD 0762/ST 69132 Expt.				PD 0786/ST 69132 Expt.			
	Percent Damaged	No. Squares	No. Larvae	No. Eggs	Percent Damaged	No. Squares	No. Larvae	No. Eggs
Cycle 0	7.7a	11b	11b	55ac	8.5bc	8b	8b	45a
Cycle 1	9.9a	13ab	13ab	48cd	9.3ab	13ab	13ab	50a
Cycle 2	7.4a	13bc	13bc	41bd	10.1ab	14a	14a	61a
PD 0762	7.6a	8d	8d	56ac	7.0c	9b	9b	59a
ST 69132	8.7a	15ac	15ac	48cd	9.3ab	17a	17a	58a
Coker 315	9.4a	17a	17a	51cd	11.6a	15a	15a	51a

^a Entry means reflect cumulative insect data summed for 25 plants in each replicate over the 16 sample dates in 1993 and 11 dates in 1994. Means within a column followed by the same letter are not significantly different based on a t-test or LSD in the PD 0762/ST 69132 and PD 0786/ST69132 experiments, respectively (P = 0.05; SAS Institute, 1990).

1993 (6/28 and 7/19) and the 7/18 sample date in 1994. The rank of genotype means (data not shown) on the 6/28 and 7/18 sample dates were consistent with the 2-yr seasonal means (Table 1), with Coker 315 suffering the most square damage and PD 0786 the least square damage. On the 7/19 date, the entry means indicated no difference in square damage among selection cycles and no significant difference between Coker 315 and PD 0786. In the PD 0762/ST 69132 population, Cycle 2 exhibited significantly reduced oviposition compared with the Cycle 0 or PD 0762, yet the Cycle 2 had more larvae than PD 0762 (Table 1). Variation for oviposition was not significant ($F = 1.87$, $df = 5,5$; $P = 0.2538$) among entries in the PD 0786/ST 69132 population yet PD 0786 had fewer larvae than ST 69132 or Coker 315 (Table 1). These data support Culp et al. (1979) in that resistant PD germplasm may experience similar oviposition as nonresistant genotypes yet still harbor significantly fewer larvae. The data demonstrate that the unreleased germplasm PD 0786 has a low level of resistance to bollworm/tobacco budworm.

Subsequent to the initiation of our study, Jenkins and McCarty (1994) reported that ST 69132 is resistant to tobacco budworm. They assessed resistance in experiments artificially and uniformly infested with tobacco budworm by comparing yields between plots with and without insecticide for tobacco budworm control. We were not able to demonstrate a significant difference in percent damaged squares between ST 69132 and non-resistant Coker 315 based on 2-yr means from seasonal totals. The discrepancy in the findings of the two studies may be related to variation in pest spectrum between Mississippi and South Carolina. A comparison of the composition of bollworm/tobacco budworm population densities from nearby cotton in our study that had not been treated with insecticide revealed that in *both years* tobacco budworm was the predominant species (65-100%) in late-June and early-July, while the bollworm was most frequently observed in mid-July through August (JAD, unpublished data). In 1993, we observed significant differences among entries for percent damaged squares on the 6/28 sample date in the PD 0786/ST 69132 population when tobacco budworm was the predominant species attacking cotton (data not shown). On that sample date, fewer damaged squares were observed on ST 69132 (9.8%) compared with Coker 315 (20.6%), supporting Jenkins and McCarty (1994) that ST 69132 has a useful level of resistance to tobacco budworm.

Putatively resistant plants were selected in our study based on a visual yield estimate at maturity when bollworm/tobacco budworm damage was allowed to exceed established thresholds for the initiation of control. Breeders frequently use a simple mass selection scheme in early segregat-

ing generations to allow evaluation of many experimental types. Our data indicate it likely that a portion of the plants selected escaped or tolerated insect damage rather than expressed resistance. Culp et al. (1979), however, were able to develop resistant types PD 695 and PD 875 using similar methodology. We also selected against frego bract (Green 1955) plants during each cycle of selection. Some producers consider frego bract undesirable based on anecdotal evidence that plants expressing this trait are less productive than normal bract plants. If the gene or genes conferring resistance in the PD germplasm are linked to the gene conditioning frego bract, this may be another reason we were unable to demonstrate a response to selection.

Reduction of pesticide use for cotton production could increase grower profits and lessen cotton's contribution of pesticides to the environment. Cotton cultivars genetically engineered to produce the delta-endotoxin from the bacterium *Bacillus thuringiensis* subsp. *kurstaki* (Berliner) (Bt cotton) provide effective control of tobacco budworm and generally bollworm and will help achieve these goals. Even though germplasm such as PD 0786 only has a low level of resistance, it might complement the Bt technology in terms of efficacy against bollworm and in resistance management. The Bt toxin is more effective against tobacco budworm than bollworm (Benedict et al. 1993). In South Carolina, we have observed that the bollworm usually is the predominant species attacking cotton during July and August (Mahaffey et al. 1994; Mann et al. 1997), and Bt cotton could suffer economic damage due to heavy infestations of bollworm. In terms of resistance management, PD 0786 harbored fewer larvae and thus when combined with Bt might place less selection pressure on the bollworm population to develop resistance.

CONCLUSIONS

In summary, we were not able to further exploit the source of bollworm/tobacco budworm resistance identified by Culp et al. (1990). Future efforts at utilizing resistance may have to include a direct measure when segregating populations are evaluated. A disadvantage of this practice is that it would severely limit population size and potentially agronomic advance. We did find that the unreleased germplasm PD 0786 has a low level of resistance as evidenced by less square damage and fewer bollworm/tobacco budworm larvae compared with non-resistant Coker 315 when produced without insecticide. PD 0762 appears to be tolerant of insect induced square loss as its reaction to bollworm/tobacco budworm based on square damage suggest it is not resistant when high densities of bollworm/

tobacco budworm occur. In addition to possibly complimenting the Bt technology, the low level of bollworm/tobacco budworm resistance of PD 0786 might be amenable for use in developing countries where pesticides are unavailable.

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