

Alabama Cotton Commission
FY 2007
Final Report

PROJECT TITLE: Production and Characterization of Bt resistance in Cotton Bollworm, *Helicoverpa zea*

PI: William J. Moar
Department of Entomology/Plant Pathology
301 Funchess Hall,
Auburn University, AL 36849

PROJECT NUMBER: 03-348AL

BACKGROUND:

Since 2004, we have selected for Bt resistance in cotton bollworm (CBW), *Helicoverpa zea*, the last major cotton caterpillar pest in which there is no Bt resistant colony, and the more difficult of the two major caterpillar pests to control in Bt and non-Bt cotton. We currently have a population of CBW with greater than 100-fold resistance to Cry1Ac (the Bt in Bollgard, and in Widestrike).

For 2007 we proposed to:

- 1) Continue laboratory selection with our Cry1Ac toxin resistant strain of CBW
- 2) Determine the characteristics in MVPII that negatively impact the resistant strain
- 3) Once 100-fold resistance is achieved, the following experiments will be conducted:
 - a) Evaluate fitness costs
 - b) Determine level of resistance needed to survive on Bollgard cotton
 - c) Select for resistance using Cry1Ac protoxin
- 4) Select for Cry2a resistance

RESULTS: Note: Research conducted from 2004-2007 (with funding from Cotton Incorporated) has recently been published in: Appl. Environ. Microbiol. 2008. Production and Characterization of *Bacillus thuringiensis* Cry1Ac-resistant cotton bollworm, *Helicoverpa zea* (Boddie) Konasale J. Anilkumar, Ana Rodrigo-Simón, Juan Ferré, Marianne Pusztai-Carey, Sakuntala Sivasupramaniam, and William J. Moar

<http://aem.asm.org/cgi/content/abstract/AEM.01612-07v1>

Laboratory selection with the Cry1Ac toxin resistant strain of CBW continued throughout 2007. Although selection pressure remained the same (500ppm Cry1Ac toxin), resistance did increase to 150-200 fold. Cry1Ac-resistant CBW had negligible cross resistance to protoxin (Cry1Ac form found in MVPII). There were no binding differences for both Cry1Ac, Cry1Aa and GalNac further suggesting that the primary mechanism of resistance to Cry1Ac toxin IS NOT an alteration in binding which is typically observed for protoxin selection and resistance, and a mechanism of resistance in PBW and TBW. Crossing studies confirm previous reports that resistance is inherited as a co-dominant trait

There were significant fitness costs associated with Cry1Ac resistance in CBW such as increased pupal mortality, a male-biased sex ratio, lower mating success, higher larval mortality, lower larval weight, longer larval developmental period, lower pupal weight, longer pupal duration, and a higher number of morphologically abnormal adults (Tables 1-3). Although several attempts were made to select for Cry2A resistance, the significant fitness costs observed with adults resulted in only 1-2 generations of survivors before the colony crashed.

Cry1Ac-resistant CBW was also tested on field-grown *Bt* (DPL-555) and non *Bt*-cotton (DPL-491) squares (Tables 4-5). AR had higher larval survivorship, number of larval instar reached, and duration of larval survival after feeding on *Bt* cotton squares. However, AR could still not complete larval development on *Bt* cotton. These results support the difficulty of maintaining Cry1Ac resistant populations of *H. zea* in the laboratory, and may help explain why field-evolved resistance has yet to be observed in this major pest of *Bt* cotton.

Table 1. Reproductive propensity (mean \pm SE) of Cry1Ac-resistant (AR) and susceptible (SC) strains of cotton bollworm, *H. zea* during selection and rearing, respectively.

Reproductive traits	N ^a	% mating	% multiple mating	Spermatophore /male
AR	2066	40.27 \pm 5.18	16.76 \pm 3.40	0.58 \pm 0.08
SC	1820	71.85 \pm 3.53	26.01 \pm 2.34	1.23 \pm 0.10
t-Test results		t _{13,1} = -6.468, p=0.000	t _{13,1} = -2.521, p=0.026	t _{13,1} =-5.628, p=0.000

Table 2. The reproductive (mean \pm SE) success in Cry1Ac-resistant (AR) and susceptible (SC) strains of cotton bollworm, *H. zea* and their reciprocal crosses

Strain/cross	N ^a	% mating	% multiple mating	Spermatophore /male
AR	960	29.48 \pm 2.98 ^a	12.04 \pm 1.93	0.41 \pm 0.06 ^a
SC	815	66.29 \pm 3.65 ^b	21.60 \pm 4.43	1.01 \pm 0.18 ^b
AR ♀ X SC ♂	140	46.34 \pm 3.98 ^b	20.19 \pm 5.52	0.95 \pm 0.29 ^{ab}
SC ♀ X AR ♂	143	40.24 \pm 7.38 ^{ab}	16.10 \pm 7.25	0.53 \pm 0.14 ^a
F-Test results		F _{3,19} =8.141 p=0.002	F _{3,19} =0.700 p=0.566	F _{3,19} =3.804 p=0.031

^aN: Number of moths

Superscript letters after means within a column indicate significant differences at p<0.05 level by Tukey's test

Table 3. Fitness parameters (mean \pm SE) for Cry1Ac-resistant (AR) and susceptible (SC) strains of cotton bollworm, *H. zea*

Life-history trait	AR		SC	
	Regular diet	Selection diet	Regular diet	Selection diet
Larval weight in 7D (mg)	109.48 \pm 3.22 ^b	91.04 \pm 3.89 ^a	144.58 \pm 3.95 ^c	131.99 \pm 4.48 ^c
Larval duration (d)	15.01 \pm 0.09 ^b	15.20 \pm 0.11 ^b	13.94 \pm 0.11 ^a	14.09 \pm 0.13 ^a
Larval mortality (%)	18.23 \pm 2.46 ^b	12.85 \pm 2.11 ^{ab}	5.04 \pm 0.63 ^a	10.07 \pm 3.91 ^{ab}
Pupal weight (mg)	347.70 \pm 4.22 ^a	355.41 \pm 3.37 ^{ab}	375.32 \pm 3.34 ^{bc}	368.4 \pm 3.67 ^{bc}
Pupal duration (d)	12.02 \pm 0.12 ^b	12.19 \pm 0.12 ^b	11.39 \pm 0.1 ^a	11.82 \pm 0.1 ^b
Malformed adults (%)	74.38 \pm 13.46 ^b	71.10 \pm 9.49 ^b	13.87 \pm 1.88 ^a	16.10 \pm 6.12 ^a
Number of eggs	NA	NA	653.74 \pm 51.58	583.26 \pm 68.53
Hatching (%)	NA	NA	85.29 \pm 2.61	86.60 \pm 3.12

Superscript letters after means within life history traits indicate significant differences at the p<0.05 level by Tukey's test

NA: not available

Table 4. Performance of Cry1Ac-resistant (AR) and susceptible (SC) strains of cotton bollworm, *H. zea* on *Bt* (DPL-555) and NBt (DPL-491)-cotton

Strain	Square	Larval weight at 7D	%3 rd instars	%4 th instars
AR	Bt	1.09 ± 0.12	20.00 ± 5.09	5.56 ± 2.22
	NBt	5.38 ± 0.43	75.56 ± 5.56	63.33 ± 5.77
SC	Bt	0.75 ± 0.09	5.56 ± 2.94	0.0 ± 0.0
	NBt	5.73 ± 0.45	80.00 ± 5.09	65.56 ± 5.88
F-Test		F _{3,202} =68.4, <i>p</i> =0.000	F _{3,11} =35.68, <i>p</i> =0.000	F _{3,11} =92.86, <i>p</i> =0.000

Table 5. Lethal time to mortality for Cry1Ac-resistant (AR) and susceptible (SC) strains of Cotton bollworm, *H. zea* on *Bt* (DPL-555) and NBt (DPL-491)-cotton

Strain	Square	LT ₅₀ (95% CI)	LT ₇₅ (95% CI)	LT ₉₀ (95% CI)	Slope ± SE	χ ²
AR	Bt	6.14 (4.81-7.30)	10.35 (8.76-12.10)	16.30 (13.60-21.24)	3.14 ± 0.44	3.15
	NBt	20.07 (17.62-23.33)	34.60 (28.79 – 46.07)	56.80 (43.28 – 89.00)	2.86 ± 0.39	4.38
SC	Bt	3.35 (1.90 – 4.49)	5.84 (3.93 – 7.36)	9.64 (7.52 – 13.48)	2.73 ± 0.64	2.09
	NBt	19.59 (17.05-23.01)	35.85 (29.28-49.55)	62.18 (45.79-104.81)	2.56 ± 0.37	4.48