2005 Beltwide Cotton Conferences, New Orleans, Louisiana - January 4 - 7, 2005

BACILLUS THURINGIENSIS CRY1AC RESISTANCE MONITORING PROGRAM FOR TOBACCO BUDWORM AND BOLLWORM IN 2004

Carlos A. Blanco, Michelle Mullen and Craig Abel
USDA - Agricultural Research Service
Stoneville, MS
Julius R. Bradley
North Carolina State University
Raleigh, NC
Peter Ellsworth
University of Arizona
Maricopa, AZ
Jeremy K. Greene
University of Arkansas
Monticello, AR
Ames Herbert
Virginia Tech
Suffolk, VA
Roger Leonard
Louisiana State University
Winnsboro, LA
Juan D. Lopez
USDA-ARS
College Station, TX
Robert Meagher
USDA Agricultural Research Service
Gainesville, FL
William Moar
Auburn University
Auburn, AL
Megha Parajulee
Texas Agricultural Experiment Station
Vernon, TX
Roy D. Parker
Texas Cooperative Extension
Corpus Christi, TX
Phillip Roberts and John Ruberson
University of Georgia
Tifton, GA
Richard Sprenkel
University of Florida
Quincy, FL
Glenn Studebaker
University of Arkansas
Keiser, AR
Antonio P. Teran
INIFAP
Ciudad Cuauhtemoc, Tamaulipas,
Michael Williams
Mississippi State University
Mississippi State, MS
John Van Duyn
North Carolina State University
Plymouth, NC

2005 Beltwide Cotton Conferences, New Orleans, Louisiana - January 4 - 7, 2005

Abstract

The susceptibility of the tobacco budworm (*Heliothis virescens* [F.]) and bollworm *(Helicoverpa zea* [Boddie]) to the *Bacillus thuringiensis* Cry1Ac protein in was tested in populations from 9 cotton-producing states in 2004. The survivorship of larvae obtained from mass mating males captured in pheromone traps near cotton fields (wild strain) with laboratory-adapted females (Cry1Ac-susceptible colony), was determined with 2 diagnostic concentrations for the first (F1) and second (F2) generation of each species plus an untreated control. Survivorship of those larvae was concurrently compared with the survivorship of the Cry1Ac-susceptible laboratory colony. Survival of 19 strains of *Heliothis virescens* and 37 strains of *Helicoverpa zea* tested between April and October, 2004 was not elevated above that in the susceptible colony for F1 or F2 generations using current methodology. However, the current method does have limitations, and additions and modifications to that methodology are discussed.

Introduction

Controversy about insect resistance management (IRM) has arisen since the deployment of transgenic cotton. At the grower level, however, acceptance of this technology has been rapid and constitutes an example of how these plants can transform the agricultural landscape. Currently in the U.S. transgenic cotton represents 73% of the planted area (Aldhous 2003) Because these cottons constantly express the Cry1Ac protein from *Bacillus thuringiensis* Berliner (Bt), the widespread and prolonged exposure to Bt proteins provides a constant selection pressure, representing one of the largest selections for resistance development in insect populations the world has ever seen (Tabashnik et al. 2003). In the U.S., an IRM strategy for Bt cotton was mandated by the Environmental Protection Agency (E.P.A.) that is based on a "high dose" expression of the protein and the implementation of a structured refuge which together mitigate the likelihood of resistance evolution (E.P.A. 2001). This strategy is believed to have helped maintain the susceptibility of target pests such as tobacco budworm and pink bollworm (*Pectinophora gossypiella* [Sauders]) to the Cry1Ac protein in current commercial varieties (Bollgard®).

The detection of resistance development to transgenic cotton plants expressing *Bacillus thuringiensis* toxins is an important consideration for the preservation of this technology. Since 1996, a yearly program has been conducted in the major cotton areas of the U.S. to monitor resistance of target insects to the *Bacillus thuringiensis* Cry1Ac protein. This program, which has been continuously expanded and improved, now covers 10 states, and involves more than 20 researchers who contribute important information to industry and the U.S. Environmental Protection Agency. Results from this program in 2004 are included in this report.

Materials and Methods

Male bollworms (*Helicoverpa zea* [Boddie]) and tobacco budworms (*Heliothis virescens* F.) captured in pheromone traps near cotton fields throughout the U.S. cotton region (Alabama, Arizona, Arkansas, Florida, Georgia, Louisiana, Texas, and Virginia) were shipped overnight to the Southern Insect Management Research Unit of the USDA Agricultural Research Service in Stoneville, MS (see Blanco et al. 2004 for detailed methodology). Mississippi and North Carolina initiated their strains from larvae. Males were mass-mated with laboratory-reared Cry1Ac-susceptible females in carton buckets at ≤30+ : 30> ratio, fed 10% sugar solution and maintained at 28 ± 2 ºC , 65 ± 10% RH, under 14:10 h L:D luminosity. Cry1Ac protein, obtained from lyophilized MVP II® insecticide, was incorporated into Nutri-Soy Wheat Germ diet at 0.05 and 1.0 µg of Cry1Ac per mL of diet for tobacco budworm and 100 and 250 µg per mL for bollworm. One neonate inoculated each cell of the treatments consisting of 16 micro-wells for control, 0.05 and 250 concentrations while 96 microwells were used for concentrations 1.0 and 100.0 Larvae on diet were kept in a room with controlled environmental conditions as previously described. Larvae were scored as dead when they did not move after probed. Developmental stage (instar) for survivors was visually estimated 7 days later. Data presented in this report have not been transformed or analyzed.

Additions and modifications made in 2004:

1) Modification of a tobacco budworm diagnostic concentration. The concentration 0.1 µg of Cry1Ac per ml of diet used in 2003 was replaced with 1.0 µg. This modification includes a concentration that causes mortality and/or growth inhibition with less ambiguity than the previous concentration (0.1 µg).

2) In order to test a higher number of larvae on 1 diagnostic concentration as suggested by Sims et al. (1996) as the effective concentration of Cry1Ac protein that is expected to reduce larval weight by 99% (EC_{99}), a Bio-BA-128

bio-assay tray (C-D International, Inc) was divided into sets of 16 microwells devoted to 0 and 0.05 treatments for tobacco budworm and 0 and 250 µg of Cry1Ac /mL treatments for bollworm. This constituted a replication per insect strain.

3) As a pilot program to learn more about the accuracy of the test and the logistics involved in its establishment, the inclusion of F2 testing for several strains was implemented. The second generation was obtained by mass mating adults (at ≈30+ : 30> ratio) resulting from rearing 150-180 F1 larvae.

Results and Discussion

Tobacco budworm. This insect is of main interest in this program, being the primary target of transgenic cottons in the geographical area that the authors of this report cover (except Arizona). After 1996, when the commercial deployment of Bollgard® cottons occurred, fewer *H. virescens* males have been captured by pheromone traps in these areas (Figure 1). The number of moth shipments received in 2004 dropped 51% compared to 2003 and the number of states that captured moths also declined by 35%. This decline is believed to be attributed to the rapid adoption of transgenic cotton in most of our geographical range, and reflects the effectiveness of the technology for the control of this pest in the field. Other means for obtaining moths or larvae should be implemented (e.g. the use of trap crops and/or attractants) to make up for the necessary numbers to conduct this program.

Eighteen strains were established from 4 different states producing 18 F1 and 15 F2 bioassays, testing 1,840 and 1,728 larvae on the 1.0 µg diagnostic concentration respectively. Of all the insects exposed to this Cry1Ac concentration, none developed beyond 2nd instar (Table 1). The lower concentration (0.05 µg) that historically has been used in the last 4 years, allowed further larval development. Therefore, the development of certain 3rd instar larvae is not of concern to this procedure with this particular concentration. The strains tested between April and October, 2004 showed similar susceptibility as the Cry1Ac-susceptible colony maintained in USDA-ARS Stoneville using current methodology. No statistical analysis has been preformed.

Bollworm. Thirty-eight strains were tested from 9 states. A total of 3,744 F1 neonates were tested in 38 assays at 100 µg of Cry1Ac per mL of diet and 768 at 250 µg. In the second generation, 43 assays tested 4,129 neonates at 100 µg and 688 at 250. Only 1 of these larvae, from a single strain (Hamburg, Arkansas obtained in 24 June 2004) developed to second instar in 7 days on the 100 µg concentration. Because the goal is to have a diagnostic concentration that would prevent all or most susceptible larvae from reaching third instar, this larva was still considered as susceptible. It is also important to mention that in the same assay, another larva but from the Cry1Ac-susceptible colony reached 2nd instar at the same concentration.

References

Aldhous, P. 2003. Damned if they do, damned if they don't. Nature 425: 656-659.

Blanco, C., L. C. Adams, J. R. Bradley, S Carroll, P. Ellsworth, J. Gore, J. K. Greene, D. Hardee, A. Herbert, D. Johnson, M. Karner, R. Leonard, B. Lewis, J. D. Lopez, Jr., R. Luttrell, M. Mullen, M. Parajulee, D. Parker, R. D. Parker, M. Roof, P. Roberts, J. Ruberson, R. Sprenkel, S. Stewart, G. Studebaker, J. R. Weeks, M. Williams and John Van Duyn. 2004. *Bacillus thuringiensis* resistance monitoring program for tobacco budworm and bollworm in 2003. Proceedings of the Beltwide Cotton Conferences, National Cotton Council, Memphis, TN.

Environmental Protection Agency. 2001. Biopesticides Registration Action Document: *Bacillus thuringiensis* Plant-Incorporated Protectants (10/16/01), posted at, http://www.epa.gov/pesticides/biopesticides/pips/bt_pips.htm>

Sims, S. R., J. T. Greenplate, T. B. Stone, M. Caprio and F. Gould. 1996. Monitoring strategies for early detection of Lepidoptera resistance to *Bacillus thuringiensis* insecticidal proteins, pp. 229-242. *In* T. M. Brown, (ed.). Molecular Genetics and Ecology of Pesticide Resistance. American Chemical Society Symposium Series.

Tabashnik, B., Y. Carrière, T. J. Dennehy, S. Morin, M. S. Sisterson, R. T. Roush, A. M. Shelton and J-Z Zhao. 2003. Insect resistance to transgenic Bt crops: Lessons from the laboratory and field. Journal of Economic Entomology 96: 1031-1038.

2005 Beltwide Cotton Conferences, New Orleans, Louisiana - January 4 - 7, 2005

Disclaimer

This research was partially funded by Monsanto Company and Dow AgroSciences LLC. Mention of trade names or commercial products in this report is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture, or by any of the educational institutions.

Acknowledgment

We wish to thank Sharlene Matten, O. P. Perera and Sakuntala Sivasupramaniam for comments made to the manuscript. The help provided by Tabetha Stokes-Gentry is greatly appreciated. The pheromone trap captures information of Washington County, Mississippi was provide by Larry C. Adams.

Figure 1. Average number of *H. virescens* males captured per pheromone traps per year in Stoneville, Mississippi.

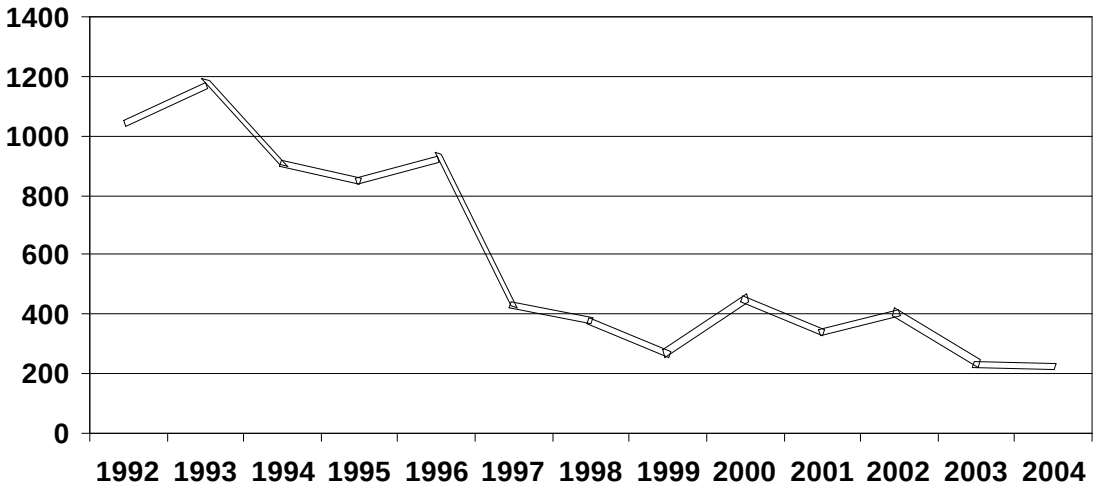

Table 1. Susceptibility of *Heliothis virescens* progenies of pheromone-captured males mass mated with laboratory-reared *Bacillus thuringiensis*-susceptible females 7 days after exposure to 2 diagnostic concentrations. [1]Micrograms (µg) of Cry1Ac per mL of insect artificial diet.

F L O R I D A (Gadsden county) males represented= 264										
	F1 STRAINS (4)					Bt-SUSCEPTIBLE COLONY				
TREAT[1]	N	DEAD	L1	L2	≥L3	n	DEAD	L1	L2	≥L3
0	96	11	2	9	74	64	7	1	19	37
0.05	96	16	22	54	4	64	20	14	21	9
1.0	576	488	87	1	0	384	365	17	2	0
	F2 STRAINS (5)					Bt-SUSCEPTIBLE COLONY				
0	64	3	2	10	49	64	1	0	0	63
0.05	64	13	30	21	0	64	24	12	28	0
1.0	480	218	262	0	0	480	465	15	0	0
G E O R G I A (counties) males represented= 300										
	F1 STRAINS (3)					Bt-SUSCEPTIBLE COLONY				
TREAT[1]	N	DEAD	L1	L2	≥L3	n	DEAD	L1	L2	≥L3
0	64	7	0	10	47	64	19	0	26	19
0.05	64	11	10	30	13	64	32	16	16	0
1.0	384	348	36	0	0	384	382	2	0	0
	F2 STRAINS (2)					Bt-SUSCEPTIBLE COLONY				
0	32	0	0	2	30	32	6	4	4	18
0.05	32	8	16	8	0	32	16	14	2	0
1.0	192	176	16	0	0	192	190	2	0	0
M I S S I S S I P P I (Washington county) males represented= 320										
	F2 STRAINS (4)					Bt-SUSCEPTIBLE COLONY				
TREAT[1]	N	DEAD	L1	L2	≥L3	n	DEAD	L1	L2	≥L3
0	96	4	0	7	85	96	3	0	0	93
0.05	96	15	15	58	8	96	8	16	64	8
1.0	576	522	54	0	0	576	528	48	0	0
CENTRAL TEXAS (counties) **males represented= 346**										
	F1 STRAINS (7)					Bt-SUSCEPTIBLE COLONY				
TREAT[1]	N	DEAD	L1	L2	≥L3	n	DEAD	L1	L2	≥L3
0	112	14	10	16	72	80	11	4	20	45
0.05	112	27	32	53	0	80	17	27	36	0
1.0	592	546	46	0	0	400	380	20	0	0
	F2 STRAINS (4)					Bt-SUSCEPTIBLE COLONY				
0	80	12	3	1	64	64	4	6	22	32
0.05	80	50	29	1	0	64	33	13	18	0
1.0	480	466	14	0	0	384	380	4	0	0

Table 2. Susceptibility of *Helicoverpa zea* progenies of pheromone-captured males mass mated with laboratory-reared *Bacillus thuringiensis*-susceptible females 7 days after exposure to 2 diagnostic concentrations. [1]Micrograms (µg) of Cry1Ac per mL of insect artificial diet.

A L A B A M A (counties)										
		F2 STRAIN					Bt-SUSCEPTIBLE COLONY			
TREAT[1]	N	DEAD	L1	L2	≥L3	n	DEAD	L1	L2	≥L3
0	16	1	4	11	0	16	5	5	5	1
100	96	94	2	0	0	96	96	0	0	0
250	16	15	1	0	0	16	16	0	0	0
A R I Z O N A (Pinal county) **males represented= 240**										
		F1 STRAINS (3)					Bt-SUSCEPTIBLE COLONY			
TREAT[1]	N	DEAD	L1	L2	≥L3	n	DEAD	L1	L2	≥L3
0	128	4	6	17	101	112	2	2	2	106
100	144	77	67	0	0	112	88	23	1	0
250	128	68	60	0	0	112	109	3	0	0
		F2 STRAINS (3)					Bt-SUSCEPTIBLE COLONY			
0	144	11	9	52	72	144	0	0	12	132
100	864	791	73	0	0	864	649	214	1	0
250	144	140	4	0	0	144	130	14	0	0
A R K A N S A S (counties) **males represented= 120**										
		F1 STRAINS (3)					Bt-SUSCEPTIBLE COLONY			
TREAT[1]	N	DEAD	L1	L2	≥L3	n	DEAD	L1	L2	≥L3
0	48	3	4	19	22	48	0	0	2	46
100	288	170	118	0	0	288	258	30	0	0
250	48	39	9	0	0	48	48	0	0	0
		F2 STRAINS (2)					Bt-SUSCEPTIBLE COLONY			
0	48	6	7	17	18	48	2	0	7	39
100	288	229	58	1	0	288	204	83	1	0
250	48	45	3	0	0	48	41	7	0	0
F L O R I D A (Gadsden county) **males represented= 600**										
		F1 STRAINS (6)					Bt-SUSCEPTIBLE COLONY			
TREAT[1]	N	DEAD	L1	L2	≥L3	n	DEAD	L1	L2	≥L3
0	160	3	2	33	122	160	9	10	14	127
100	960	667	293	0	0	960	607	353	0	0
250	160	135	25	0	0	160	156	4	0	0
		F2 STRAINS (2)					Bt-SUSCEPTIBLE COLONY			
0	32	4	0	6	22	32	0	0	2	30
100	192	152	40	0	0	192	110	82	0	0
250	32	32	0	0	0	32	32	0	0	0
G E O R G I A (counties) **males represented= 60**										
		F1 STRAINS (3)					Bt-SUSCEPTIBLE COLONY			
TREAT[1]	N	DEAD	L1	L2	≥L3	n	DEAD	L1	L2	≥L3
0	32	0	0	1	31	16	2	0	0	14
100	192	115	77	0	0	96	70	26	0	0
250	32	27	5	0	0	16	13	3	0	0

Table 2 continues..

L O U I S I A N A (counties) **males represented= 60**										
		F1 STRAINS (3)					Bt-SUSCEPTIBLE COLONY			
TREAT[1]	N	DEAD	L1	L2	≥L3	n	DEAD	L1	L2	≥L3
0	16	0	0	6	10	16	1	1	6	8
100	96	68	28	0	0	96	95	1	0	0
250	16	14	2	0	0	16	16	0	0	0
		F2 STRAINS (2)					Bt-SUSCEPTIBLE COLONY			
0	16	8	0	5	3	16	0	0	1	15
100	96	91	5	0	0	96	55	41	0	0
250	16	16	0	0	0	16	16	0	0	0
M I S S I S S I P P I (Washington county) males represented= 300										
		F2 STRAINS (5)					Bt-SUSCEPTIBLE COLONY			
TREAT[1]	N	DEAD	L1	L2	≥L3	n	DEAD	L1	L2	≥L3
0	96	7	2	8	79	96	8	0	22	66
100	576	523	53	0	0	576	466	110	0	0
250	96	91	5	0	0	96	87	9	0	0
NORTH CAROLINA (counties)										
		F2 STRAINS (2)					Bt-SUSCEPTIBLE COLONY			
TREAT[1]	N	DEAD	L1	L2	≥L3	n	DEAD	L1	L2	≥L3
0	32	2	0	4	26	32	8	2	18	4
100	192	169	23	0	0	192	178	14	0	0
250	32	32	0	0	0	32	32	0	0	0
NORTHWEST TEXAS (counties) **males represented= 310**										
		F1 STRAINS (4)					Bt-SUSCEPTIBLE COLONY			
TREAT[1]	N	DEAD	L1	L2	≥L3	n	DEAD	L1	L2	≥L3
0	96	1	0	21	74	96	14	2	25	55
100	576	415	161	0	0	576	497	79	0	0
250	96	86	10	0	0	96	90	6	0	0
CENTRAL TEXAS (counties) **males represented= 600**										
		F1 STRAINS (5)					Bt-SUSCEPTIBLE COLONY			
TREAT[1]	N	DEAD	L1	L2	≥L3	n	DEAD	L1	L2	≥L3
0	80	1	1	9	69	80	6	5	18	51
100	480	330	150	0	0	480	458	22	0	0
250	80	75	5	0	0	80	80	0	0	0
		F2 STRAINS (7)					Bt-SUSCEPTIBLE COLONY			
0	128	12	4	57	55	128	11	6	22	89
100	768	612	156	0	0	768	627	141	0	0
250	128	120	8	0	0	128	97	31	0	0
SOUTHEAST TEXAS (counties) **males represented= 420**										
		F1 STRAINS (5)					Bt-SUSCEPTIBLE COLONY			
TREAT[1]	N	DEAD	L1	L2	≥L3	n	DEAD	L1	L2	≥L3
0	96	4	2	22	68	96	13	0	2	81
100	336	171	165	0	0	336	312	24	0	0
250	96	92	4	0	0	96	90	6	0	0
		F2 STRAINS (5)					Bt-SUSCEPTIBLE COLONY			
0	160	11	16	61	72	160	13	1	29	117
100	960	877	83	0	0	960	659	301	0	0
250	160	145	15	0	0	160	136	24	0	0

Table 2 continues..

	V I R G I N I A (counties) **males represented= 510**									
	F1 STRAINS (5)					Bt-SUSCEPTIBLE COLONY				
TREAT[1]	N	DEAD	L1	L2	≥L3	n	DEAD	L1	L2	≥L3
0	112	3	2	7	100	112	7	0	18	87
100	672	375	297	0	0	672	598	74	0	0
250	112	89	23	0	0	112	109	3	0	0
	F2 STRAINS (1)					Bt-SUSCEPTIBLE COLONY				
0	16	0	0	0	16	16	1	0	0	15
100	96	66	30	0	0	96	66	30	0	0
250	16	15	1	0	0	16	16	0	0	0