MEASURING GENERATION SURVIVAL OF BOLLWORM ON BOLLGARD AND BOLLGARD II COTTON: IMPLICATIONS FOR RESISTANCE MONITORING

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Abstract

Helicoverpa zea (Boddie) larvae were collected from brown-silk stage Bt-corn on commercial farms in four different production areas of Arkansas. Larvae were taken directly from the corn ears and exposed to a low dose of Cry1Ac incorporated into meridic diet or placed on diet with no Cry1Ac toxin. Detailed observations were made on survival and growth rate of the larvae. Reproductive capacity was measured in resulting inbred laboratory colonies and those crossed with LabZA, a longtime laboratory strain with known susceptibility to Bt toxins. Subsequent first generation progeny were exposed to Cry1Ac toxin in diet incorporation assays and plant-tissue assays with conventional, Bollgard and Bollgard II cotton. Cohorts of individual colonies with high survival at seven days on Bollgard or Bollgard II cotton were further exposed to Bt cotton tissues (leaves, squares and bolls) throughout the larval period. Inbred colonies exhibited low reproductive capacity, but those crossed with LabZA had higher reproductive rates and measured survival to pupation on Bollgard and Bollgard II cotton. Survival to pupation of colonies reared only on Bollgard and Bollgard II cotton ranged from 0 to 4%. Paired cohorts reared on conventional cotton had 80 to 87% survival to pupation. Estimated moth emergence patterns for each study site using *H. zea* developmental times in the literature and observed development on cotton and Bt-plant materials indicated that the number of annual generations from crop hosts in Arkansas may range from five in the southwest to three in the northeast. Cotton was a poor host with expanded generation time. The influence of cotton on the annual host sequence and the additional increased development time due to Bt exposure suggest that only a fraction of the third generation will be available to damage cotton in northeast Arkansas. This illustrates the potential impact new Bt corns may have on population ecology of the bollworm and the importance of increased corn acreage to the abundance of this polyphagous pest.

Introduction

The great polyphagy of *Helicoverpa zea* (Boddie) is well recognized in agriculture as the insect has a number of common names including bollworm on cotton, corn earworm on corn, sorghum headworm on sorghum, soybean podworm on soybean, tomato fruitworm on tomato and others. This wide host range and the sequence of crops utilized by the insect over a single growing season have meaningful influence on potential resistance to Bt toxins expressed in Bt cotton and Bt corn. This polyphagy creates seasonal developmental scenarios where some generations may not be exposed to the transgenic toxins in cotton and corn. Also, the use of similar Bt toxins in both Bt corn and Bt cotton may expose populations to multiple selection bouts within a given year.

We have been involved in studies to measure variability in response of *H. zea* populations to Bt toxins for several years (Luttrell et al. 1999, Ali et al. 2006, Ali and Luttrell 2007, Ali and Luttrell 2009b). Others have explored the potential interactions between selection and exposure to Bt toxins in both Bt cotton and Bt corn. Gore et al. (2003) examined the developmental rate of larvae collected on sweet corn and then reared a generation on a number of agronomic hosts. Those reared on corn and meridic diet had much faster development than those reared on cotton. Those reared on corn has higher mortality in subsequent generation exposure to Bt cotton that those reared on soybean and grain sorghum. Jackson et al. (2008) found that a significant fraction of *H. zea* in mid- to late-season originated from corn in the Midsouth. Caprio et al. (2009) studied long-term fecundity of *H. zea* in simulation studies involving different crops and sequences of generation utilization of different crops. They indicated that fecundity of the individual is influenced by temporal stability of the habitat and the likelihood that an individual's offspring will move into different habitats as well as the measured fecundity of an individual within a given habitat (crop).

In 2009, we collected larvae from Bt corn fields in different production regions of Arkansas to further explore the impacts of Bt exposure and crop sequence on possible Bt selection for resistance, fitness costs associated with Bt exposure and overall impact of mortality and delayed development on cotton risk of infestation by *H. zea*.

Materials and Methods

At four different locations (replicates) on each of four commercial farms in Arkansas, *H. zea* larvae were collected from Bt corn and placed on an untreated meridic diet or a diet containing 10 ug/ml Cry1Ac. Test locations were the Greg Williams Farm near Gin City, the Worth Matteson Farm near Foreman, the Mike Brown Farm near Pickens and the David Wildy Farm near Leachville. Sample dates were late June to early July. The source of Cry1Ac was lyophilized MPVII powder obtained from Monsanto Company, St. Louis, MO. The dose (concentration) used was based on previous assays and was intended to increase mortality about 50%. Densities and instar of larvae were recorded in the field, and detailed observations of each field-collected larva were made in the Cralley-Warren Laboratory at the University of Arkansas. Data were obtained on survival, length of larval and pupal stages, pupal weight, sex and date of moth emergence. Survivors were used to establish four types of laboratory colonies from each of the four field sites: **a)** a control inbred with survivors from the untreated diet (CI), **b)** a treated inbred with survivors from the treated diet (TI), **c)** a cross of males from the treated diet with females from LabZA, a susceptible laboratory strain maintained at the University of Arkansas (TM x LabZA), and **d)** a cross of females from the treated diet with males from LabZA (TF x LabZA). Laboratory survival and reproduction of each colony was monitored and compared to that of LabZA. Detailed records of daily cohorts of eggs were maintained and samples of individual cohorts were exposed to a diet containing 100 ug/ml of Cry1Ac and plant tissue (upper leaf) assays with conventional, Bollgard and Bollgard II cotton. Sixteen larvae were used in each individual assay. The diet assay included a 0 concentration as a control, and LabZA was used as an experimental control. Assays were repeated with cohorts from each colony as long as females were producing eggs. The plant assays were continued to pupation or death with individual cohorts that had survival greater than 75% at seven days on Bollgard or survival greater than 50% on Bollgard II. Fresh plant tissue was provided every few days as needed. Larvae were reared for two weeks on upper leaves, one week on squares, one week on small bolls and the remainder of the larval period on large bolls collected from field grown plots of conventional, Bollgard and Bollgard II cotton. Procedures were identical to those reported by Ali and Luttrell (2008).

All data on survival, development and reproductive capacity were studied by descriptive statistics, analysis of variance and Tukey's mean separation procedures (JMP 2008). Developmental times were compared to degree day (DD) accumulations at each field site to examine possible impacts on number and timing of field generations. Weather records for Gin City were obtained from daily weather records for Plain Dealing, Louisiana. Those for Foreman were from weather records at DeKalb, Texas, and those for Pickens were from weather records at Monticello, Arkansas. Weather records from Jonesboro were used for estimated heat unit accumulations at the Leachville field site. Cotton development was based on DD60 accumulations referenced by COTMAN (Danforth and O'Leary 1998). Historical cutout dates for south and north Arkansas were those listed by Studebaker (2010). Insect development was based on the phenology models on the University of California IPM website (www.ipm.ucdavis/edu) and developmental times published by Butler (1976) and Hartstack et al. (1976). Emergence patterns for the second generation on corn were calculated from the age (instar) distribution of larvae collected at each field site and the predicted date of spring emergence from the local weather records. Using this point of seasonal reference, dates of first generation and subsequent third and later generations were estimated using: a) a model system based on diet-based development (873 DD), b) a model system based on diet-based development during the first field generation, corn-based development (992 DD) during the second field generation, cotton-based development (1279 DD) during the third generation and diet-based development for each subsequent generations, c) a model system using diet-based development during the first field generation, our observed emergence on untreated diet during the second generation and our observed survival and emergence for larvae fed conventional cotton during the during the third generation, and d) a model system using diet-based development during the first field generation, our observed emergence on treated diet during the second generation and our observed survival and emergence for larvae fed Bollgard cotton during the third generation. Generations beyond the third field generation were assumed to develop as projected by the diet-based development model (873 DD). The minimum temperature for development of *H. zea* was assumed to be 54.7^0F (Harstack et al. 1976).

Results

Insect infestations varied slightly among the populations, but were generally similar. Densities were about a two corn cobs per larva or about a larva every other plant (Table 1). Larval size was about third instar at all locations. Larvae placed on the 10 ug Cry1Ac diet had higher mortality, increased escape through the cardboard lids on the

diet cups, about a 50% reduction in survival to pupation, a longer larval period of about seven days, a shorter pupal period and smaller pupae than those placed on the untreated diet.

Reproduction in the laboratory colonies created by inbreeding the field colonies (CI or TI) or crossing males (TM x LabF) and females (TF x LabM) of the field colonies with LabZA varied (Tables 2 and 3). The number of cohorts or days of egg production among the different colonies ranged from 12 to 22. Females from Test 1 and Test 2 produced more eggs and larvae than those from Test 3 and Test 4 (Table 3). Colonies from field males crossed with LabZA had higher reproductive rates per female than the other colonies, especially the treated inbred colonies. The average number of eggs per female from field colonies (56.55) was about 1/5 that observed with LabZA females (264). A similar difference was noted in numbers of larvae produced per female.

Table 1. Population traits of larvae collected from corn and placed on diet containing 0 or 10 ug Cry1Ac/ml.

	Mean (SEM) Across All Four Locations	
	0 ug Cry1Ac/ml Diet	10 ug Cry1Ac/ml Diet
Corn Cobs Per Larva	1.98 (0.15) a	2.11 (0.11) a
Instar at Collection	3.18 (0.09) a	3.24 (0.04) a
% Mortality of Larvae	11.39 (2.63) b	33.31 (2.14) a
% of Larvae that Escaped	3.75 (2.21) b	22.33 (3.52) a
% Survival to Pupation	79.21 (5.25) a	41.32 (3.44) b
Days Before Pupation	12.17 (0.29) b	19.40 (0.20) a
Days in Pupal Stage	13.24 (0.23) a	11.77 (0.11) b
Pupal Weight (grams)	461.16 (5.43) a	390.82 (3.49) b
Sex Ratio (% Female)	0.47 (0.05) a	0.51 (0.02) a

Table 2. Eggs and larvae per female from laboratory colonies created from the field collections.

	Cohorts	Egg/Female	Larvae/Female
T1CI	13	7.94 e	2.14 e
T1TF x LABM	14	53.56 c-e	27.12 b-e
T1TI	19	5.08 e	0.01 e
T1TM x LABF	12	107.48 c	107.48 ab
T2CI	16	4.49 e	0.00 e
T2TF x LABM	16	16.50 de	5.83 de
T2TI	19	13.03 de	3.59 de
T2TM x LABF	15	121.30 bc	82.60 a-c
T3CI	22	61.12 c-e	103.62 c-e
T3TF x LABM	21	27.00 de	16.96 c-e
T3TI	18	32.16 c-e	5.36 c-e
T3TM x LABF	22	151.27 b	33.72 b-e
T4CI	17	4.97 e	0.14 e
T4TF x LABM	16	81.32 c-e	70.48 b-e
T4TI	20	15.76 de	0.82 e
T4TM x LABF	21	227.32 a	131.76 a

Table 3. Eggs and larvae per female from different types of laboratory colonies.

	Cohorts	Egg/Female	Larvae/Female
All Field Colonies	17.5	56.55	29.23
LabZA	22	264	237.6
All Test 1	58	43.51 a	34.19 a
All Test 2	66	42.65 a	33.65 a
All Test 3	83	33.39 b	28.33 b
All Test 4	74	35.37 b	29.23 b
All CI	68	19.63 b	26.48 ab
All TF x LABM	67	44.59 b	30.10 ab
All TI	76	16.51 b	2.45 b
All TM x LABF	70	151.84 a	88.89 a

Mortality at the 100 ug Cry1Ac/ml dose was less than that previously observed (Ali and Luttrell 2009) and there were few differences among the colonies in mortality at seven days (Table 4). All colonies had mortality levels similar to that observed for LabZA. The lowest mortality was from colony T3CI. The lowest mortality plus stunting response in diet incorporation assays was from T3TM x LABF, the treated males from Test 3 crossed with LabZA females.

Table 4. Response of laboratory colonies exposed to 100 ug Cry1Ac in diet incorporation assays.

Colony	Number Assays	% Corr. Mort. (100 ug Cry1Ac/ml)	Range	% Corr. Mort. + L1 Larvae (100 ug/ml)
LAB ZA	14	29.31 (14.4) ab	(6.67 - 56.25)	100.00 (0.00) a
T1TF X LABM	12	10.96 (4.45) b	(0.00 - 46.75)	98.88 (0.75) ab
T1TM INBREED	2	9.38 (3.13) b	(6.25 - 12.50)	100.00 (0.00) a
T1TM x LABF	13	8.02 (3.61) b	(0.00 - 31.25)	89.70 (5.17) ab
T2TF x LABM	4	12.81 (2.47) b	(6.67 - 18.75)	96.77 (1.87) ab
T2TM INBREED	2	8.33 (8.33) b	(0.00 - 16.67)	100.00 (0.00) a
T2TM x LABF	18	17.29 (3.76) ab	(0.00 - 60.00)	95.32 (1.89) ab
T3CI	4	4.69 (1.56) b	(0.00 - 6.25)	96.88 (1.80) ab
T3TF x LABM	7	35.12 (11.34) a	(6.67 - 100.0)	99.11 (0.89) ab
T3TI	2	22.60 (14.90) ab	(7.69 - 37.50)	92.86 (7.14) ab
T3TM x LABF	11	5.68 (2.97) b	(0.00 - 31.25)	86.13 (6.07) b
T4TF x LABM	13	16.43 (4.00) b	(0.00 - 37.50)	90.12 (5.19) ab
T4TM x LABF	14	20.24 (3.84) ab	(0.00 - 50.00)	90.54 (4.08) ab
all	117	15.21 (1.55)	(0.00 - 100.0)	93.18 (1.44)

Using the criteria of less than 25% mortality on Bollgard tissue at seven days or less than 50% mortality on Bollgard II tissue at seven days, 24 of the 74 cohort colonies tested were continued in plant assays until pupation. Most (n=22) were colonies from treated males crossed with LabZA (TM x LabZA). Two were from treated females crossed with LabZA (TF x LabZA). No inbred colony (CI or TI) had mortality rates less than the selection criteria.

Table 5. Numbers of cohort colonies continued to pupation in plant assays.

	Number of Plant Assays	
	7 Day	Continued to Pupation
T1TF x LabZA	1	1
T1TM x LabZA	12	9
T2TF x LabZA	3	1
T2TM (Inbred)	2	0
T2TM x LabZA	15	9
T3CI x LabZA	3	0
T3TF x LabZA	5	0
T3TI x LabZA	2	0
T3TM x LabZA	7	3
T4TF x LabZA	10	0
T4TM x LabZA	14	1
All	74	24

No differences were observed among colony types in mortality on conventional, Bollgard or Bollgard II cotton at seven days. Colonies derived from Test 1 and Test 2 field collections had less mortality than those derived from Test 3 and Test 4 field collections (Table 6).

Table 6. Mortality of larvae from different colony types and different tests in cotton assays at seven days.

	% Corrected Mortality – Fed Cotton Tissue for 7 Days			
	No. Tests	Conventional	Bollgard	Bollgard II
CI	3	8.9 a	48.9 a	83.3 a
TF x LABZA	19	7.4 a	57.4 a	73.9 a
TI	2	0.0 a	56.7 a	84.7 a
TM x LABZA	50	11.1 a	43.8 a	62.2 a
Test 1	13	7.4 a	25.2 b	49.2 b
Test 2	20	9.6 a	30.4 b	51.9 b
Test 3	17	7.4 a	52.4 a	72.9 a
Test 4	14	12.7 a	71.5 a	83.8 a

Among the colonies chosen for continued plant assays to pupation, there were generally no differences among colony types or colonies originating from different collection sites in mortality on cotton plant tissue at seven days or at pupation. Colonies from Test 3 at Pickens had higher mortality at seven days on Bollgard plant tissue. At pupation, survival rates on conventional cotton averaged about 80%. Those on Bollgard were less than 5%, but some survival was observed from colonies originating from all test sites except for those from Test 3 at Pickens. At pupation, low levels of survival (less than 2%) were observed on Bollgard II cotton in colonies with treated males crossed with LabZA (TM x LabZA) from the Test 2 collection site (Foreman).

Table 7. Mortality in different colony types and collection sites that were continued to pupation in plant assays.

	No. Tests	Conventional	Bollgard	Bollgard II
		7 Day Mortality		
TF x LABZA	2	6.7 a	40.0 a	50.0 a
TM x LABZA	22	8.8 a	20.0 a	40.8 a
		Mortality at Pupation		
TF x LABZA	2	22.5 a	95.8 a	100.0 a
TM x LABZA	22	18.1 a	97.8 a	99.5 a
		7 Day Mortality		
Test 1	10	7.0 a	19.3 b	43.3 a
Test 2	10	11.1a	16.2 b	36.5 a
Test 3	3	3.9 a	45.8 a	50.3 a
Test 4	1	16.0 a	8.0 b	40.0 a
		Mortality at Pupation		
Test 1	10	16.7 a	96.3 a	100.0 a
Test 2	10	21.6 a	98.5 a	98.9 a
Test 3	3	13.3 a	100.0 a	100.0 a
Test 4	1	20.0 a	96.0 a	100.0 a

Survival rates of larvae to pupation in colonies reared on meridic diet in the laboratory ranged from 62 to 92%. The inbred colonies from the field collections (CI and TI) had lower survival in the second generation in the laboratory than LabZA or the crosses between field colonies and LabZA (TM x LabZA and TF x LabZA). Time to pupation was similar among all colonies reared on meridic diet and averaged 16.6 days.

Table 8. Survival to pupation and larval developmental time for different colonies reared on meridic diet.

Second Generation (Post Diet Assay) Rearing on Meridic Diet

Colony	No. Cohort Colonies	% Survival to Pupation	Time to Pupation (d)
All Field Female Derived Colonies	9	81.56 (3.97) a	16.11 (0.73) a
All Field Male Derived Colonies	13	83.03 (3.30) a	16.31 (0.61) a
Both Sexes Field Derived Colonies	3	62.61 (6.87) b	19.00 (1.26) a
LabZA	2	91.67 (8.41) a	17.50 (1.55) a
All Colonies	27	80.91 (2.55)	16.63 (0.43)

Time to pupation for larvae reared on Bollgard plant tissue was 49.3 days, about seven days longer that those reared on conventional cotton tissue (42.0 days) and about a month longer than those reared on meridic diet in the laboratory (16.6 days). LabZA developed faster on conventional cotton (31.6 days) than male and female crosses with LabZA (41.5 and 45.4 days). Female crosses with LabZA had longer larval development times than males crossed with LabZA (~ four day difference).

Table 9. Pupal weight and larval developmental time for colonies fed different types of cotton tissues.

Survivors Reared Only on Cotton

Cotton Host	Colony	No. Survivors	Pupal Weight (g)	Days to Pupation
Conventional	LabZA	16	285.6 (16.9) b	31.6 (1.2) c
Conventional	Female Colonies	40	349.0 (10.8) a	45.5 (0.8) a
Conventional	Male Colonies	285	337.6 (3.4) a	41.6 (0.3) b
Conventional	All	341	336.37 (3.7)	42.00 (0.3)
Bollgard	Female Colonies	2	356.6 (26.3) a	48.7 (0.8) a
Bollgard	Male Colonies	11	328.4 (12.4) a	49.3 (0.8) a
Bollgard	All	13	333.6 (11.2)	49.3 (0.7)

Patterns of estimated moth emergence are presented in Figures 1-4 for Gin City, Foreman, Pickens and Leachville using **a)** a diet based larval development model, **b)** a corn and cotton larval development model for the second and third generations, **c)** actual observed emergence of larvae from untreated diet in our studies for the second generation and emergence of survivors on conventional cotton in our studies for the third generation, and **d)** actual observed emergence of larvae from treated diet in our studies for the second generation and emergence of survivors on Bollgard cotton in our studies for the third generation. At the Gin City site, the number of generations ranged from 5 to 3.9. Using the simulated developmental times and progressively increased developmental time with cotton and Bt exposure in models A-D, more than one generation was eliminated at each field site. At Pickens (Figure 3) and Leachville (Figure 4) only three generations would be completed under the simulated development that included increased exposure to Bt on corn during the second field generation and development on Bollgard cotton in the third generation.

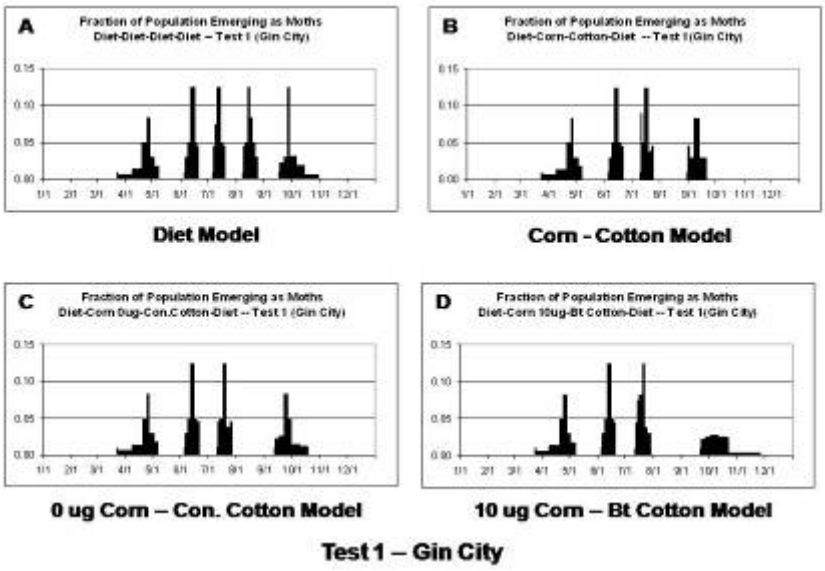

Figure 1. Moth emergence patterns for Gin City using different models of larval developmental time.

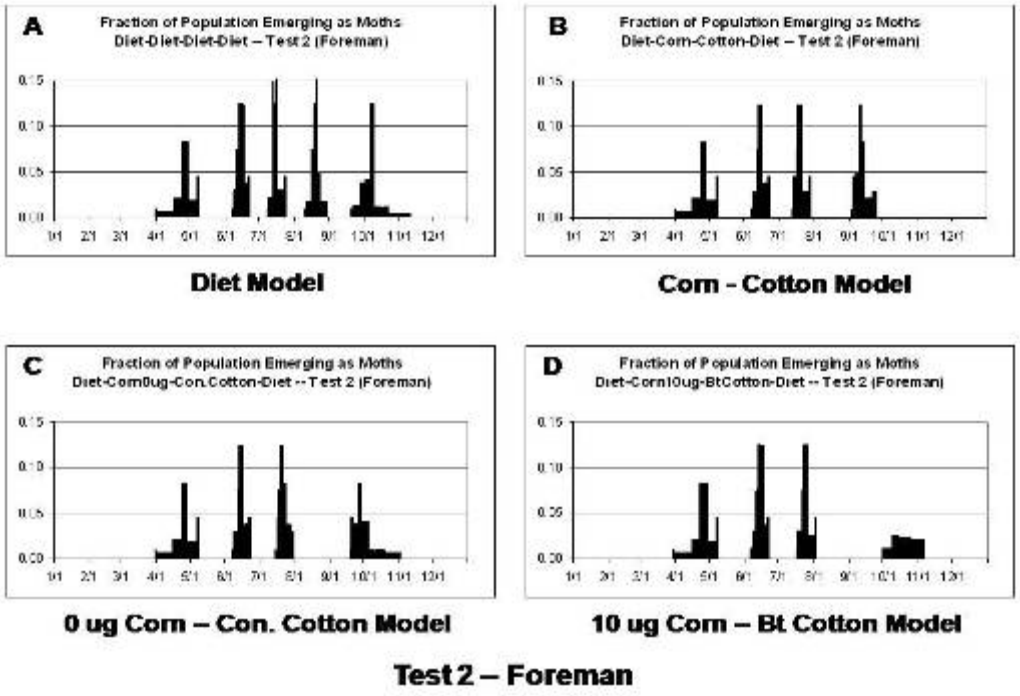

Figure 2. Moth emergence patterns for Foreman using different models of larval developmental time.

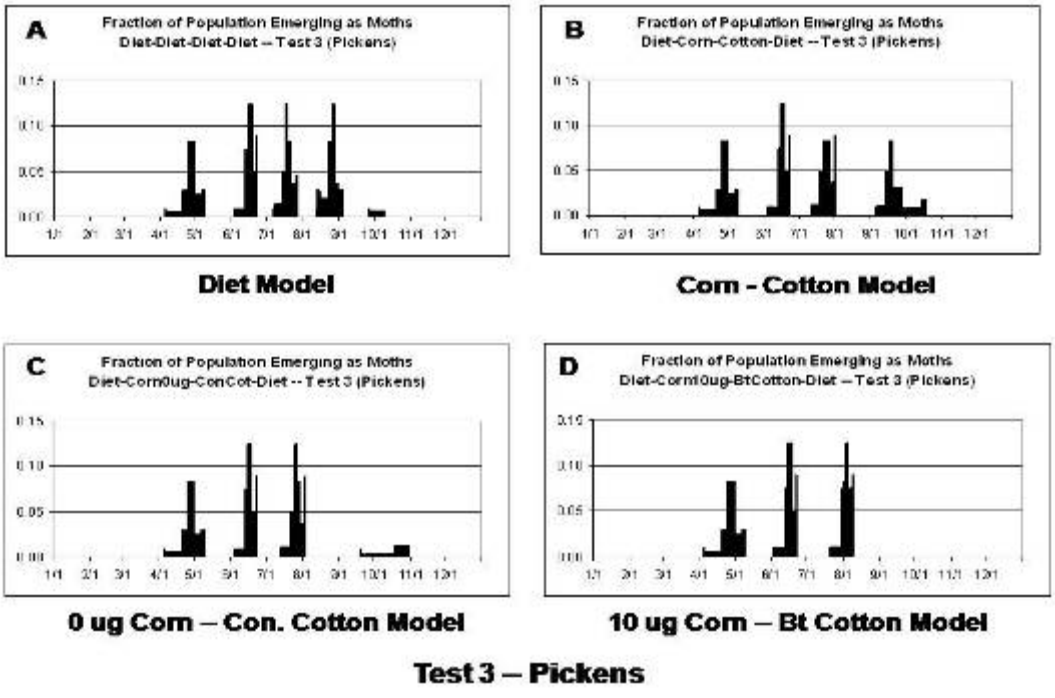

Figure 3. Moth emergence patterns for Pickens using different models of larval developmental time.

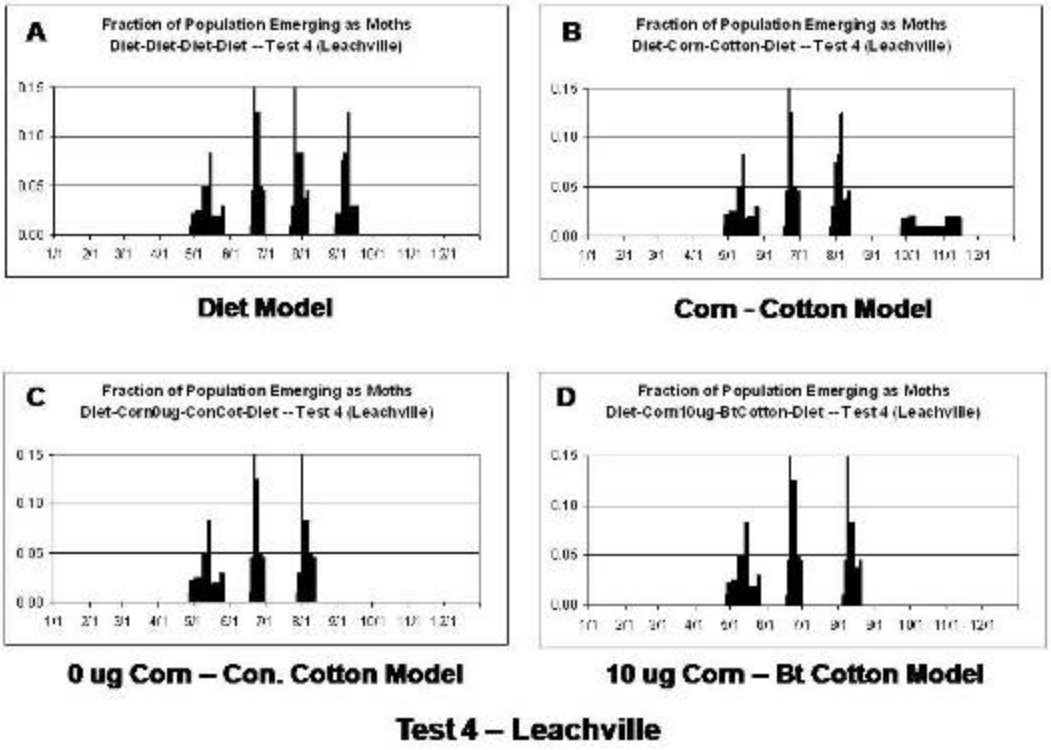

Figure 4. Moth emergence patterns for Leachville using different models of larval developmental time.

Assuming that most bollworm on cotton were third generation larvae originating from moths that developed as larvae on corn or Bt corn during the second generation, we calculated the fraction of the third generation that would reach third instar before cotton cutout. Estimated cotton cutout dates were those listed in Studebaker (2010) or northeast, central and southeast Arkansas. Under average cutout dates for southeast Arkansas and an additional 350 DD60s, all locations in Arkansas would be exposed to the entire third generation of larvae. However, under average cutout dates for northeast Arkansas and an additional 350 DD60s, only 90% of the third generation would reach third instar before cutout at Pickens and only 40% would reach third instar before cutout at Leachville.

Table 10. Number of *H. zea* generations for the different test sites and different larval development models.

Number of *H. zea* Generations Using 2009 Weather Data

	Diet Model	Corn-Cotton Model	Bt Corn 0ug - Con. Cotton Model	Bt Corn 10ug - Bt Cotton Model
Test 1 - Gin City	5.0	4.0	4.0	3.9
Test 2 - Foreman	5.0	4.0	4.0	3.7
Test 3 - Pickens	4.1	4.0	3.2	3.0
Test 4 - Leachville	4.0	3.7	3.0	3.0

Table 11. Fraction of third generation larvae reaching size of large larvae before cotton cutout.

Fraction of Third-Generation Large Larvae Before Average Cutout + 350 DD (Historical Cutout Date for N.E. Arkansas)

	Diet Model	Corn-Cotton Model	Bt Corn 0ug - Con. Cotton Model	Bt Corn 10ug - Bt Cotton Model
Test 1 - Gin City	1.0	1.0	1.0	1.0
Test 2 - Foreman	1.0	1.0	1.0	1.0
Test 3 - Pickens	1.0	1.0	1.0	0.9
Test 4 - Leachville	1.0	1.0	1.0	0.4

Discussion

Levels of Bt susceptibility observed in this study were similar to those previous observed but greater than those reported for some of the less susceptible field colonies tested over the last decade (Ali and Luttrell 2009b). Additional exposure of *H. zea* larvae from Bt corn to Cry1Ac resulted in reduced survival and increased time to pupation. Field collected insects exhibited strong reproductive fitness costs and subsequent tests with progeny were limited to those crossed with LabZA, the susceptible laboratory stain. This may have direct implications to the experimental procedures previously used to study levels of Cry1Ac and Cry2Ab resistance in *H. zea* (Luttrell et al. 1999, Ali et al. 2006, Ali and Luttrell 2007, Ali and Luttrell 2009b). Strong reproductive fitness costs and laboratory rearing of field insects for multiple generations prior to testing could dramatically impact the resistance gene frequencies in the test insects and result in under-estimates of original resistance levels. The founder effects measured in this study may be useful in *a posteriori* examinations of the previous resistance monitoring reports.

Survival of larvae to pupation on Bollgard and Bollgard II cotton was less than 5% but measurable and generally similar to that previously reported by Ali and Luttrell (2009a). The greatly expanded development time has important implications to field ecology of this polyphagous pest. Cotton, as noted by Gore et al. (2003) and many others, is a poor host for *H. zea*. Additional delays in the development of surviving individuals fed Bt cotton or Bt corn further impacts seasonal abundance of this multi-voltine pest and may allow cotton to escape some of the historical economic damage associated with longer-season cotton production and previous plant cultivars that did not express insecticidal toxins. Alternatively, the expected commercialization of new Bt corns that have higher activity against *H. zea* may further reduce the threat of damaging populations on cotton prior to cotton cutout, especially in northeast Arkansas where the growing season is shorter and efficient management of the harvestable crop is a priority.

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