Genetic Variation for Resistance to *Bacillus thuringiensis* Toxins in *Helicoverpa zea* (Lepidoptera: Noctuidae) in Eastern North Carolina

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J. Econ. Entomol. 99(5): 1790–1797 (2006)

ABSTRACT To evaluate resistance to *Bacillus thuringiensis* Berliner (Bt) toxins, adult female bollworms, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), were collected from four light trap locations in two eastern North Carolina counties from August to October during 2001 and 2002. Females were allowed to oviposit, and upon hatching, 24 neonates from each female (F_1 lines) were screened for survival and growth rate on each of three diets: non-Bt diet, diet containing 5.0 μg/ml Cry1Ac toxin, or diet containing 5.0 μg/ml Cry2Ab toxin. These screens were designed to identify nonrecessive Bt resistance alleles present in field populations of bollworm. Of 561 and 691 families screened with both Cry1Ac- and Cry2Ab-containing diets in 2001 and 2002, respectively, no F_1 lines were identified that seemed to carry a gene conferring substantial resistance to either Cry1Ac or Cry2Ab. Adults from F_1 lines with growth scores in the highest (R) and lowest (S) quartiles were mated in four combinations, RxR, SxR, RxS, and SxS. Differences in growth rates of larvae from these crosses demonstrated that there is substantial quantitative genetic variation in eastern North Carolina populations for resistance to both Cry1Ac and Cry2Ab toxins. These findings, in addition to results suggesting partially dominant inheritance of resistance to Cry1Ac and Cry2Ab, are critically important for determining appropriate resistance management strategies that impact the sustainability of transgenic cotton, *Gossypium hirsutum* (L.).

KEY WORDS *Bacillus thuringiensis*, bollworm, resistance management, resistance monitoring

Transgenic cotton, *Gossypium hirsutum* (L.), containing genes from the soil bacterium *Bacillus thuringiensis* Berliner (Bt), have been widely planted in recent years, comprising ≈80% of the cotton acreage in North Carolina (Williams 2005). The majority of this acreage is currently planted to Bollgard (Monsanto Company, St. Louis, MO), which produces the Cry1Ac δ-endotoxin. The remainder is made up of Bollgard II (Monsanto Company), which produces Cry1Ac and Cry2Ab, or WideStrike (Dow AgroSciences, LLC, Indianapolis, IN), which expresses Cry1Ac and Cry1F. Although transgenic cotton provides complete control of the tobacco budworm, *Heliothis virescens* (F.), laboratory and field studies have demonstrated that Bollgard cotton does not adequately control bollworm in all cases (Burd 2001, Jackson et al. 2004, Mahaffey et al. 1995). Thus, resistance management issues associated with the deployment and sustainability of Bt cotton have become a concern.

For resistance evolution to be managed using the high-dose refuge strategy, Bt cotton must express a high dose of toxin, inheritance of resistance must be effectively recessive in the field, frequency of resistance genes in the population must be low, and most individuals that develop in the Bt crop must mate with individuals that develop in non-Bt crops (Bates et al. 2005). However, studies have suggested that cotton producing only Cry1Ac is not a high dose for bollworm (Mahaffey et al. 1995) and that inheritance of resistance to Cry1Ac is dominant or incompletely dominant (Burd et al. 2000). In addition, Burd et al. (2003) estimated the frequency of Bt resistance alleles in the general bollworm population and determined that it was not as low as had been assumed.

Because Bt resistance alleles were detected in the general population of bollworm in North Carolina during 2000 (Burd et al. 2003), this study was first designed to determine whether a substantial shift in the frequency of resistance genes would be observed in the North Carolina bollworm population over a 3-yr period. Second, tests were initiated to verify whether individuals that responded moderately well to discriminating doses of Cry1Ac and Cry2Ab toxins carried minor resistance genes. If no alleles conferring resistance to Cry1Ac or Cry2Ab were discovered, we sought to determine whether minor resistance genes would impact larval growth and survival on these toxins. And third, stable carbon isotope analyses were used to determine whether host use impacted genetic variation for Bt resistance in bollworm. Because bollworm is a very polyphagous pest and development on

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Bollgard cotton is not uncommon, these analyses were conducted to determine whether average growth ratings from F_1 screens on Cry1Ac-containing and Cry2Ab-containing artificial diet would differ between families whose maternal parent completed larval development on C_3 or C_4 hosts.

Materials and Methods

F_1 Screens. From August to October during 2001 and 2002, adult female bollworms were collected from light traps at four locations in eastern North Carolina; three light traps were located in Washington Co., and the fourth trap was located in Martin Co. These traps were located in a diverse agroecosystem near crop hosts such as cotton; corn, *Zea mays* L.; soybean, *Sorghum bicolor* (L.) Moench.; and peanut, *Arachis hypogaea* L. In total, 561 and 691 females (lines/families) were collected in 2001 and 2002, respectively, and they were used for the bioassays. Females were placed individually into 300-ml clear plastic cups that were covered with cheesecloth to provide a substrate for oviposition. Females were retained in rearing facilities at North Carolina State University at 27–30°C, 55–60% RH, and a photoperiod of 14:10 (L:D) h. Cheesecloth was checked daily for the presence of eggs.

Upon hatch, 24 neonates from each family line were placed onto each of three diets: non-Bt (NBT) and Cry1Ac- and Cry2Ab-containing diets. All diets were prepared by the methods of Burton (1970). Both Bt diets contained 5.0 μg of toxin per milliliter of diet, which was the discriminating dose used by Burd et al. (2003). Cry1Ac (MVPII, Mycogen Corp., San Diego, CA) was obtained as a gift. Cry2Ab was acquired by producing lyophilized corn tissue containing the Cry2Ab toxin and grinding the tissue into powder; Monsanto Company determined the concentration of Cry2Ab within the corn powder by using a toxin-specific enzyme-linked immunosorbent assays as described by Sims et al. (1996). Diet was poured into 24-well plastic bioassay plates (Corrigan and Company Inc., Jacksonville, FL) and refrigerated before use. A single neonate (<12 h old) was placed into each well using a fine camel's-hair brush, and Mylar film was heat sealed onto bioassay plates. Two holes were punched through the film over each well with no. 2 insect pins to allow air exchange. All plates were observed after 7 d when larvae were scored based on instar size, as determined based on head capsule and body size (Neunzig 1969). Instar was based on head capsule size; ratings within an instar were dependent upon the relationship of head capsule size to body size. For example, a large head capsule on a small body would be an early instar, whereas, a small head capsule on a large body would be a late instar. Instar sizes were converted to an ordinal ranking system, where 1, first instar; 2, early second; 3, mid-second; 4, late second; 5, early third; 6, mid-third; 7, late third; 8, early fourth; 9, mid-fourth; 10, late fourth; 11, early fifth; 12, mid-fifth.

Selection of Survivors. In 2001, survivors from the F_1 screens were used for subsequent experiments, by using lines whose performance ranked in the upper or lower quartile for either of the Bt diets. Larvae from the non-Bt diet were reared to the adult stage. Reciprocal F_1 crosses were made between adults from lines that ranked in the highest (R) and lowest (S) quartiles for each diet. RxR and SxS crosses also were conducted. Neonates from successful F_1 crosses were placed on the appropriate Bt diet to determine inheritance of resistance. Surviving larvae were weighed after 10 d. These data were not reported for 2002 because low numbers of viable eggs were obtained from these crosses.

Host Use Effect on Genetic Variation. More than 25 crops grown in the cotton belt have been recorded as hosts for bollworm (Stadelbacher et al. 1986, Kogan et al. 1989, Sudbrink and Grant 1995). Because of its host diversity, we chose to determine whether host use impacted genetic variation for Bt resistance in bollworm. A subsample of 74 adult females collected in 2001 was analyzed to determine stable carbon isotope ratios for each moth. Plants using the C_3 photosynthetic pathway, such as cotton, are more depleted in ^{13}C relative to ^{12}C than C_4 plants, such as corn (Hardwick 1965). Because this subsample of moths was collected during the period in which survivors from Bollgard cotton would be eclosing, we wanted to determine whether average growth ratings from F_1 screens on Cry1Ac- and Cry2Ab-containing artificial diet would differ between families whose maternal parents completed larval development on C_3 or C_4 hosts.

In 2001, adult females whose progeny were tested in the original Bt performance screen were placed individually into glass scintillation vials and frozen. A subsample of 37 moths whose progeny was in the upper quartile with respect to survival and growth rate on Bt diet was selected for stable carbon isotope analysis. In addition, 37 females whose progeny were in the lower quartile with regard to performance on Bt diet were selected for analysis. These selected females were collected between mid-August and mid-September, the period in which adults would be emerging from Bt cotton fields (Jackson et al. 2004). One forewing from each moth was removed and placed into a 2.5-ml plastic vial. The wing was then cut into three pieces and placed into a 0.2-cm^2, tared tin capsule that was combusted to CO_2 by using a Carlo Erba NA 1500 CHN analyzer at the University of Georgia stable isotope research laboratory (Athens, GA). The $\delta^{13}C$ measurements were made on a Finnigan-MAT Delta C mass spectrometer (Thermo Electron Corporation, Waltham, MA) using a Conflo II Interface. Companion standards with known isotope ratios were tested along with the experimental samples. Females from the 2002 experiment were not analyzed for isotopic ratios because minimal variation in average ratings among families.

Data Analysis. PROC UNIVARIATE was used to determine average ordinal ranking for each single family line on each of the three diets (SAS Institute 1990). Only survivors were used in determining the average ordinal ranking of each family. To control for vigor effects, average ratings for all families on either

Table 1. Correlation of selected traits among bollworm families selected on each of three diets (Cry1Ac, Cry2Ab, and NBT)

Comparison			2001		2002	
			r^a	P^b	r^a	P^b
Avg ratings on Cry1Ac	versus	avg ratings on Cry2Ab	0.318	<0.0001	0.578	<0.0001
Avg ratings on Cry1Ac	versus	avg ratings on NBT	−0.052	0.2590	0.348	<0.0001
Avg ratings on Cry2Ab	versus	avg ratings on NBT	0.124	0.0044	0.423	<0.0001
% survival on Cry1Ac	versus	% survival on Cry2Ab	0.485	<0.0001	0.612	<0.0001
% survival on Cry1Ac	versus	% survival on NBT	0.065	0.1241	0.054	0.1628
% survival on Cry2Ab	versus	% survival on NBT	0.041	0.3277	0.067	0.0833
% survival on Cry1Ac	versus	avg ratings on Cry1Ac	0.284	<0.0001	0.083	0.0383
% survival on Cry2Ab	versus	avg ratings on Cry2Ab	0.416	<0.0001	0.156	0.0001
% survival on NBT	versus	avg ratings on NBT	0.450	<0.0001	0.045	0.2423
Avg corrected ratings on Cry1Ac	versus	avg corrected ratings on Cry2Ab	0.453	<0.0001	0.586	<0.0001
Avg corrected ratings on Cry1Ac	versus	avg ratings on NBT	−0.437	<0.0001	−0.405	<0.0001
Avg corrected ratings on Cry2Ab	versus	avge ratings on NBT	−0.358	<0.0001	−0.365	<0.0001

[a] Pearson correlation coefficient.
[b] Probability > | r | under Ho: Rho = 0. See note under Date Analysis concerning adjustment of *P* values to increase power.

Bt diet were corrected according to the highest rated family on NBT diet (Burd et al. 2003). Average ratings on Bt diets were corrected according to the following formula: average corrected rating = [average rating (Bt)/(average rating (NBT)/highest average rating (NBT))]. PROC CORR was used to determine any correlation between instar size and percentage of survivorship on any of the three diets. All correlation tests used the Pearson correlation coefficient to determine significance. Original *P* values are presented in Table 1; however, an independent test, the sequential Bonferroni technique (Holm 1979) as described by Rice (1989), was used to enhance power and to eliminate the possibility of incorrectly rejecting one or more true null hypotheses. In 2001, no significant correlation was changed by the above-mentioned technique. The only significant correlation that would have changed in 2002 was the percentage of survival on Cry1Ac versus average rating for lines on Cry1Ac. Thus, no inferences are made for this comparison based on lack of significance. Mean larval weights from selection experiments were separated using Fisher protected least significant difference test with PROC GLM (SAS Institute 1990). Data from the stable carbon isotope analyses were correlated (PROC CORR) to the average growth rating of the subsequent progeny on both Cry1Ac and Cry2Ab diets.

Results

F_1 Screens. Distributions of average ratings for each family line on NBT diet during each year of the study, along with percentage of survival of these families, are shown in Fig. 1. The average ratings were ≈9.4 and 6.7 for 2001 and 2002, respectively. These ratings corresponded to a mid-to-late fourth instar in 2001 and a late third instar in 2002. The 2002 distribution of families on NBT diet was shifted to the left compared with 2001 distributions. This may have been due to a change in the corn–soybean blend used in preparing the artificial diet or to a general lower vigor of the moths in 2002. The age class distributions for 2002 larvae on Cry1Ac and Cry2Ab diets also were shifted in a similar manner. Therefore, relationships of performance distributions on Bt diets to those on NBT diet were similar among years.

The distributions of average ratings for family lines on Cry1Ac-containing diet during each year of the study, along with percentage of survival of these families, are shown in Fig. 1. The average ratings for all larvae on Cry1Ac-containing diet were 4.4 and 2.6 for 2001 and 2002, respectively. Ratings corresponded to a late second to early third instar in 2001 and an early-to-mid second instar in 2002. Relationships of distributions on Cry1Ac-containing diet to that of the NBT diet were similar across years; the average performance ratings of families on Cry1Ac-containing diet were 47 and 39% of the average ratings of families on NBT diet in 2001 and 2002, respectively.

Also illustrated in Fig. 1 are the distributions of average ratings for family lines on Cry2Ab-containing diet during each year of the study as well as percentage of survival of these families. The average ratings for all larvae on Cry2Ab-containing diet were 4.9 for 2001and 2.6 for 2002. These ratings corresponded to an early third instar in 2001 and an early-to-mid second instar in 2002. As with Cry1Ac, relationships of distributions on Cry2Ab-containing diet to that of the NBT diet were similar across years; average performance ratings of families on Cry2A-containing diet were 52 and 39% of the average ratings of families on NBT diet in 2001 and 2002, respectively.

There was a significant correlation between average ratings of family lines on Cry1Ac and Cry2Ab diets during both years of the study (Table 1), indicating that families with larger than average individuals on Cry1Ac had larger than average individuals on Cry2Ab as well. In 2001, no significant correlation existed between average ratings when comparing Cry1Ac versus NBT; however, a significant positive correlation existed when comparing these ratings in 2002. Significant correlations existed between average ratings when comparing Cry2Ab versus NBT in 2001 and 2002.

Correlation analyses for average ratings that were corrected for vigor when comparing either Bt diet versus NBT diet are also shown in Table 1. The positive correlation between Cry1Ac and Cry2Ab diets remained when examining corrected ratings during both

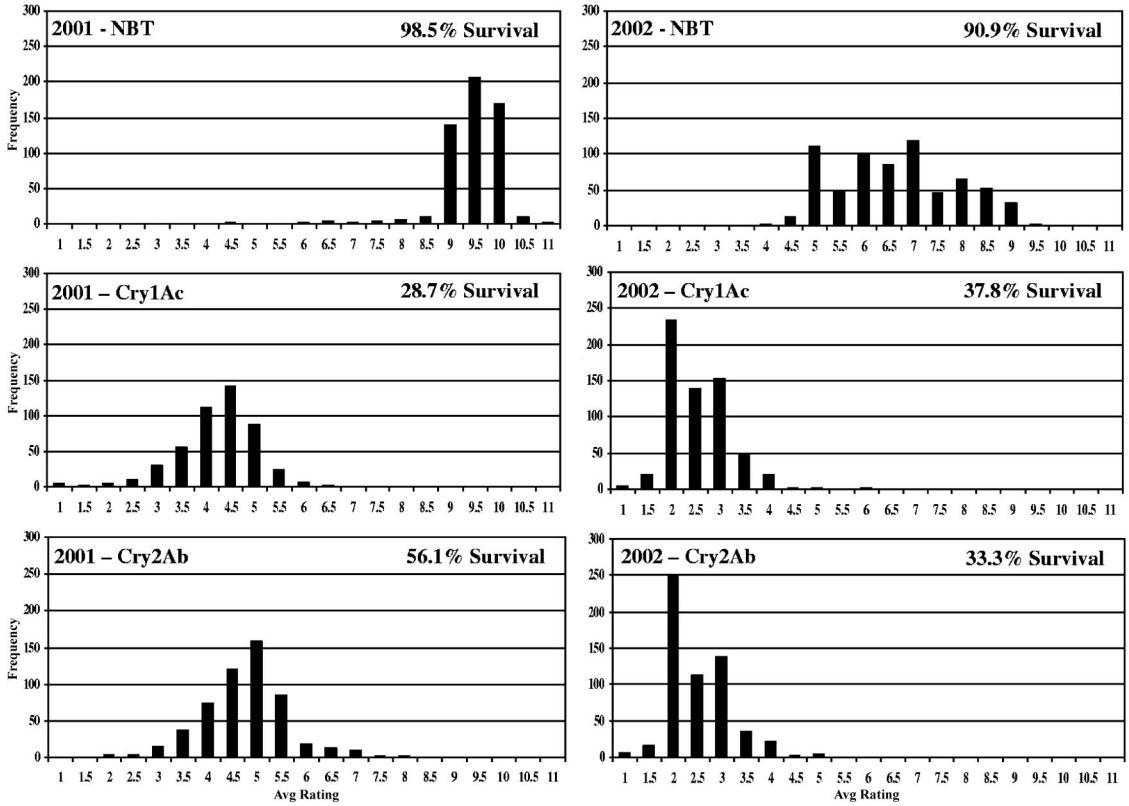

Fig. 1. Distributions of average ratings for 7-d-old larvae of *H. zea* family lines on Cry1Ac, Cry2Ab, and NBT diets in 2001 and 2002.

years of the study. However, in 2001, the insignificant correlation between ratings of families on Cry1Ac and NBT diets became a highly significant negative correlation when corrected for vigor; in 2002, the highly significant positive correlation between family ratings on Cry1Ac and NBT diets became a highly significant negative correlation. When comparing average ratings on Cry2Ab and NBT, significant positive correlations between ratings of families on Cry2Ab and NBT diets became increasingly significant as a negative correlation when corrected for vigor in both years.

There was also a significant positive correlation for percentage of survival of families when comparing Cry1Ac versus Cry2Ab in 2001 and 2002 (Table 1), indicating those families with higher survival on one Bt diet had higher survival on the other Bt diet as well. There was no significant correlation when comparing percentage of survival for families on either Cry1Ac or Cry2Ab versus those on NBT diet during either year of the study (Table 1). A significant positive correlation was also found between percentage of survival and average ratings for families on Cry1Ac, Cry2Ab, and NBT diets (Table 1) in 2001, suggesting that as average family rating increased so did percentage of survival for families on these diets. However, although these same correlations remained positive in 2002, only the correlation with percentage of survival on Cry2Ab versus average family rating on Cry2Ab was significant (Table 1).

Selection of Survivors. Because no major Bt resistance genes were detected in 2001 or 2002, tests were conducted to determine whether there was genetic variation among families whose average ratings were in the upper and lower quartiles. To determine whether single family lines with growth in the highest 25% of all 561 single family lines on Cry1Ac- or Cry2Ab-containing diet were genetically different from lines with growth in the lowest 25%, families with upper and lower quartile ratings were used to perform reciprocal crosses. Mean ratings on Cry1Ac-containing diet for single family lines used to perform reciprocal crosses were 5.4 for upper quartile lines and undefined for lower quartile lines, because there were no survivors on Cry1Ac-containing diet. Mean growth ratings on Cry2Ab-containing diet for lines used for reciprocal crosses were 6.0 for the upper quartile lines and 2.9 for the lower quartile lines.

Reciprocal crosses were established using individuals from each line that had developed on the non-Bt diet so that there would be no maternal effects carried over from the stress of the Bt diets. Seven and eight successful crosses were obtained for Cry1Ac and Cry2Ab lines, respectively. Successful crosses for Cry1Ac screening were as follows: two resistant

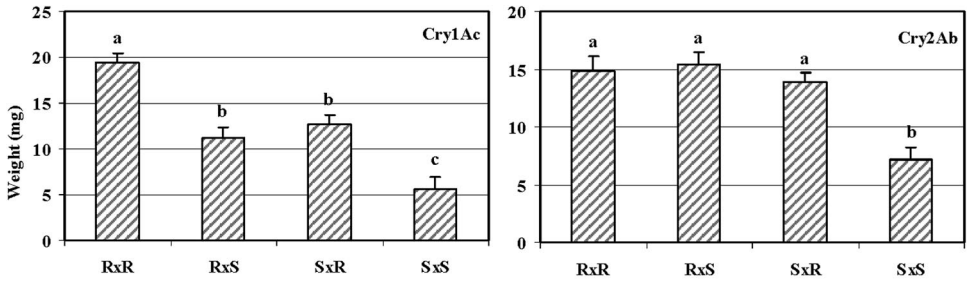

Fig. 2. Mean larval weight in milligrams for *H. zea* upper quartile line (RxR), lower quartile line (SxS), and reciprocal crosses between these lines (RxS and SxR) taken after 10 d on 5.0 μg/ml Cry1Ac- or Cry2Ab-containing artificial diet.

crosses (RxR), two resistant female × control male crosses (RxS), two control female × resistant male crosses (SxR), and one control cross (SxS). Cry2Ab crosses that were successful were two RxR, two RxS, three SxR, and one SxS.

Average weights of larvae from RxR, RxS, SxR, and SxS crosses after rearing on Cry1Ac-containing diet for 10 d are shown in Fig. 2. There was a significant difference in average larval weights on Cry1Ac-containing diet among F_2 crosses ($F = 46.64$; df = 3, 3; $P = 0.0112$). F_2 cross weights were highest for the RxR cross and lowest for the SxS cross. No difference existed in weights of larvae on Cry1Ac-containing diet between the reciprocal crosses. A significant difference also was observed for weights of larvae on Cry2Ab-containing diet among F_2 crosses ($F = 27.23$; df = 3, 3; $P = 0.0112$). The RxR and reciprocal crosses produced average larval weights that were higher than that of the SxS cross on Cry2Ab-containing diet.

The distributions of log-transformed larval weights on Cry1Ac- and Cry2Ab-containing diets for each of the four crosses are presented in Fig. 3, along with percentage of survival of F_2 individuals. RxR distributions were shifted farther to the right than all other crosses, particularly the SxS cross on both Cry1Ac- and Cry2Ab-containing diets. Percentage of survival was higher for RxR and reciprocal crosses than for the SxS cross on either diet.

Host Use Effect on Genetic Variation. Approximately 34% of the F_0 moths possessed isotopic ratios consistent with C_4 hosts. Correlation analyses for average ratings for families on Cry1Ac, Cry2Ab, and NBT diets versus $\delta^{13}C$ ratios of the corresponding female parent are shown in Fig. 4. Progeny of individuals that developed on C_4 hosts as larvae had an average rating (SE) on Cry1Ac of 4.38 (0.09), whereas those that developed on C_3 hosts as larvae had an average rating of 3.64 (0.26). For Cry2Ab, average ratings for individuals that developed as larvae on C_3 and C_4 hosts were 4.69 (0.14) and 4.74 (0.10), respectively. However, several families whose maternal parent developed on a C_3 host had an average rating of zero, which indicates that there were no survivors for that family. All C_3 and C_4 families on NBT diet performed similarly with respect to average ratings.

Discussion

Based on previous studies from Burd et al. (2000), inheritance for resistance to Cry1Ac toxin in bollworm is dominant or incompletely dominant. Therefore, any individuals carrying resistance alleles were likely to survive when screened on a discriminating dose of Cry1Ac toxin in artificial diet. This type of inheritance enables heterozygotes to survive when selected on Bt diet. Heterozygotes also would be the most probable carrier of resistance alleles in field populations. In addition, the most probable matings in the general population would likely occur between heterozygote individuals and homozygote susceptible individuals. One-half of the offspring from this cross would be heterozygote and the other homozygote susceptible. Based on the above-mentioned information, a line was considered resistant if 50% of the screened F_1 individuals were similar in size to their NBT counterparts.

Burd et al. (2003) reported an estimated gene frequency for resistance to Cry1Ac to be 0.00043 after finding one resistant individual out of 583 collected and screened in 2000 (assuming four alleles per moth). In addition, the frequency of alleles conferring resistance to Cry2Aa was estimated to be 0.00039, where a single resistant individual was detected out of 646 screened (assuming four alleles per moth). During 2001, 561 females were screened against Cry1Ac- and Cry2Ab-containing diet, whereas 691 females were screened against the same diets in 2002. Results from these studies suggested that none of the individuals tested during these 2 yr were carriers of major alleles imparting resistance to either Cry1Ac or Cry2Ab. However, as described by Burd et al. (2003), many families had above-average growth on Cry1Ac and Cry2Ab diets. Although this accelerated growth was not attributed to major resistance genes (F_1 survivors not as large as those on NBT diet), it could be attributed to minor resistance genes or major resistance genes with lower levels of dominance.

Because Bollgard cotton does not produce a high dose for bollworm, larvae with minor resistance genes would be selected for and could, over time, decrease the efficacy of Bollgard cotton as well as second-generation, pyramided-gene cotton. The 2001 study demonstrated that single family lines with growth in the highest 25% of all 561 single family lines on Bt

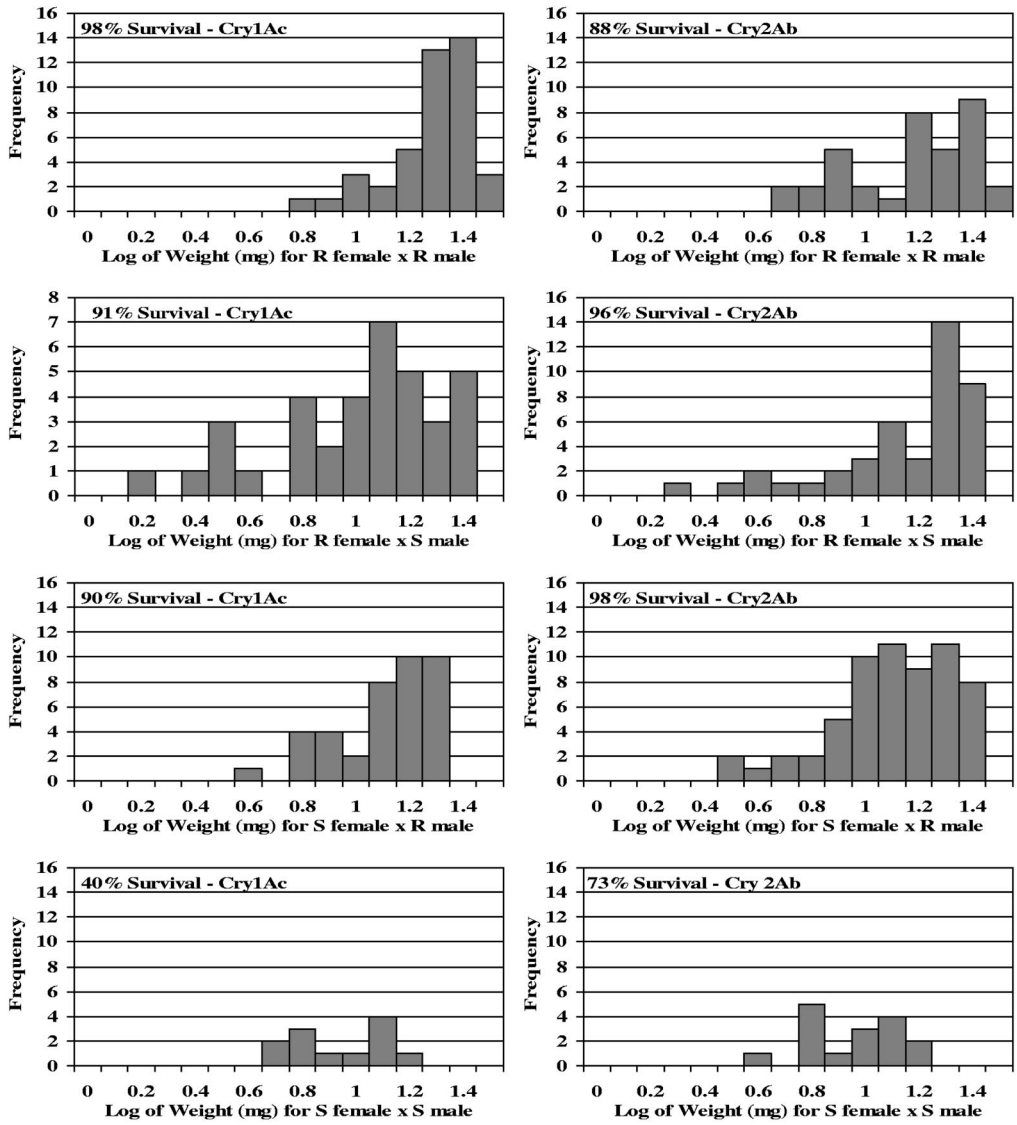

Fig. 3. Distribution of larval weights ($\log_e$) for upper quartile lines, lower quartile lines, and reciprocal F_1 crosses between these lines taken after 10 d on 5.0 μg/ml Cry1Ac- or Cry2Ab-containing artificial diet. Percentage survival for each group is shown on each graph.

toxins were genetically different from lines whose growth was in the lowest 25%. That larvae from the S female × R male crosses performed better than the larvae of the SxS crosses proves that the difference was not due to a maternal effect. Given that there is heritable variation for minor genes, monitoring programs for bollworm and other pests exposed to moderate doses of Bt toxins should not only search for major genes.

Results from selection experiments testing inheritance of resistance to Cry1Ac are consistent with those reported by Burd et al. (2000). These studies indicated that resistance to Cry1Ac is inherited as a dominant or incompletely dominant trait. This experiment demonstrated that resistance to Cry2Ab is also inherited as a dominant or incompletely dominant trait. However, the dominance described here could be characterized as functional dominance based on the discriminating dose of toxins used in the studies (Tabashnik et al. 2002, Burd et al. 2003). It should be noted, however, that previous work by Burd (2001) showed that resistance to Cry1Ac also was dominantly inherited at 100 μg/ml diet.

To determine whether above-average growth rates of survivors on Bt diets were influenced by survival of F_0 females on Bt cotton, stable carbon isotope analyses were performed on two subsamples of moths: 1) those whose progeny's growth rate was in the upper quartile, and 2) those whose progeny's growth rate was in the lower quartile. If individuals completing develop-

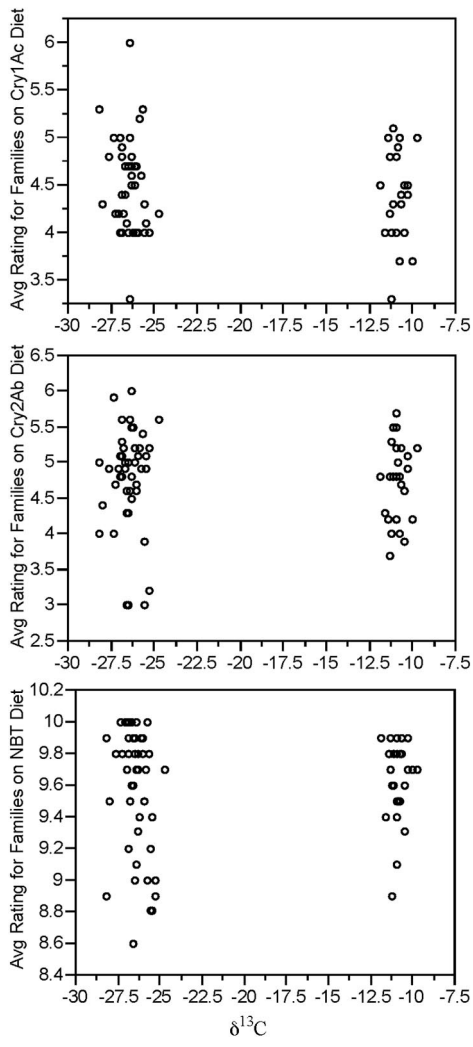

Fig. 4. Correlation graphs for average ratings for families on Cry1Ac, Cry2Ab, and NBT artificial diets versus $\delta^{13}C$ levels of the maternal parent collected from mid-August to mid-September in 2001.

ment on Bt cotton were screened, then moths that fed as larvae on C_3 host plants would have greater survival and an increased growth rate in the presence of Cry1Ac-containing diet than those from C_4 hosts. Analyses demonstrated that progeny from an individual that developed as a larva on a C_3 host are likely to perform similarly in the presence of a Bt toxin compared with individuals whose maternal parent developed as a larva on C_4 hosts. These results suggest that the bollworm adults that originated from C_3 hosts most likely developed on soybean, *Glycine max* (L.) Merrill, peanuts, non-Bt cotton, or broadleaf weeds, rather than on Bt cotton. Also, because 34% of the individuals developed on C_4 hosts, and field corn is the most prominent C_4 host during this period, field corn may serve as an important source of nonselected adult moths for mating with those emerging from Bt cotton.

Comparisons of frequency distributions of average ratings of families on each Bt toxin and the relationships of distributions on Bt and non-Bt diet among the 2 yr of this study and the study described by Burd et al. (2003) demonstrated that there was no substantial change in the shape of the growth distributions for single family lines on either Cry1Ac or Cry2Ab over the 3-yr period. This indicates that if there had been an increase in the frequency of major or minor resistance genes, it had been too small to detect, even with data on >500 families in each season. In addition, females collected from the same area in eastern North Carolina have been examined for the presence of Bt resistance alleles from 2003 to 2005 in a USDA–ARS resistance monitoring program. To date, no other evidence of major Bt resistance genes has surfaced for North Carolina bollworm populations (Blanco et al. 2004, 2005). The results from our 3-yr gene frequency estimates and those of Blanco et al. (2004, 2005) may allow us to quantify certain parameters that are typically assumed when modeling evolution of Bt resistance in field populations of bollworms. These results may be useful in modeling efforts used to make resistance management decisions so not to limit the sustainability of these or future Bt technologies.

Acknowledgments

Special thanks to Rogan Jackson, Margery Ambrose, Maria Marcus, Ryan Kurtz, and Carlton Purvis for technical assistance. We express appreciation to Cotton, Inc. for providing a graduate research assistantship for R.E.J. and to Monsanto Company for providing partial project funding.

References Cited

Bates, S. L., J.-Z. Zhao, R. T. Roush, and A. M. Shelton. 2005. Insect resistance management in GM crops: past, present and future. Nat. Biotechnol. 23: 57–62.

Blanco, C. A., L. C. Adams, J. Gore, D. Hardee, M. Mullen, J. R. Bradley, J. Van Duyn, P. Ellsworth, J. Greene, D. Johnson, et al. 2004. *Bacillus thuringiensis* monitoring program for tobacco budworm and bollworm in 2003, pp. 1327–1331. *In* Proceedings of the Beltwide Cotton Conference, 5–9 January 2004, San Antonio, TX. National Cotton Council of America, Memphis, TN.

Blanco, C. A., M. Mullen, C. Abel, J. R. Bradley, P. Ellsworth, J. K. Greene, A. Herbert, R. Leonard, J. D. Lopez, R. Meagher, et al. 2005. *Bacillus thuringiensis* Cry1Ac resistance monitoring program for tobacco budworm and bollworm in 2004, pp. 1210–1218. *In* Proceedings of the Beltwide Cotton Conference, 4–7 January 2005. National Cotton Council of America, Memphis, TN.

Burd, A. D., F. Gould, J. R. Bradley, Jr., J. W. Van Duyn, and W. J. Moar. 2003. Estimated frequency of nonrecessive Bt resistance genes in bollworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) in eastern North Carolina. J. Econ. Entomol. 96: 137–142.

Burd, A. D. 2001. The influences of environmental factors on susceptibility of *B.t.* cottons to bollworm, *Helicoverpa zea*, and factors affecting resistance to *B.t.* toxins for bollworm. Ph.D. dissertation, North Carolina State University, Raleigh, NC.

Burd, T., J. R. Bradley, Jr., J. W. Van Duyn, and F. Gould. 2000. Resistance of bollworm, *Helicoverpa zea*, to Cry1Ac

toxin, pp. 923–926. *In* Proceedings of the Beltwide Cotton Conference, 5–8 January 2000, San Antonio, TX. National Cotton Council of America, Memphis, TN.

Burton, R. L. 1970. A low cost artificial diet for the corn earworm. J. Econ. Entomol. 63: 1969–1970.

Hardwick, D. F. 1965. The corn earworm complex, p. 246. Mem. Entomol. Soc. Can. 40.

Holm, S. 1979. A simple sequentially rejective multiple test procedure. Scand. J. Stat. 6: 65–70.

Jackson, R. E., J. R. Bradley, Jr., J. W. Van Duyn, and F. Gould. 2004. Comparative production of bollworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), from transgenic cottons expressing either one or two *Bacillus thuringiensis* Berliner proteins with and without insecticide oversprays. J. Econ. Entomol. 97: 1719–1725.

Kogan, M., C. G. Helm, J. Kogan, and E. Brewer. 1989. Distribution and economic importance of *Heliothis virescens* and *Helicoverpa zea* in North, Central, and South America and of their natural enemies and host plants, pp. 241–297. *In* E. G. King and R. D. Jackson [eds.], Proceedings of the Workshop on Biological Control of *Heliothis:* Increasing Effectiveness of Natural Enemies, New Delhi, India. Office of International Cooperation and Development, U.S. Dep. Agric., Washington, DC.

Mahaffey, J. S., J. R. Bradley, Jr., and J. W. Van Duyn. 1995. B. t. cotton: field performance in North Carolina under conditions of unusually high bollworm populations, pp. 796–798. *In* Proceedings of the Beltwide Cotton Conference, 4–7 January 1995, San Antonio, TX. National Cotton Council of America, Memphis, TN.

Neunzig, H. H. 1969. The biology of the tobacco budworm and the corn earworm in North Carolina with particular reference to tobacco as a host. North Carolina Agricultural Experiment Station. Tech. Bull. No. 196.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43: 223–225.

SAS Institute. 1990. SAS/STAT user's guide, vol. 2. SAS Institute, Cary, NC.

Sims, S. R., S. A. Berberich, D. L. Nida, L. L. Segalini, J. N. Leach, C. C. Ebert, and R. L. Fuchs. 1996. Analysis of expressed proteins in fiber fractions from insect-protected and glyphosate-tolerant cotton varieties. Crop Sci. 36: 1212–1216.

Stadelbacher, E. A., H. M. Graham, V. E. Harris, J. D. Lopez, J. R. Phillips, and S. H. Roach. 1986. *Heliothis* populations and wild host plants in the southern U.S., pp. 54–74. *In* S. J. Johnson, E. G. King, and J. R. Bradley, Jr. [eds.], Theory and tactics of *Heliothis* population management. Southern Coop. Ser. Bull. 316.

Sudbrink, D. L., and J. F. Grant. 1995. Wild host plants of *Helicoverpa zea* and *Heliothis virescens* (Lepidoptera: Noctuidae) in eastern Tennessee. Environ. Entomol. 24: 1080–1085.

Tabashnik, B. E., Y.-B. Liu, T. J. Dennehy, M. A. Sims, M. S. Sisterson, R. W. Biggs, and Y. Carrière. 2002. Inheritance of resistance to Bt toxin Cry1Ac in a field-derived strain of pink bollworm (Lepidoptera: Gelichiidae). J. Econ. Entomol. 95: 1018–1026.

Williams, M. R. 2005. Cotton insect loss estimates – 2004, pp. 1105–1160. *In* Proceedings of the Beltwide Cotton Conference, 4–7 January 2005, New Orleans, LA. National Cotton Council of America, Memphis, TN.

Received 20 February 2006; accepted 28 June 2006.