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GENETIC VARIATION FOR RESISTANCE TO CRY1AC AND CRY2AB IN BOLLWORM, *HELICOVERPA ZEA*, IN NORTH CAROLINA

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Abstract

Adult female bollworm moths were collected from various light trap locations in eastern North Carolina in late summer 2001. Female moths were allowed to oviposit, and upon hatch, 24 neonates from each female (F_1 families) were screened for growth rate on each of three diets: non-*B. t.*, Cry1Ac, and Cry2Ab; *B. t.* diets contained 5.0 µg toxin per milliliter of diet. Adults from F_1 families with growth scores in the highest (H) and lowest (L) quartiles were mated in 4 combinations, HxH, LxH, HxL, and LxL. Differences in growth rates of larvae from these crosses demonstrated that there is substantial quantitative genetic variation for resistance to both CryIAc and Cry2Ab.

Introduction

Bollgard cottons have been widely planted in recent years, comprising approximately 67% of the cotton acreage in North Carolina in 2001 (Bacheler per. comm.). These transgenic cottons are particularly active against heliothine pests, such as tobacco budworm, *Heliothis virescens* (Fab.), and bollworm, *Helicoverpa zea* (Boddie); however, recent laboratory and field studies have demonstrated that Bollgard cottons do not adequately control bollworm and that major resistance genes can be found in North Carolina bollworm populations (Jackson et al. 2000, 2001; Burd et al. 1999, 2001; Lambert et al. 1996, 1997; Mahaffey et al. 1994, 1995). Because of this, strict attention has been devoted to resistance management issues associated with the deployment and sustainability of *B. t.* cottons (Tabashnik 1994; Matten 2001).

B. t. cottons must express a high dose of toxin, inheritance of resistance must be incompletely or completely recessive, and frequency of resistance genes in the population must be low, in order for resistance evolution to be controlled (Scientific Advisory Panel 1998; Union of Concerned Scientists 1998); however, recent studies have suggested that none of these assumptions are true for bollworm (Burd et al. 1999, 2000, 2001). Current resistance management strategies are dependent upon these assumptions; therefore, it is critical that these unknowns be resolved so that appropriate resistance management strategies can be implemented.

The objectives of the study were 1) to extend results of Burd et al. (2001) by determining if individuals that responded moderately well to discriminating doses of Cry1Ac and Cry2Ab toxins carried minor resistance genes that could decrease the efficacy of Bollgard cotton, and 2) determine if there had been a substantial shift in the frequency of resistance genes in the bollworm population from the 2000 to 2001 growing season.

Materials and Methods

From August-October 2001, adult female bollworm moths were collected from light traps at various locations in eastern North Carolina. A total of 561 female moths (lines) were used for the bioassays. Adult females were placed individually into 10-oz. clear plastic cups that were covered with cheesecloth to provide a substrate for oviposition. Moths were retained in rearing facilities at North Carolina State University at 27-30°C, 55-60% relative humidity, and a 14:10 light:dark photoperiod. Cheesecloths were checked daily for the presence of eggs.

Upon hatch, 24 neonates from each female line were placed onto each of three diets: non-*B. t.* (NBT), Cry1Ac-containing, and Cry2Ab-containing diets. Both *B. t.* diets contained 5.0 µg toxin per milliliter of diet. Cry1Ac was obtained from Mycogen, Corp. in a formulation of MVP®. Cry2Ab was acquired by producing lyophilized corn tissue containing the Cry2Ab toxin and grinding the tissue into powder; Monsanto Agric. Co. determined the concentration of Cry2Ab within the corn powder. Diet was poured into 24-well plastic bioassay plates and refrigerated before use. A single neonate was placed into each well using a fine camel hair paintbrush. Mylar film was heat-sealed onto bioassay plates to prevent escape of larvae. Two holes were punched through the film over each well with No. 2 insect pins to allow air exchange. All plates were observed after seven days when larvae were scored based on instar size. Instar size was determined based on head capsule and body size (Neunzig 1969). Instar sizes were converted to an ordinal ranking system as shown in Table 1. Data were analyzed using PROC UNIVARIATE (SAS Institute 1990).

For those lines whose performance ranked in the upper or lower quartile for either of the *B. t.* diets, larvae from the non-*B. t.* diet were reared to the adult stage. Reciprocal crosses were made between adults from lines that ranked in the highest (H) and lowest (L) quintiles for each diet. HxH and LxL crosses were also conducted. Neonates from successful crosses were placed on the appropriate *B. t.* diet and weighed after 10 days.

Results

Distribution of average ratings for each female line on NBT diet is shown in Figure 1. The average rating was approximately 9.4, which ranged between mid 4th and late 4th instar.

The distribution of average ratings for female lines on Cry1Ac-containing diet is shown in Figure 2. The average rating for all larvae on Cry1Ac-containing diet was 4.4, which ranged between late 2nd and early 3rd instar. To determine if single female lines with growth in the highest 25% of all 561 single female lines on Cry1Ac were genetically different from lines with growth in the lowest 25%, lines within these ranges were used (that portion of the line on NBT diet) to perform reciprocal crosses. The upper quartile rating for growth on Cry1Ac-containing diet was 5.0 (early 3rd instar), while the lower quartile rating was 4.0 (late 2nd instar). Mean ratings for single female lines used to perform reciprocal crosses were 5.4 for upper quartile lines and undefined for lower quartile lines since there were no survivors on Cry1Ac-containing diet.

Figure 3 illustrates the distribution of average ratings for female lines on Cry2Ab-containing diet. The average rating for all larvae on Cry2Ab-containing diet was 4.9, which is almost an early 3rd instar. As with those single female lines on Cry1Ac, upper and lower quartile lines on Cry2Ab-containing diet were saved to perform reciprocal crosses. The upper quartile growth rating for lines on Cry2Ab was 6.0 compared to 4.0 for lower quartile lines. Mean growth ratings for lines within these ranges used for reciprocal crosses were 6.0 for the upper quartile lines and 2.9 for the lower quartile lines.

Seven and eight successful crosses were obtained for Cry1Ac and Cry2Ab, respectively, for use in the reciprocal cross study. Successful crosses for Cry1Ac screening were as follows: 2 resistant crosses (HxH), 1 resistant female x control male cross (HxL), 2 control female x resistant male crosses (LxH), and 2 control crosses (LxL). Cry2Ab crosses that were successful were: 2 resistant crosses, 2 resistant female x control male crosses, 3 control female x resistant male crosses, and 1 control cross.

Average weights of larvae from HxH, HxL, LxH, and LxL crosses after rearing on Cry1Ac-containing diet for 10 days are shown in Figure 4. The average (SE) larval weight for the HxH lines was 19.4 (0.99) milligrams compared to 6.3 (0.86) milligrams for the LxL lines. Average larval weights for the H female x L male and L female x H male crosses were 15.2 (1.51) and 12.7 (0.97) milligrams, respectively. Average larval weights from crosses of HxH, HxL, LxH, and LxL crosses after rearing on Cry2Ab-containing diet are shown in Figure 5. The average (SE) larval weight for the HxH, HxL, LxH, and LxL crosses were 14.6 (1.38), 14.6 (1.07), 13.9 (0.78), and 7.95 (1.19), respectively.

Discussion

Burd et al. (2001) tested field-collected single female lines on CryIAc and on Cry2Aa. In that study, adults from the line that grew best on CryIAc were mated to adults from a line that performed very poorly on CryIAc, and progeny were tested for growth on CryIAc to confirm that the rare (0.00043) best-performing line had heritable CryIAc resistance. Although that work proved the existence of a rare major gene for resistance, no crosses were conducted to determine if smaller differences among other female lines were genetically based. Because Bollgards cotton do 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 cottons. The present study demonstrated that single female lines with growth in the highest 25% of all 561 single female lines on *B. t.* toxins were genetically different from lines whose growth was in the lowest 25%. The fact that larvae from the L female X H male crosses performed better than the larvae of the LxL 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 *B. t.* toxins should not only search for major genes.

A comparison of figures 2 and 3 from the present study and the two related figures in Burd et al. (2001) show no substantial change in the shape of the growth distributions for single female families on either CryIAc or Cry2A. This indicates that if there has been an increase in the frequency of minor resistance genes it has been too small to detect, even with data on over 500 females in each season. Furthermore, Burd et al. (2001) found 1 female line that did better than all other lines on CryIAc and Cry2Aa due to a genetic factor. We found no lines of this type. Had alleles for this major genetic factor increased substantially between the 2000 and 2001 season, we would have expected to find at least one such line. Again, our data indicate that resistance does not seem to be increasing rapidly in field populations of bollworm.

The reciprocal cross study (Figure 4) suggests that resistance to Cry1Ac toxin is dominantly or incompletely dominantly inherited, which is consistent with previous studies (Burd et al. 2000, 2001). Results from the reciprocal cross study on Cry2Ab-containing diet (Figure 5) indicate that inheritance of resistance to Cry2Ab is dominant as well.

The results provided herein may enable us to characterize and quantify certain parameters involved in modeling the evolution of *B. t.* resistance in bollworm. As suggested in this study and previous experiments, higher than anticipated resistance gene frequencies, in addition to dominant or incompletely dominant inheritance, demand proper deployment of *B. t.* cottons and adequate refuges to sustain the use of the *B. t.* technology.

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Literature Cited

Bacheler, J. S. 2002. Personal communication.

Burd, A. D., J. R. Bradley, Jr., and J. W. Van Duyn. 1999. Performance of selected *Bt* cotton genotypes against bollworm in North Carolina. Proc. Beltwide Cotton Conf. 931-934.

Burd, A. D., J. R. Bradley, Jr., J. W. Van Duyn, and F. Gould. 2000. Resistance of bollworm, *Helicoverpa zea*, to Cry1A(c) toxin. Proc. Beltwide Cotton Conf. 923-926.

Burd, A. D., J. R. Bradley, Jr., J. W. Van Duyn, and F. Gould. 2001. Estimated frequency of non-recessive *B.t.* resistance genes in bollworm, *Helicoverpa zea*. Proc. Beltwide Cotton Conf. 820-822.

Jackson, R. E., J. R. Bradley, Jr., A. D. Burd, and J. W. Van Duyn. 2000. Field and greenhouse performance of bollworm on Bollgard II cotton genotypes. Proc. Beltwide Cotton Conf. 1048-1052.

Jackson, R. E., J. R. Bradley, Jr., J. W. Van Duyn, and A. D. Burd. 2001. Efficacy of Bollgard and Bollgard II cottons against bollworm, *Helicoverpa zea* (Boddie), in field and greenhouse studies. Proc. Beltwide Cotton Conf. 815-818.

Lambert, A. L., J. R. Bradley, Jr., and J. W. Van Duyn. 1996. Effects of natural enemy conservation and planting date on the susceptibility of *Bt* cotton to *Helicoverpa zea* in North Carolina. Proc. Beltwide Cotton Conf. 931-935.

Lambert, A. L., J. R. Bradley, Jr., and J. W. Van Duyn. 1997. Interactions of *Helicoverpa zea* and *Bt* cotton in North Carolina. Proc. Beltwide Cotton Conf. 870-873.

Mahaffey, J. S., J. S. Bacheler, J. R. Bradley, Jr., and J. W. Van Duyn. 1994. Performance of Monsanto's transgenic *B.t.* cotton against high populations of lepidopterous pests in North Carolina. Proc. Beltwide Cotton Conf. 1061-1063.

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. Proc. Beltwide Cotton Conf. 795-798.

Matten, S. R. 2001. EPA update on insect resistance management for Bollgard cotton. Proc. Beltwide Cotton Conf. 840-844.

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. Bul. No. 196. 63 pp.

Scientific Advisory Panel, Subpanel on *Bacillus thuringiensis* (bt) Plant-Pesticides, February 9-10, 1998. 1998. Transmittal of the final report of the FIFRA Scientific Advisory Panel, Subpanel on *Bacillus thruingiensis* (Bt) Plant-Pesticides and Resistance Management, Meeting held on February 9-10, 1998. (Docket Number: OPPTS-00231).

SAS Institute. 1990. SAS/STAT User's Guide, Vol. 2. SAS Institute, Cary, NC, 795 pp.

Tabashnik, B. E. 1994. Evolution of resistance to *Bacillus thuringiensis*. Ann. Rev. Entomol. 39: 47-79.

Union of Concerned Scientists. 1998. Now or never: serious new plans to save a natural pest control. M. Mellon and J. Rissler, eds. pp. 149.

Table 1. Rating scale used to convert Helicoverpa zea instar size to appropriate ordinal ranking after seven days on artificial diet.

Larval Size	**Ordinal Ranking**
1st instar	1
Early 2nd instar	2
Mid 2nd instar	3
Late 2nd instar	4
Early 3rd instar	5
Mid 3rd instar	6
Late 3rd instar	7
Early 4th instar	8
Mid 4th instar	9
Late 4th instar	10
Early 5th instar	11

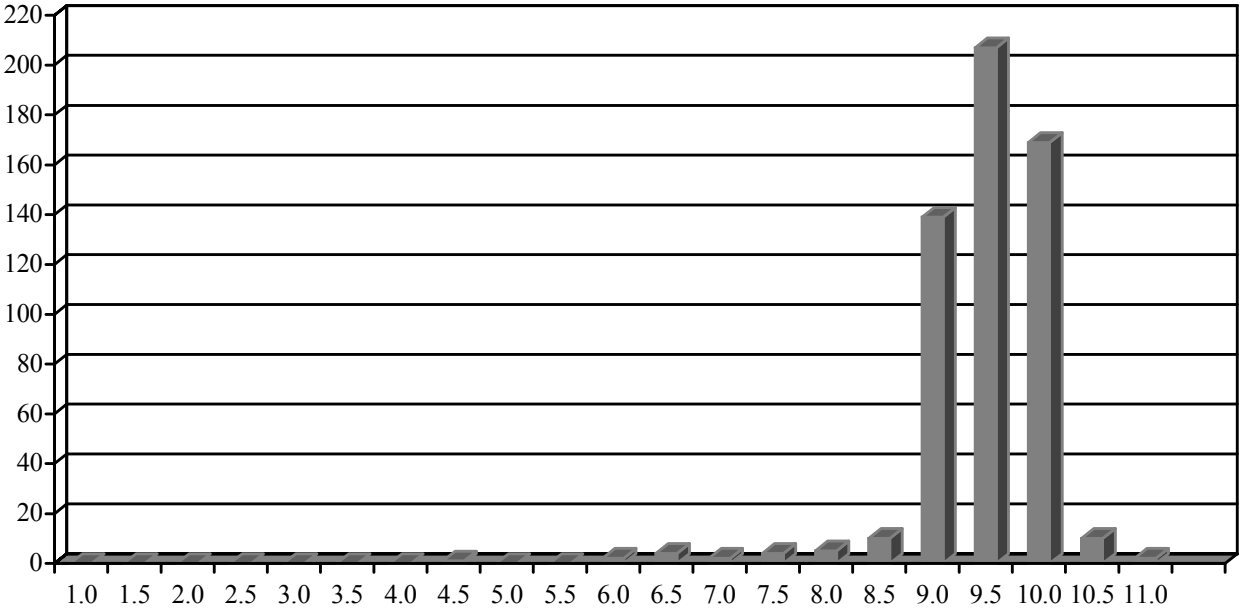

Figure 1. Distribution of average rating for *Helicoverpa zea* female lines on NBT diet. Mean rating is approximately 9.4, which corresponds to a mid 4th instar.

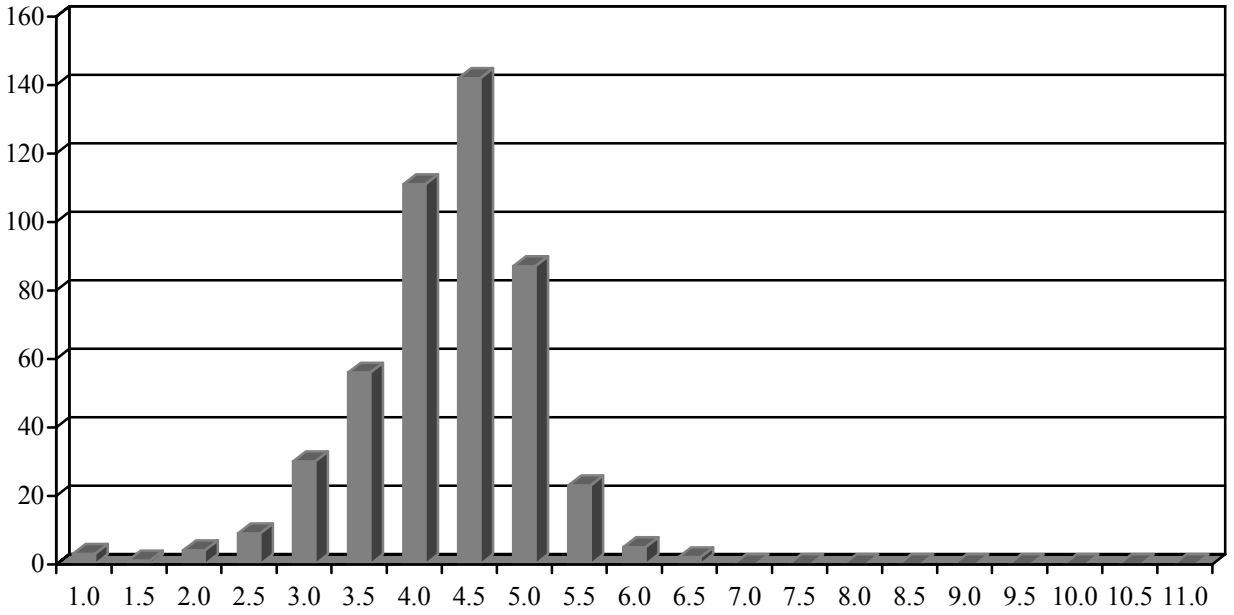

Figure 2. Distribution of average rating for *Helicoverpa zea* female lines on Cry1Ac-containing diet. Mean rating is approximately 4.4, which corresponds to a late 2nd instar.

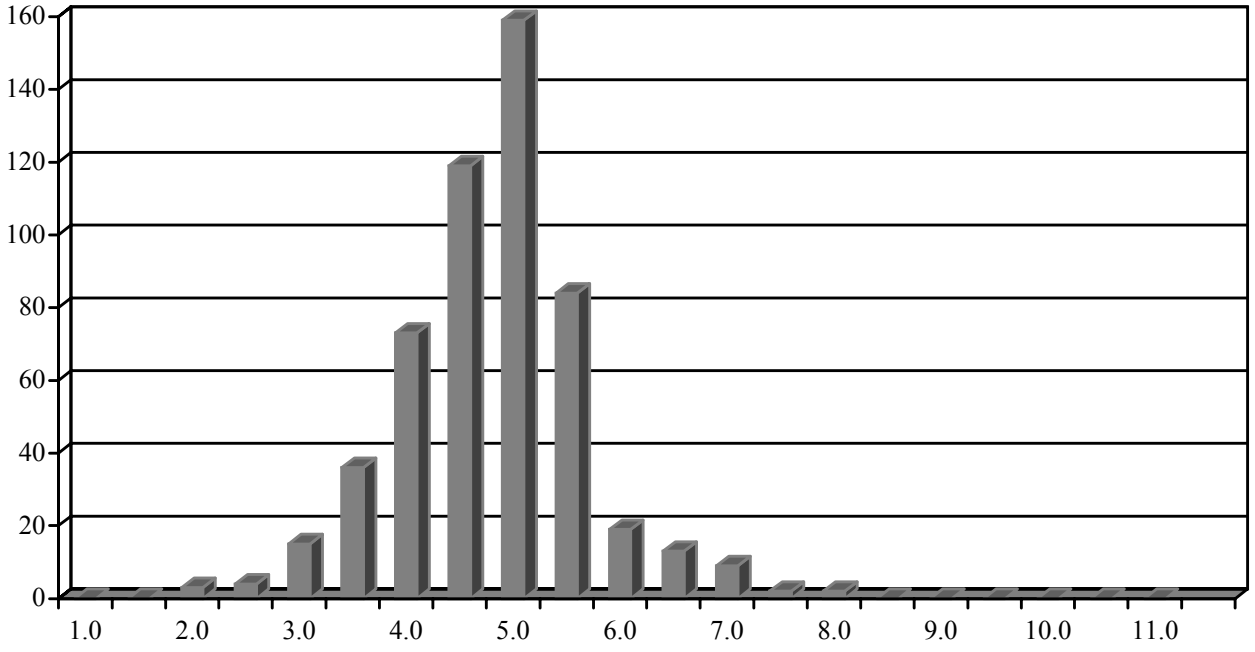

Figure 3. Distribution of average rating for *Helicoverpa zea* female lines on Cry2Ab-containing diet. Mean rating is approximately 4.9, which corresponds to an early 3rd instar.

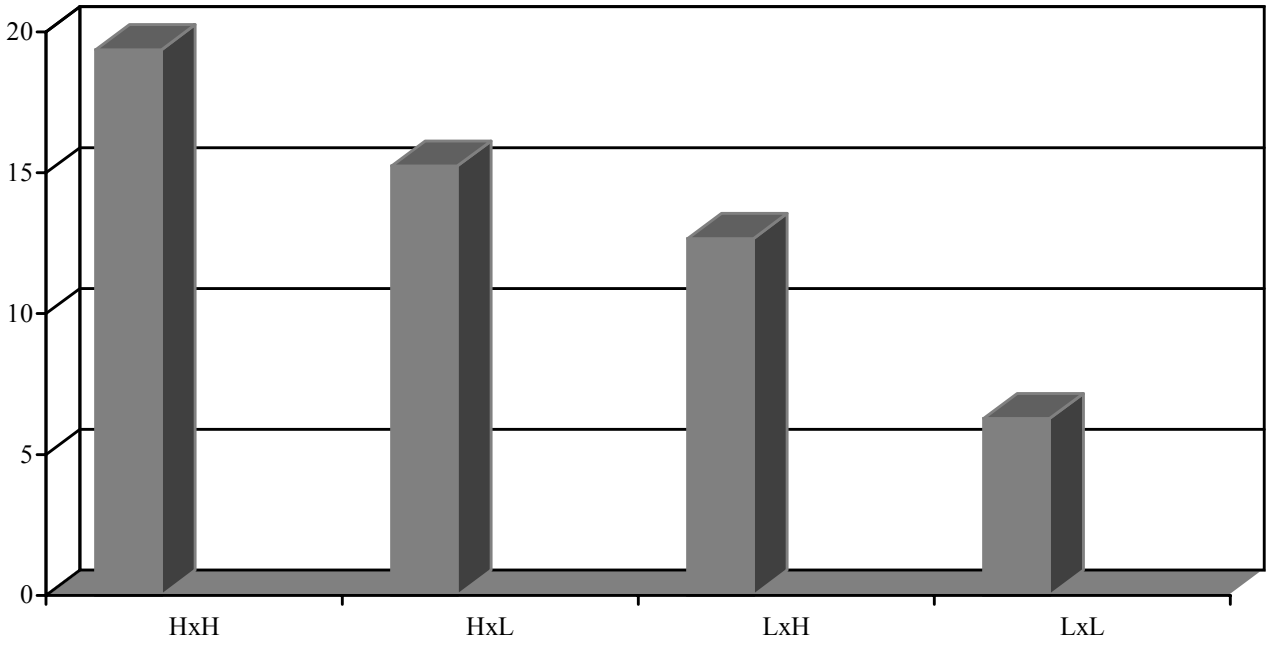

Figure 4. Mean larval weight in milligrams for *Helicoverpa zea* resistant line (HxH), control line (LxL), and reciprocal crosses between these lines (HxL and LxH) taken after ten days on 5.0 μg/ml of Cry1Ac-containing diet.

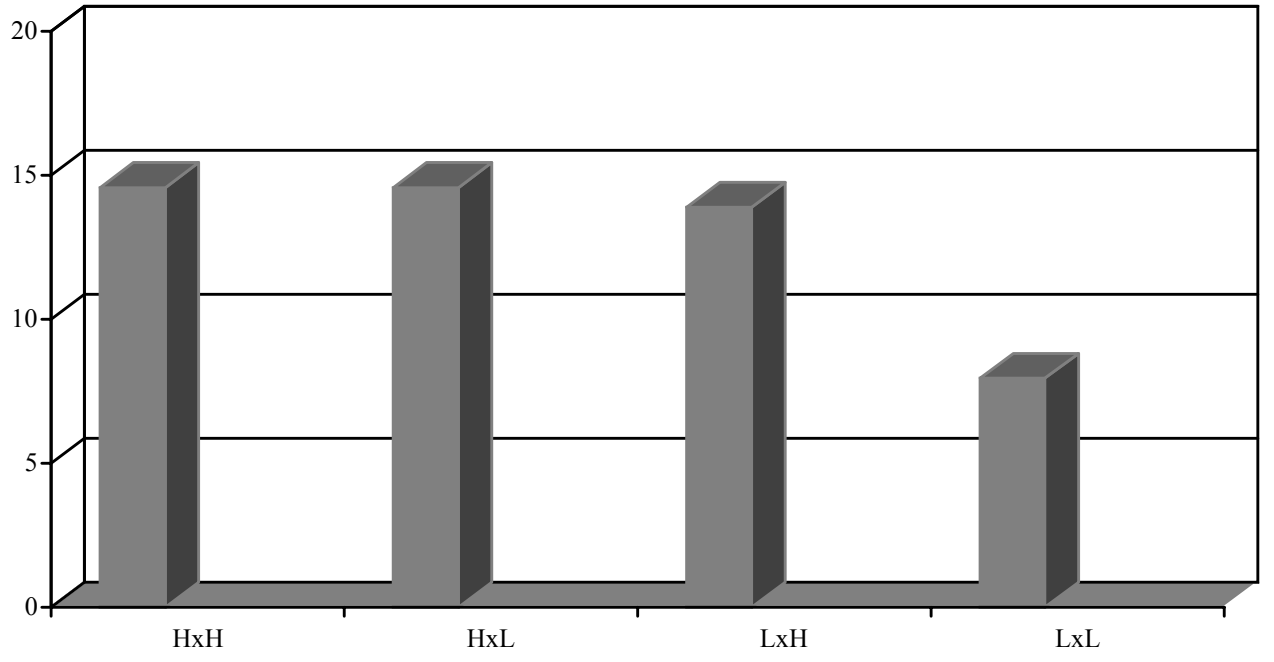

Figure 5. Mean larval weight in milligrams for *Helicoverpa zea* resistant line (HxH), control line (LxL), and reciprocal crosses between these lines (HxL and LxH) taken after ten days on 5.0 μg/ml of Cry2Ab-containing diet.