ESTIMATED FREQUENCY OF NON-RECESSIVE *B.T.* RESISTANCE GENES IN BOLLWORM, *HELICOVERPA ZEA*

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Abstract

In summer 2000, adult female bollworm moths were collected from various light-trap locations near Tidewater Research Station, Plymouth, NC. Female moths were allowed to lay eggs; and at hatch, 24 larvae from each female were screened on each of three diets: non-*B.t.*, CryIA(c), and CryIIAa at 5.0 *ug* toxin per milliliter of diet. These screens were performed to isolate any resistance genes present in the field populations of bollworm. We found 1 individual out of 583 screened that appeared to carry a major gene for resistance to CryIA(c). Assuming four genomes per individual, then the gene frequency is 1/2332 or 0.00043. Other females appeared to have minor genes for CryIA(c) resistance or major genes with lower levels of dominance. We also found 1 individual out of 646 screened that appeared to carry a major gene for resistance to CryIIAa. The gene frequency for CryIIAa resistance would be estimated at 1/2584 or 0.00039. Again, other females seemed to carry minor resistance genes. Along with results reporting partially dominant inheritance of CryIA(c) resistance, these estimates may be important when instituting refuge areas for the sustainability of transgenic cotton.

Introduction

Transgenic cottons, specifically those containing the *B.t.* endotoxin, have received widespread planting in recent years. These *B.t.* cottons may provide an enhanced method of management in areas where heliothines (budworm and bollworm) are key target pests. Many researchers have focused on resistance management concerns associated with the deployment of transgenic cottons (Tabashnik 1994; Kennedy and Whalon 1995). In order for these *B.t.* cottons to be successful in controlling resistance evolution in heliothine pests, they must express a high dose of toxin. Recent laboratory and field studies have shown that this is not the case for the bollworm (Burd et al. 1999; Lambert et al. 1996, 1997; Mahaffey et al. 1994, 1995). Also, recent laboratory studies performed at NCSU have shown that resistance to the CryIA(c) endotoxin for the bollworm may be inherited as a single dominant or partially dominant trait (Burd et al. 2000); this violates another assumption for the sustainability of *B.t.* cotton. It is imperative that we determine which assumptions hold true and which do not so that appropriate management strategies for the deployment and sustainability of *B.t.* cotton may be developed. Determining these unknowns will allow for the conceptualization and implementation of refuge strategies that take full advantage of this new technology.

This current study focuses on the assumption that the initial gene frequency for *B.t.* resistance in bollworm is present at extremely low levels. Based on lab experiments from 1999, we were able to screen the progeny of field collected female bollworm moths on a discriminating dose of CryIA(c) and CryIIAa toxins. This paper reports estimates of frequencies for resistance genes for bollworm populations in eastern North Carolina.

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Materials and Methods

In August-October 2000, adult female bollworm moths were collected from light-traps at 4 locations in eastern North Carolina near Tidewater Research Station. A total of 646 female moths (lines) were used for the bioassays. Progeny from the first 63 female lines bioassayed were placed on a lower concentration of CryIA(c) diet than the remaining females used and were not included in the overall analysis for the CryIA(c) lines. Therefore, only 583 female lines were used for the analysis of the CryIA(c) families. Adult females were placed individually in 8 ounce clear plastic cups and covered with cheesecloth to provide a substrate for egg laying. Moths were kept in rearing facilities at North Carolina State University at 27-30°C, 55-60% relative humidity and a photoperiod of 14:10 light:dark hours. Cheesecloths were checked daily for the presence of eggs.

At larval hatch, 24 neonates from each female line were placed onto three different diets. These diets were non-*B.t.* (NBT), CryIA(c)-containing diet and CryIIAa-containing diet. The concentration of both *B.t.* toxins was 5.0 *ug* per milliliter of diet. CryIIAa was purified from the NRD-12 isolate of *Bacillus thuringiensis* subsp. *kurstaki* and was expressed in a recombinant *Escherichia coli* strain (Moar et al. 1994). CryIA(c) was obtained as a gift from Mycogen Corp. in a formulation of their product, MVP. The diet was poured into 24-well plastic bioassay plates and then stored in the refrigerator before use. A single neonate was placed into each well with a fine camel hair paint brush; and plates were heat-sealed with Mylar film. All plates were scored after seven days. Larvae were scored based on instar size. Instar size was determined based on head capsule and body size (Neunzig 1969). All instar sizes were converted to an ordinal ranking system as seen in Table 1. These data were analyzed using PROC UNIVARIATE (SAS Institute 1990).

Survivors from the original bioassays were used for subsequent selection experiments. Any lines that performed just as well on either *B.t.* diet as they did on NBT diet were saved and screened again. These lines were mass mated among themselves and selected on the appropriate *B.t.* diet the next generation. Several lines that had no survivors on either *B.t.* diet but performed well on NBT diet were also saved. These were used as control lines for subsequent tests. Reciprocal crosses were made between these control lines and the survivors from selected lines to determine possible inheritance of resistance. Larvae from these crosses and also from the selected and control lines were placed on the appropriate *B.t.* diet and weighed after 10 days.

Results

Figure 1 shows the distribution of average ratings for female lines on CryIA(c) diet. A total of 583 female lines were screened on CryIA(c) diet. The average rating for all larvae on CryIA(c) diet was approximately 4.0, which corresponds to a late 2nd instar. Bioassays on CryIA(c) revealed one family with most individuals as large as control larvae. As seen in figure 1, the average rating of this single family was 7.4 which falls between a late 3rd and early 4th instar. Ten out of twenty-four larvae survived on the *B.t.* diet; and these surviving larvae ranged from mid 3rd to mid 4th instar. The controls (NBT) for this family ranged from late 3rd to mid 4th instar with a mean rating of 8.25.

The distribution of average ratings for each female line on CryIIAa diet is illustrated in figure 2. A total of 646 female lines were screened on CryIIAa diet. The average rating for all larvae on CryIIAa diet was approximately 3.0 which corresponds to a mid 2nd instar. Bioassays from CryIIAa lines also revealed one family with most individuals as large as controls. The average rating for this family was 6.5 (figure 2) which is between a mid 3rd and late 3rd instar. Nineteen out of twenty-four larvae from this family survived on the CryIIAa diet; and they ranged from early

2nd instar to mid 4th instar. The controls (NBT) for this family ranged from late 3rd to mid 4th instar with a mean rating of 8.55.

Figure 3 illustrates the distribution of average ratings for each female line on NBT diet. The average rating was approximately 9.0 which corresponds to a mid 4th instar.

Figure 4 illustrates weights from the selected family that performed best on CryIA(c) in the original bioassay. These weights were taken from the second generation of larvae (the original field collected females would be their grandmothers). Figure 4 also shows weights for second-generation control larvae (individuals from a particular family line that died on both toxin diets but not on NBT diet). Weights of reciprocal crosses between this selected line and the control line are also shown. As seen in figure 4, the average larval weight for the selected line was 0.028 grams. The average weights for the selected female x control male and the control female x selected male crosses were 0.016 grams and 0.014 grams, respectively. All control larvae once again died on the 5.0 *ug/*ml CryIA(c) diet.

Discussion

Based on our lab studies from 1999 (Burd et al. 2000), we concluded that inheritance for resistance to CryIA(c) toxin was dominant or incompletely dominant. Therefore, any individuals carrying genes for resistance were likely to survive when screened on a discriminating dose of this toxin in artificial diet. This type of inheritance enables heterozygotes to survive when selected on *B.t.* diet. Statistically, the most probable carrier for this resistance gene would be a heterozygote in current field populations. Also the most probable mating would occur between heterozygote individuals and homozygote susceptible individuals. With this in mind, the likely offspring form this cross would be ½ heterozygote and ½ homozygote susceptible. From this, we assumed that if a screened line had 50% of the individuals that were the same size as their NBT counterparts then this would be considered a resistant line.

Screening 583 total females on CryIA(c) diet allowed us to characterize 2332 genomes because each mated female carries 2 of her own alleles and 2 from her male counterpart. With this in mind, our estimated gene frequency for resistance to CryIA(c) toxin would be 1/2332 or 0.00043. It should be noted that this estimate is conservative. As seen in figure 1, many female lines had higher than average growth on CryIA(c) diet (i.e. the entire family reached 3rd instar but was not quite as large as the controls). This may be due to the presence of minor genes for CryIA(c) resistance or major genes with lower levels of dominance. In either case this would serve to increase our estimate of the initial gene frequency for CryIA(c) resistance. The one family which survived on the CryIA(c) had a total of 10 out of 24 individuals survive. This is consistent with the 50% that we expected. Also, all survivors were consistent in size with their NBT counterparts.

Since 646 female lines were screened on CryIIAa diet, then the number of genomes we actually tested was 2584. Therefore, our estimated gene frequency for resistance to CryIIAa toxin would be 1/2584 or 0.00039. Again, this is a conservative estimate based on the fact that other female lines performed above average and may have been carrying minor genes or major genes with lower levels of dominance. This would also increase our estimate of initial gene frequency for CryIIAa resistance. The one family that survived from the CryIIAa bioassays had 19 out of 24 survivors. This is greater than the 50% that we expected; however, there were 6 of these individuals that were mid 2nd instar and below. Therefore, only 13 out of 24 individuals were as large as the control counterparts.

Results from selection experiments and reciprocal cross experiments (figure 4) reported in this paper are consistent with those reported last year (Burd et al. 2000). Both of these studies indicated that resistance to CryIA(c) is inherited as a dominant or incompletely dominant trait. Along with the results from our current gene frequency estimates, this may allow us to quantify certain parameters that are typically assumed when modeling evolution of *B.t.* resistance in field populations of bollworms. If the initial gene frequencies for resistance are indeed this high coupled with partially dominant inheritance, then properly structured refuge systems become critically important for transgenic *B.t.* cotton technology to be sustainable.

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

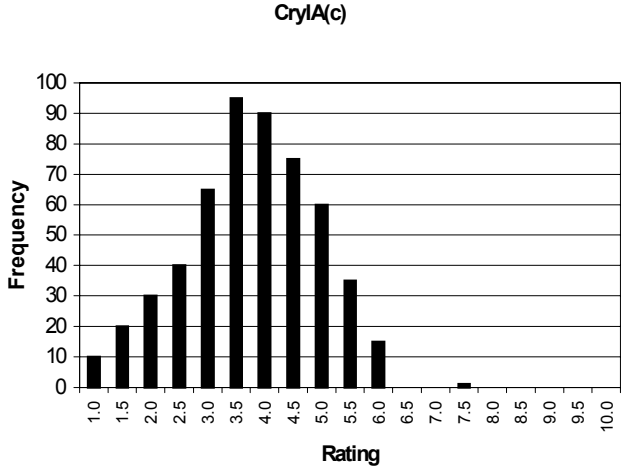

Figure 1. Distribution of average rating for *Helicoverpa zea* female lines on CryIA(c) diet. Mean rating is approximately 4, which corresponds to a late 2nd instar.

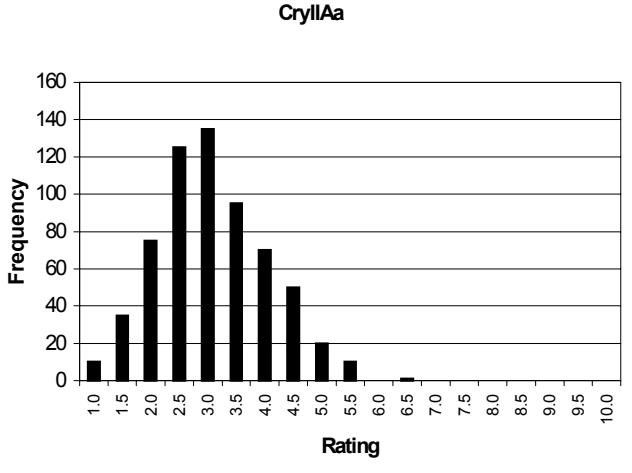

Figure 2. Distribution of average rating for *Helicoverpa zea* female lines on CryIIAa diet. Mean rating is approximately 3.0 which corresponds to a mid 2nd instar.

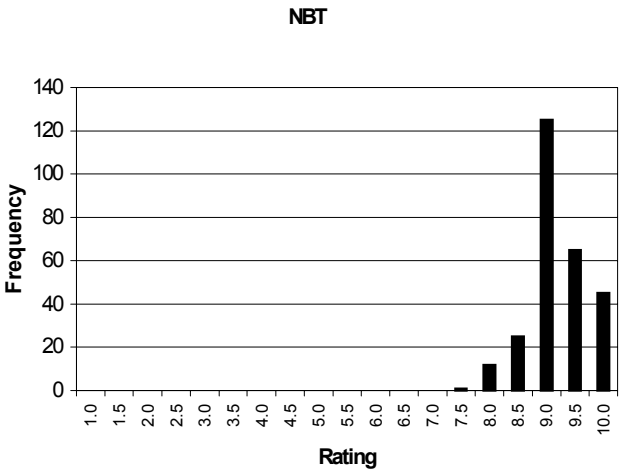

Figure 3. Distribution of average rating for *Helicoverpa zea* female lines on NBT diet. Mean rating is approximately 9.0 which corresponds to a mid 4th instar.

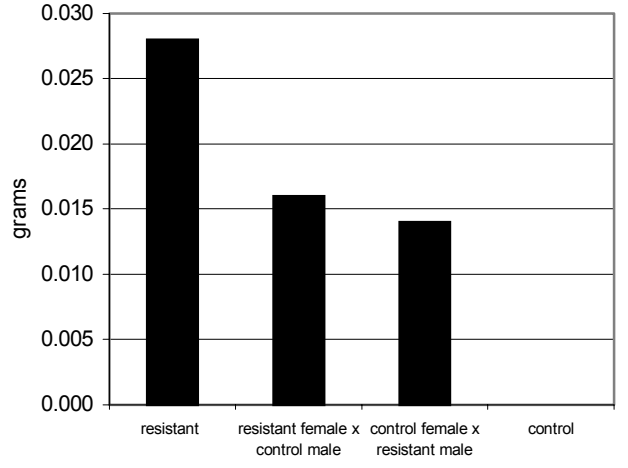

Figure 4. Weight in grams for *Helicoverpa zea* resistant line, control line and reciprocal crosses between these two lines taken after ten days on 5.0 *ug*/ml of CryIA(c) diet.