HERITABLE TOLERANCES OF Cry1Ac IN LEPIDOPTERAN PESTS OF COTTON

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Abstract

Studies were conducted to determine if cotton bollworms (CBW) and beet armyworms (BAW) possess the genetic variation necessary to respond to selection for improved tolerance of the Bt toxin Cry1Ac. BAW individuals that were the fastest to reach pupation on Cry1Ac-treated diet (approx. the first 20% to pupate) produced offspring that developed signficantly faster on Cry1Ac diet than their parental-control strain. In addition, after four generations of mating, this group developed significantly faster on transgenic-Bt cotton leaves (cv. NuCOTN 33B) than the control strain. Full-sib families of CBW exhibited significant amounts of heritable variation for tolerance of Cry1Ac (54% of the total variation). Follow-up tests with these families further supported the heritable nature of Cry1Ac tolerance in CBW.

Introduction

The tolerances of Cry1Ac in different species of insects feeding on Bt cotton may be important in determining how well resistance management strategies work in delaying resistance in these species. One important step is to determine if the genetic potential exists in these species to become more tolerant of Cry1Ac. In this paper, we focus on two species known to be more tolerant of Cry1Ac than the tobacco budworm: the cotton bollworm (CBW), *Helicoverpa zea*, and the beet armyworm (BAW), *Spodoptera exigua*. Our purpose was to determine if the genetic potential to become more tolerant of Cry1Ac is present in these species.

Materials and Methods

MVPII powder containing the Cry1Ac protein was incorporated into an artificial diet (Raulston and Lingren 1972) at a concentration of 1.0 μg/ml Cry1Ac. Neonate larvae were placed into 30 ml cups containing approx. 10 ml of Cry1Ac diet. Larvae from the same groups were also placed on non-Cry1Ac diet to act as a control.

During the 1st generation of testing BAW, the number of days to pupation for each individual was recorded. The pupae resulting from these individuals were placed into three groups (see Fig. 1): (1) the Fast group was composed of individuals that had pupated by 14-16 d; (2) the Intermediate group was composed of individuals that pupated by 17-20 d; and (3) the Slow group was composed of individuals that pupated after 21 d. Adults from these groups were mated to produce the 2nd generation and their offspring were compared to the parental-control strain (Cry1Ac and non-Cry1Ac diet). However, the Slow group did not produce sufficient larvae for testing during the 2nd generation. The number of days to pupation were compared for the second-generation larvae resulting from the remaining three groups. In addition, the Fast group was maintained for two more generations for comparison with the parental-control colony on transgenic-Bt cotton leaves (cv. NuCOTN 33B) and non-Bt cotton leaves (cv. DP5415). Larval growth on both types of cotton leaves was analyzed after 10 d of feeding.

Sixteen full-sib families were created from a CBW colony infused with wild males collected during 1998 (maintained by F. Davis, USDA-ARS, Starkville, MS). Larvae from all families were tested for 14 d on Cry1Ac diet and control diet. After 14 d, weights of individuals were taken. Heritability was estimated from REML estimates of variance components. Follow-up tests were made during the 2nd generation by grouping families based on their performance during the 1st generation (Four groups: 1 = smallest 4 families; 2 = families ranked 5th-9th in their mean weight; 3 = families ranked 10th-13th; 4 = families ranked 14th-16th).

Results

Days to pupation for BAW during the 1st generation ranged from 14 – 28 d when larvae were reared on 1.0 μg/ml Cry1Ac (Fig 1). All individuals had pupated by 14 d on the control diet. Individuals from the Fast group produced 2nd-generation offspring that pupated significantly earlier on the Cry1Ac diet than individuals from the control and Intermediate groups (Fig. 2; K-W ANOVA, $H = 114.34$, $df = 2$, $P < 0.0001$). There were no differences in the time to pupation when these groups were tested on the control diet ($P > 0.8$).

After two more generations of mating, the Fast group was compared to its control group in tests involving NuCOTN 33B leaves and DP5415 leaves. There were no significant differences between the Fast and control strains after 10 d of feeding on non-Bt cotton leaves (Fig. 3; $P = 0.659$). However, larvae from the Fast strain were significantly larger than individuals from the control strain after 9 d of feeding on Bt cotton leaves (Fig. 3; $P = 0.0195$). Replications from different locations also affected the relative differences between the BAW strains.

During the leaf tests, comparisons between the Fast and control groups also were made on artificial diets. There were no differences between the two groups of BAW on control diet after 12 d of feeding ($P = 0.099$, with slightly better

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growth in control group). However, larvae from the Fast group were significantly larger than larvae from the control group after 12 d of feeding on Cry1Ac diet (average weights = 151.3 and 75.3 mg, for Fast and control, respectively; $P <$ 0.0001).

Full-sib families of CBW significantly differed in their performance on Cry1Ac diet. The average log weights (mg) for these families on Cry1Ac varied by more than an order of magnitude (Range in log weights (mg) = 0.049 – 1.121) and was not correlated with their performance on control diet. Broad-sense heritability was estimated as 0.536 ($P < 0.001$). This suggests that as much as 54% of the total variation in 14 d log weights is attributable to heritable variation. Follow-up tests revealed that as the average weight of grouped families increased, so did the performance of their offspring (Fig 4; $P < 0.001$).

Discussion

Our results indicate that heritable variation to tolerate Cry1Ac is present in both BAW and CBW. Even after one generation of minimal selection, the Fast group of BAW was able to develop more rapidly on Cry1Ac diet. After two more generations (with out further selection), the Fast group grew more rapidly on the leaves of Bt cotton than its control strain. Therefore, BAW possesses the genetic potential to respond to selection for improved tolerance of Cry1Ac.

Other studies have reported a great deal of variation in the performances of field colonies of CBW tested for tolerance of Cry1Ac (Luttrell et al. 1999, Sumerford and Solomon 1999). The CBW population examined in this paper exhibited considerable variation in Cry1Ac tolerance. The strong linear relationship between the performance of 2nd generation offspring and their parents (Fig. 4) supports the heritability estimate of the full-sib family analysis, and supports the conclusion that CBW populations have the genetic potential to respond to selection for improved tolerance of Cry1Ac. As a consequence, previously reported small shifts in the tolerances of CBW field populations (Sumerford et al. 1999), may be indicative of small genetic changes in CBW tolerance of Cry1Ac and need to be further examined.

References

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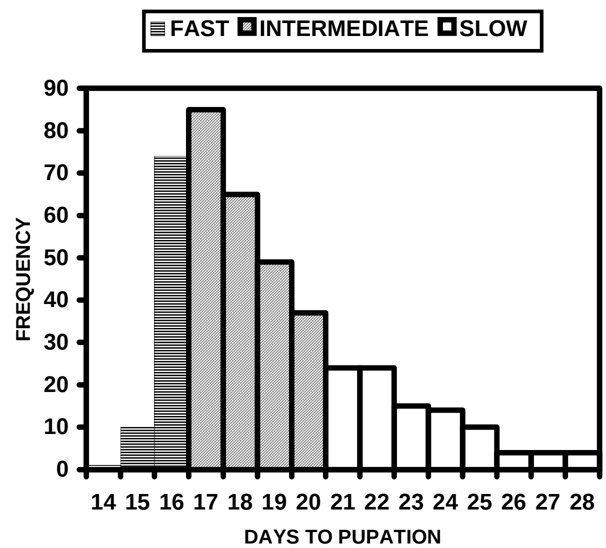

Figure 1. Frequency distribution of days to pupation on Cry1Ac diet for the 1st generation of BAW.

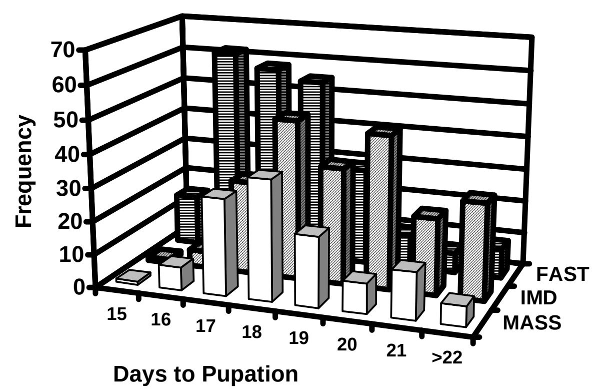

Figure 2 Frequency distributions of days to pupation for developmental groups in the 2nd generation of BAW testing on Cry1Ac diet.

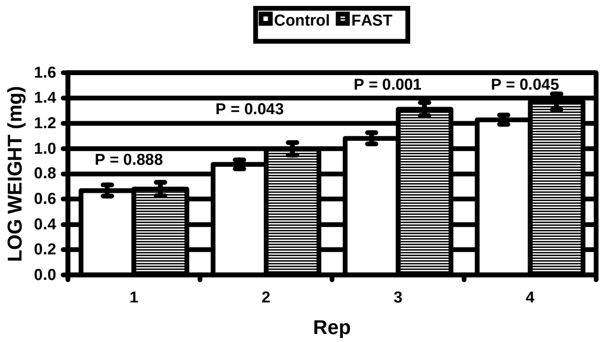

Figure 3. Average log weight (mg) of larvae from the Fast and Control strains of BAW when tested on NuCOTN 33B leaves.

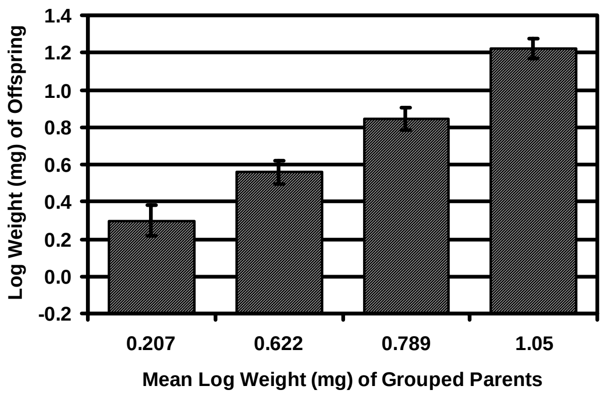

Figure 4. Mean log weight (mg) of 2nd generation CBW offspring resulting from mass mating families which had similar mean log weights (mg) during the 1st generation.