

Alabama Cotton Commission
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PROJECT TITLE: Production and Characterization of Bt resistance in Cotton Bollworm, *Helicoverpa zea*

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OBJECTIVES:

Resistance to the Bt Cry1Ac protein (MVPII) found in Bollgard (Bt) cotton by cotton bollworm, *Helicoverpa zea*, was achieved in the laboratory to a level of 10-20 fold. However, this population crashed in May 2004, as has most/all other CBW populations selected for Cry1Ac (MVPII) resistance in the US. The inability to establish a highly Cry1Ac-resistant and stable CBW population is thought to be caused primarily by inbreeding, although other factors are possible. One such factor could be that when insects are selected with Cry1Ac using the formulation MVPII containing only 19.1% AI, over 80% of the selection against CBW using MVPII are non-Bt toxin components. Not only could selection be difficult to achieve, but resistant mechanisms arising from selection may not be resistant mechanisms specific to Bt. Additionally, because the Bt (Cry1Ac) that is present in Bollgard (Bt) cotton is not in the same form as that found in MVPII, laboratory selection using MVPII is not indicative of what is occurring in the field. The objective of this proposal is to use the form of Bt Cry1Ac found in Bollgard (Bt) cotton to select for Bt resistance, and to compare these results to a colony selected using MVPII. After resistance has been achieved, further experiments can be conducted to characterize this resistance such as cross resistance studies with other Bt proteins and pyrethroids, fitness costs.

Sweet corn will be grown at the Prattville Agricultural Research Unit throughout 2005 to collect CBW larvae in order to increase heterogeneity of the selected colony as well as to potentially collect Bt resistant individuals coming off of Bollgard cotton.

2005 Results

Field:

Over 4,000 CBW larvae have been extracted from silking corn August-November, 2005. Larvae were (and currently are being) reared to pupation in the laboratory on artificial diet. Pupae were sent to USDA, Stoneville for evaluation as well as in our laboratory. Resistance monitoring results from USDA are not yet available. In our laboratory observations with field collected CBW larvae, although the larvae, pupae and adults appeared healthy and "normal", few fertile eggs were laid and were in insufficient quantity to establish a colony.

Laboratory

A susceptible laboratory strain of cotton bollworm, *Helicoverpa zea*, (SS) was established from a Monsanto laboratory colony. The baseline susceptibility (LC₅₀) value of SS to MVP II and Cry1Ac toxin were averaged over three generations conducted with a minimum of three replications per generation. The LC₅₀ for MVP II was 24.13 µg/g of diet, (95% FL 16.34-35.62 µg/g; slope ± SE: 1.73 ± 0.24) and LC₅₀ for activated toxin was 8.89 µg/g diet, (95% FL 5.83-12.53 µg/g; slope ± SE: 1.61 ± 0.41). These values indicated a 2.7 fold difference in susceptibility between the two sources of Cry1Ac. Subsequently, two Cry1Ac-resistant strains of cotton bollworm were selected using MVP II (MR) or activated Cry1Ac toxin (AR). Larvae that molted into second instar within seven days of selection were reared until pupation on regular diet (containing no Cry1Ac). Selections were conducted on every generation of the AR (currently at generation 9) and MR strain. The MR strain selection was discontinued after 7 generations of selection owing to insufficient larval numbers (discussed below). Significant variation in tolerance

was observed in both resistant strains compared to the unselected strain (SS). Resistance increased from 12.12 to 35.91 fold after 4 and 7 generations of selection in the AR strain, respectively (Table 1). However, resistance did not increase in the MR strain from 16.61 fold, even after selecting at higher concentrations for 3 more generations (Table 1 and Table 3). The AR strain required only two generations of selection to obtain more than fifty per cent survivors for the first two increases in Cry1Ac concentration (Table 2) with each increase representing a 2.5-fold increase in concentration. On the other hand, the MR strain required a minimum of three generations of selection at a given concentration to obtain fifty per cent survivorship and the selection pressure was increased by only 2 times (Table 3).

Fitness costs

The percent egg hatch (84 - 87%) was not different between the SS and the AR strains. However, adult moths of the MR strain after the first generation of selection at 500 and 1,000 µg Cry1Ac/g diet laid eggs that had very poor (<30 %) hatching. Hatching percentage was severely affected after the 8th generation of selection (Table 3), in which less than one per cent hatching (only 62 larvae from over 7000 eggs) was observed.

The dissection of dead moths revealed that, 73.7 per cent of females were unmated in the MR strain; twice the level compared to the percentage of unmated females in both the SS and the AR strains (Table 4). Additionally, the sex ratio (females:male) for the MR strain (0.9) was substantially lower compared to the sex ratio of AR (1.18) and SS (1.19) (Table 4). These results suggest a reduction in the male's ability to find, mate and/or transfer the sperm packet to the female.

Table 1. Development of Cry1Ac resistance in cotton bollworm, *Helicoverpa zea*

| Generations | LC₅₀ (µg/g) | Fiducial limits | Slope ± SE | Resistance ratio * |
|--------------------|-------------------------------|---------------------------|-------------------|---------------------------|
| AR Strain | | | | |
| 4 | 107.64 ± 10.71 | 75.37 – 155.56 | 1.42 ± 0.3 | 12.12 |
| 7 | 319.22 ± 17.60 | Not detected [#] | 1.89 ± 0.13 | 35.91 |
| MR Strain | | | | |
| 4 | 384.30 ± 66.09 | 282.31 – 568.12 | 1.79 ± 0.27 | 16.61 |
| 7 | 291.40 ± 11.15 | 155.16 – 455.54 | 1.67 ± 0.41 | 12.60 |

*: Resistance ratio = ratio of LC₅₀ of resistant strain to that of unselected strain (SS)

[#]: 95% confidence interval could not be determined as there were not many data points for prediction

Table 2. Selection details for AR strain selected using activated Cry1Ac

| Generations | Concentration (µg/g) | Number of larvae selected | Per cent survivors |
|--------------------|-----------------------------|----------------------------------|---------------------------|
| 1 | 50 | 2000 | 5.45 |
| 2 | 80 | 3500 | 12.82 |
| 3 | 80 | 2000 | 60.29 |
| 4 | 200 | 1000 | 8.67 |
| 5 | 200 | 1000 | 62.71 |
| 6 | 500 | 950 | 8.96 |
| 7 | 500 | 1000 | 32.37 |
| 8 | 500 | 336 | 34.82 |
| 9 | 500 | | |

Table 3. Selection details for MR strain selected using MVP II

| Generations | Concentration ($\mu\text{g/g}$) | | Number of larvae selected | Percent survivors | Remarks |
|-------------|-----------------------------------|----------------|---------------------------|-------------------|-------------------|
| | Cry1Ac | Inert material | | | |
| 1 | 100 | 423.56 | 2000 | 10.13 | |
| 2 | 200 | 847.12 | 3500 | 4.64 | |
| 3 | 500 | 2117.8 | 4000 | 3.23 | Poor hatching |
| 4 | 500 | 2117.8 | 2000 | 20.71 | |
| 5 | 500 | 2117.8 | 1000 | 40.27 | |
| 6 | 1000 | 4235.6 | 2000 | 8.19 | Poor hatching |
| 7 | 1000 | 4235.6 | 1900 | 20.34 | Reduced fecundity |
| 8 | 1000 | 4235.6 | 2000 | 8.47 | Poor hatching |
| 9 - 11 | No selection | | Very few number of larvae | | |

Table 4. The sex ratio and mating status in different strains of cotton bollworm, *H. zea*

| Strains | N | Sex ratio* | Number of spermatophores (% females) | | | | | |
|---------|----|------------|--------------------------------------|-------|-------|------|------|------|
| | | | 0 | 1 | 2 | 3 | 4 | 5 |
| SS | 79 | 1.19 | 37.2 | 37.20 | 18.60 | 4.65 | 0.00 | 2.32 |
| AR | 85 | 1.18 | 39.13 | 36.96 | 21.17 | 2.17 | | |
| MR | 40 | 0.90 | 73.68 | 10.50 | 15.78 | | | |

* : number of females for every male

Discussion

This research demonstrates that CBW can develop resistance to Cry1Ac insecticidal proteins quicker (3 times faster) when selected using activated toxin compared to MVP II. Thirty six-fold resistance in the AR strain achieved in just 7 generations of selection is considered as relatively rapid. Potential reasons for this relatively rapid rate of resistance development could be, 1) Selecting only larvae that had molted, thereby eliminating a higher percentage of susceptible insects in each generation and 2) Use of activated Cry1Ac toxin. A relatively rapid rate of resistance development has also been observed in *Spodoptera exigua* using Cry1C activated toxin

Resistance development in the MR strain was slower and did not increase beyond 16-fold even after selecting for three more generations at higher concentrations. The fitness of this strain was adversely affected in terms of both fecundity and fertility. We believe that the 80.9% inert ingredients in the MVP II formulation might have an effect with the fitness of this strain, especially when selecting at 1 mg/g of Cry1Ac concentration. In addition, MVP II is quite different to the Cry1Ac insects ingest when feeding on Bollgard[®] which raises concerns about using MVP II as a source of Cry1Ac for selection against CBW.