The Potential for Transgenic Cotton Plants to Select for Resistance in *Helicoverpa armigera.*

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Cotton plants expressing the Cry1Ac toxin from Bt are near to commercial release. These plants have been developed to aid in the control of heliothis caterpillars (both *Helicoverpa armigera* and *H. punctigera*), the primary targets of insecticide usage in cotton production. While it was hoped that these plants would give high enough expression of toxin to control heliothis throughout the growing season, it is now clear that some larvae can survive to pupation late in the season. The decline, however, is more of a problem for resistance management, because of the chance that at some stage during the growing season, the level of Bt toxin in the plants will be at a level that allows preferential survival of resistant, but not susceptible, individuals.

We are examining the potential of transgenic Bt cotton plants to select for resistance in *H. armigera*. In particular, we need to understand what environmental factors influence the expression of the Bt toxin. At the beginning of our study in 1993, nothing was known about the cause of the decline in toxin levels, nor were techniques available that would enable us to quantify those levels. Thus, we have had to focus on developing the necessary techniques to study the performance of transgenic plants grown under field conditions.

Changes in Efficacy of Plants during the Season

Little is known about the underlying causes of the decline in efficacy of Bt cottons. Reduced toxicity of the leaves could be due to down regulation of the genes controlling expression of the crystal protein genes, sequestration of the Bt toxin by plant secondary compounds, or mobilisation, transportation and metabolism of the Bt toxin protein by the plant.

We have successfully developed a leaf bioassay test that can measure relative changes in toxicity of Bt leaves. The bioassay (called the leaf mush test) involves mixing Bt leaves and non-Bt leaves in known proportions. In glasshouse-reared plants we observe a 3-fold decline in toxicity of the plants from young plants, (before squaring) compared with mature plants (with open bolls). This decline is only small, so it suggests that the level of Bt toxin in young cotton plants may not be much above the level needed to kill heliothis.

We began field experiments during summer 1994/95 to test the feasibility of monitoring efficacy of field grown plants using the leaf mush test. Leaves were collected from a plot of CS50 (Cry1Ab) plants grown opposite the Myall Vale Research Station by Dr G.P. Fitt. Leaf samples were taken from 20 plants on 22 December 1994, 2 February and 17 March 1995.

Bioassays were performed for each of these sample periods. Thus for each plant, we obtained three estimates of the LC50 - the amount of Bt leaf that was mixed with non-Bt leaf to give 50% kill of susceptible neonate larvae. The ratios of the LC50 for the two time periods (Dec-Feb) and (Dec-Mar) were calculated as an average over all plants. The results were:

Time Period	Mean Ratio of LC 50
Dec c.f. Feb	1.5
Dec c.f. Mar	2.6

There was a detectable change in LC50 in plants sampled during the three stages (late Dec, early Feb, mid- Mar). By March the decline in LC50 was of the order of 2.6 fold. This change is of the same order of magnitude reported for plants grown in the glasshouse. We are repeating these experiments using field grown plants containing the Cry1Ac toxin.

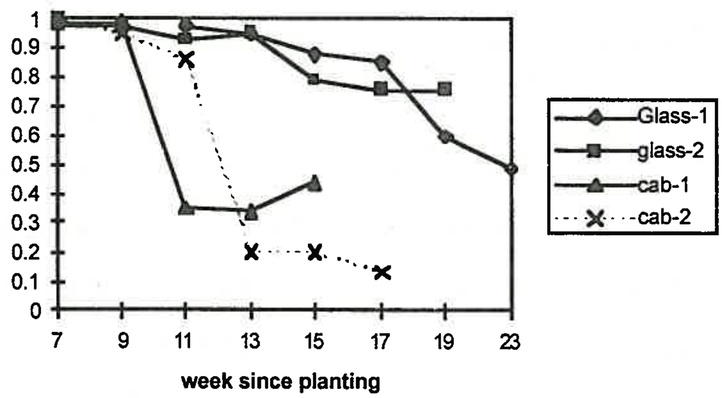
Factors Observed to be Associated with Decline in Plant Efficacy.

We have begun a series of experiments to determine if we can influence the efficacy of Bt plants. While some of the experimental conditions are quite artificial, they are giving us insights into the factors that may be important in the field.

(a) *Growing Conditions.* The first stage of these experiments was to define the variation between plants for expression of Bt, and to compare results for plants grown in either the glasshouse or a growth cabinet. A leaf at the third node was sampled every 2nd week from 7-19th weeks post planting.

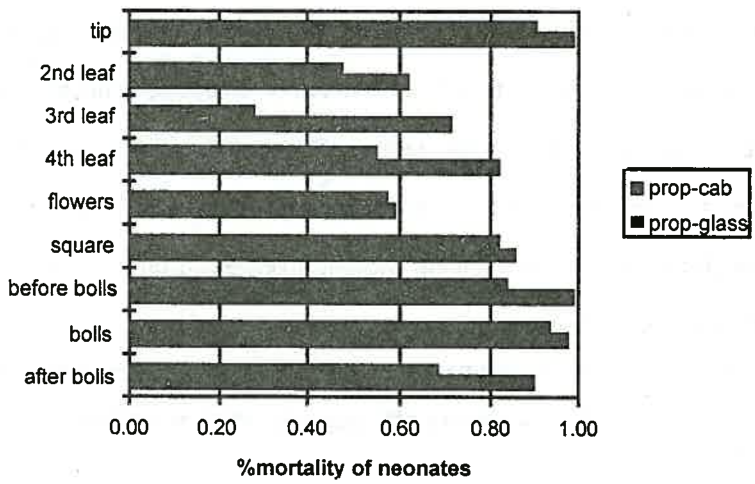
Before 1st squares, there was little variation in efficacy of Bt plants, 97% of larvae placed on Bt- plants grown either in the glasshouse or cabinets were killed. Squares first appeared between 11 and 14 weeks post planting. At this time, the efficacy of the plants in the cabinets dropped off within two weeks to only 34% mortality, compared with 86% for plants grown in the glasshouse. The average results for the four replicates (2 replicates for cabinets and glasshouses) throughout the experiment are illustrated in Figure 2. The cause of the differences between plants grown in glasshouses versus cabinets is being investigated further but may be associated with differences in light intensity .

Fig 2. Average efficacy of plants in replicates



(b) *Variation between tissues*. Plant tissue from all parts of the mature cotton plants, 17+ wk from planting, was sampled and neonate larvae placed on the tissue. Mortality of larvae was recorded after six days. Again, plants were grown in both a growth cabinet and a glasshouse. Considerable variation in efficacy was observed among tissues. The lowest efficacy was observed in leaf tissue in the 2-4th node and in the flowers, the tissue normally used to monitor plant performance. High efficacy was observed at the extremes of the plant - the tip, the bolls and leaves adjacent to the bolls. Results observed elsewhere would suggest that higher mortality would be expected when neonates are fed on plant tissue from the bottom of the plant even in control plants.

Fig 3. Tissue variation in efficacy in Bt cotton



(c) *Plant Secondary Compounds?* Another factor observed to influence the efficacy of the Bt plants was the age of the control leaves used in the bioassays. In our test, we mix Bt leaves with non-Bt leaves in known proportions. We observed different results when we used control leaves from young non-Bt plants compared with older non-Bt plants. Differences in LC50s were examined using L22 cotton variety containing the Cry1Ac toxin that was 7 wk from planting. These leaves were mixed with control leaves, 7 wk or 11 wk from planting. Results were:

Age of Control leaves	LC_{50}	95% CI
7 wk	0.56	0.34, 0.90
11 wk	2.24	1.67, 3.00

Thus, the estimated LC50 of our Bt plants increased by 2.5 fold when older control leaves were used. These results suggest that something in normal cotton leaves can make the Bt less available to the insect as the plant ages. Thus, the decline in Bt efficacy during the season may not be due entirely to a reduced expression of the toxin gene in the plant.