

How do susceptible *Helicoverpa* larvae behave on Bollgard II?

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Shortly after the widespread adoption of Bollgard II cotton in Australia there were reports that *Helicoverpa* larvae were surviving in these crops at levels that exceeded the recommended threshold levels for control.

In response to these reports, Crop Consultants Australia members were surveyed for data from the 2005–06, 2006–07, and 2007–08 seasons (see the June 2009 issue of *The Australian Cottongrower* for a full report). This work showed that in any year an average of 15 per cent of the Bollgard II area planted carried larvae at above threshold levels, and that survivors were present in some fields in all cotton growing regions. Survival always occurred during flowering, and could follow low or high egg pressure but was always within the range of one to three larvae per metre.

Within a region, survivors were localised to particular fields, and within a field the distribution of larvae could be uniform or patchy. But in general only between 20–60 per cent of plants in a field sustained damage from surviving larvae. Medium-sized larvae were most commonly found on bolls, but were also found in disproportionately large numbers on flowers of Bollgard II cotton. These have relatively low levels of Bt in their components.

In Australia a relatively high proportion of *H. armigera* and *H. punctigera* carry a

gene that can allow them to survive Cry2Ab toxin. But there is currently no evidence that larvae surviving on Bollgard II are resistant. For both species, larvae collected as eggs from various crops are just as likely to carry Cry2Ab resistance genes as survivor larvae collected from Bollgard II plants.

We set out to improve our understanding of how the altered behavioural patterns of Bt-susceptible *Helicoverpa* spp. larvae might lead to their survival on Bollgard II. This work compliments a study on appropriate economic thresholds for controlling *Helicoverpa* spp. larvae in Bollgard II (in a separate article in next issue). The complete data sets for both of these articles can be accessed at: http://www.cottoncra.org.au/content/General/Research/Projects/1_01_42.aspx

Have larvae evolved behaviour to enable survival on Bollgard II?

Given that surviving larvae are often found in flowers it is possible that there has been selection within *Helicoverpa* populations for individuals that are more likely to prefer flowers, parts of which have low expression of Bt toxins, which would enhance their chances of survival.

We tested this possibility by using simple choice tests in the laboratory to examine the distribution of larvae among leaves, squares, bolls and flowers of Bollgard II cotton plants. We compared the responses of

larvae from colonies that were comprised entirely of survivors from Bollgard II crops (called ‘selected’ larvae) versus colonies that were comprised entirely of survivors from non-Bt crops (‘non-selected’ larvae).

If for example, larvae had evolved a feeding preference in response to selection from Bollgard II we might expect the selected larvae to show a preference for flowers because components may have relatively low levels of Bt toxin.

Figure 1 shows that the larvae from the two colonies demonstrated similar preferences among plant structures. This result suggests that there has been no clear, genetically determined, change in preference behaviour as a result of selection pressure for survival on Bollgard II cotton that reduces their exposure to toxic compounds.

Do larvae shift distribution within plants of non-Bt and Bollgard II cotton?

We used a glasshouse experiment to test if the behaviour of larvae on Bollgard II cotton was different to that of larvae on non-Bt cotton. We caged moths onto non-Bt plants and Bollgard II plants and confirmed that in both cases they laid most of their eggs on the terminal parts of the plant. The hatched neonates ‘sampled’ or ‘tasted’ the plants and kept moving until they found something suitable for feeding.

On the Bollgard II plants, this tasting often resulted in death, but some neonates did survive and these were mostly concentrated on fresh flowers. If these larvae then survived to 2nd instar they were able to feed on small bolls.

The neonate and young larvae on Bollgard II cotton moved more by spinning and trying to escape from the cage compared to those on the non-Bt cotton. Up to 0.8 per cent of the eggs laid on Bollgard II plants survived the larval phase to pupae but they developed more slowly than the larvae on non-Bt cotton. These results suggest that the normal feeding and movement behaviour of larvae may increase their chances of encountering flowers on which larvae may have better survival.

Based on these results we designed a choice experiment in the laboratory using the same methods outlined above to ex-

FIGURE 1: Choice tests using Bollgard II cotton show that larvae from colonies that were selected and non-selected did not show any differences in their distribution on different parts of the plant

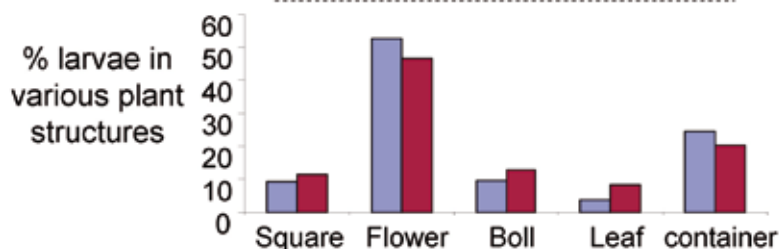
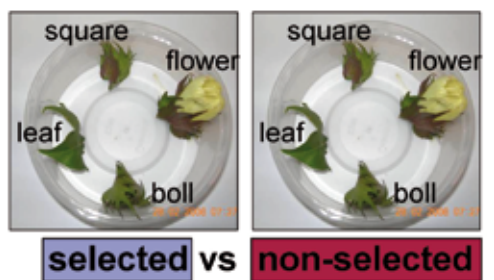
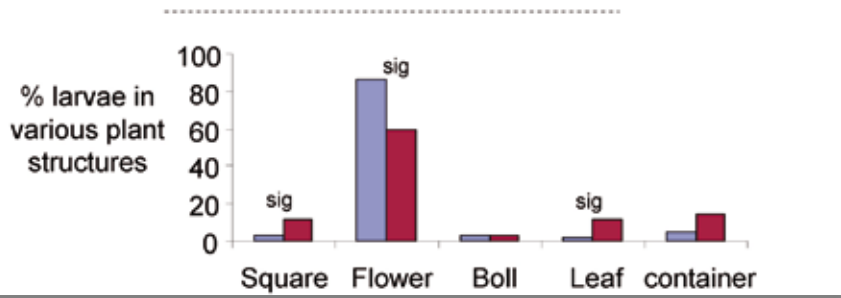
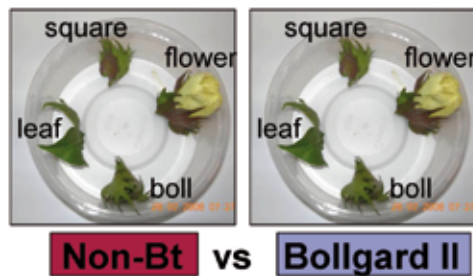


FIGURE 2: Choice tests showed that susceptible larvae were found more often on flowers and less often on leaves and squares of Bollgard II cotton compared to non-Bt cotton



amine the distribution of susceptible larvae among leaves, squares, bolls and flowers of non-Bt cotton versus Bollgard II cotton. Figure 2 shows that Bt-susceptible larvae were more likely to be found on flowers, than on other structures, on Bollgard II cotton than on non-Bt cotton. This suggests that larvae on Bollgard II may be distributed on structures on which they are more likely to survive.

Next we went to the field and tested if the movement and feeding preferences of larvae were different when released onto isolated individual plants of Bollgard II versus isolated individual plants of non-Bt cotton. We recorded the location and behaviour of the released larvae (either 3rd instars or neonates) every 30 mins for six hours after release.

This experiment was repeated across

two seasons. We found that, regardless of age, larvae on Bollgard II spent significantly less time feeding and more time crawling or resting than they did on non-Bt cotton. Additionally, on Bollgard II fewer larvae were observed on leaves and more larvae were observed on flowers than on non-Bt cotton.

These findings confirm those from the laboratory and glasshouse studies which suggested that on Bollgard II plants, larvae can select structures on which they are more likely to survive, and that the normal behaviour of larvae results in patterns of activity and feeding on Bollgard II plants, which in turn can increase their chances of encountering plant structures on which they can survive.

Do larvae shift dispersal along rows?

In the work above the focus was between structures on individual plants. But in the field, plants overlap and larvae can move along and even in some cases across rows. A logical conclusion would be that larvae on Bollgard II plants would be more likely to move, and possibly encounter a neighbouring plant, and they should move further from a release plant on Bollgard II cotton than non-Bt cotton.

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We investigated this in the field by releasing 3rd instar larvae onto the central plant of eight metre rows of non-Bt cotton and Bollgard II cotton and then relocating them at two, 24 and 48 hours after release to record how far they had moved.

After two hours most larvae had not moved very far from the release plant, but, by 24 hours after release, larvae on Bollgard II had moved significantly further than those on non-Bt plants, and this difference was even more apparent 48 hours after release. So larvae can distinguish between suitable (non-Bt) and non-suitable (Bt cotton) food and move away from unsuitable food, resulting in greater dispersal when plants contain Bt toxin.

Do larvae shift distribution among plants expressing different levels of Bt toxin?

Based on the results of all the experiments above it would seem logical that larval movement may mean that *Helicoverpa* move away from unsuitable plants, and so logically if they encounter a suitable plant they should then settle and feed on it. A suitable plant could be one with lower expression of Bt toxins, or a rogue non-expressing plant.

We tested this by looking at larval move-

ment when they were offered various combinations of suitable or non-suitable food. We did this by placing 3rd instar larvae on the central plant of triplets that were made up of various combinations of non-Bt cotton (C) and Bollgard II cotton (B): C-C-C, B-C-B, B-B-B or C-B-C. We observed larvae 24, 48, and 72 hours after release.

This experiment was also designed to explain the patterns of distribution of damage in commercial fields with survivors, specifically those larvae that penetrated fruit on only 30–50 per cent of plants.

Across all time points we found that significantly more larvae were found on the centre release plant if it was non-Bt cotton whether the neighbouring plants were non-Bt cotton or Bollgard II. In contrast, if the central release plant was Bollgard II most of the larvae moved off it, whether the neighbouring plants were non-Bt cotton or Bollgard II. We can infer from this result that if larvae found themselves on a poorly expressing Bollgard II plant then they would be unlikely to move from this plant onto the neighbouring plants.

In the Bollgard II only triplets, larvae were distributed roughly evenly across the three plants with 30 per cent on the central plant. In the Bollgard II triplets

with non-Bt cotton either side only about 15 per cent of larvae were found on the release plant. We can infer from this result that if larvae found themselves on a well expressing Bollgard II plant then they would be likely to move from this plant onto the neighbouring plants but would be more likely to settle on the neighbouring plants if they were poor expressers.

These findings suggest that between plant movement occurs frequently in Bollgard II fields and that larvae encountering lower expressing plants are likely to remain and feed there, increasing their chances of surviving to adults.

Possible explanations for survival of susceptible larvae on Bollgard II plants

In all of the studies we collected plant material to investigate relationships between the behaviour of larvae and within plant variation in Bt expression. We also conducted several other experiments specifically to investigate field variation in Bt expression on larval survival, and within season variation to identify shifts over time that might coincide with the presence of larvae in Bollgard II fields.

In all of these studies we were interested in relative differences in expression rather than absolute levels and used a standard ELISA method. When we compared these data to other studies that measured Bt expression using ELISA methods we found that toxin levels vary considerably within plants, among plants, among fields and within seasons.

We also found that Bt expression does not necessarily correlate well with insect bioassays of either behaviour or survival. Nevertheless, this variation does provide the basis for explaining the survival of Bt susceptible larvae on Bt crops.

Survival can only occur in Bt crops if hatching neonates have access to food that does not kill them. Larvae hatching on flowers may have a higher chance of survival but the probability of being laid on a flower is low because there are fewer of them than other structures, and because they are ephemeral.

But neonates could hatch, feed insufficiently to ingest a lethal dose of Bt or perhaps regurgitate the Bt food, and then move, which will increase their chances of encountering a more suitable food, such as a flower. This could be on the plant that they hatched on or a neighbouring plant with poorer expression where they can establish before moving onto areas with higher expression in a less-vulnerable life-stage. So even in situations of good expression it is

FIGURE 3: Susceptible larvae placed on central plants of trios of non-Bt cotton (C) or Bollgard II cotton (B) were less likely to leave if the initial plant was non-Bt cotton – if the initial plant was Bollgard II cotton they were more likely to settle on a neighbouring plant if it was non-Bt cotton

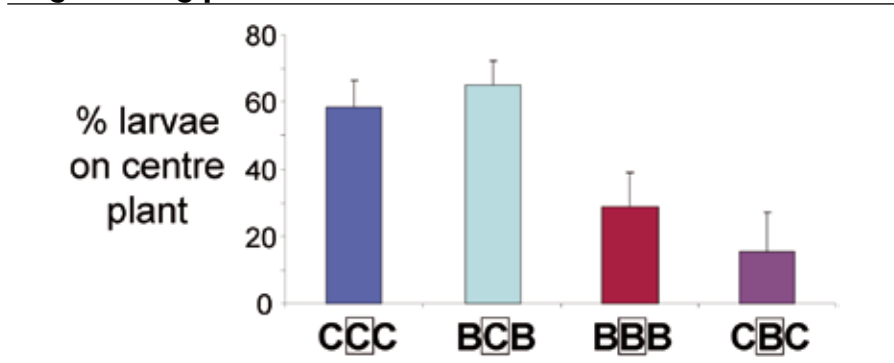
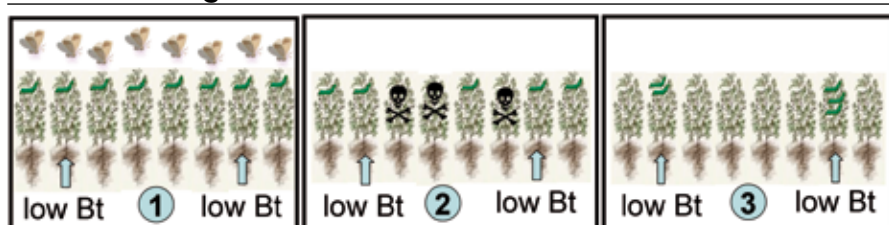


FIGURE 4: A possible model for the survival of susceptible larvae on Bollgard II cotton



(1) At times some Bollgard II plants express levels of Bt that are not sufficient to control larvae. Larvae hatched on these plants remain.
 (2) Larvae on neighbouring Bollgard II plants that express well feed and die or feed and move to the nearest low Bt plant (3).

possible that some larvae, normally a very small proportion, could survive.

Alternatively, or in combination, if expression is variable between plants and within plants, within a row of cotton there may be some plants which express relatively low levels of Bt. Usually these plants control larvae but occasionally if whole fields, or parts of fields, are stressed, the expression of plants across these areas may decrease to the point where a Helicoverpa egg laid on one of these plants

may have an increased survival as a larva (Figure 3). A Helicoverpa egg laid on an adjacent plant that is expressing well, would hatch a neonate that will feed and die or feed and move on until it found a low expressing plant (Figure 4).

CONCLUSIONS

- Larvae exhibit strong behavioural responses to the Bt proteins in Bollgard;
- Normal larval behaviour coupled with the sometimes temporal and spatial variability of Bt toxin expression in Boll-

gard II cotton, can result in a proportion of bollworm populations becoming established; and,

- No evidence exists to suggest that larvae have evolved a behaviour to overcome Bt-cotton.

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