

Surviving *Helicoverpa* larvae in Bollgard II: Survey results

Gavin Whitburn¹ (University of Queensland) and Sharon Downes¹

Ingard was released for commercial production in Australia in the 1995–96 season. It expressed a *cry1Ac* gene that led to plants producing the toxin Cry1Ac which kills *Helicoverpa*. Younger plants controlled larvae but a decline in toxin expression later in the season meant insecticide use was necessary after egg pressure.

In 2004–05 Ingard was superseded by Bollgard II, which expresses Cry2Ab throughout the growing period in addition to the original Cry1Ac toxin which declined over time. This two toxin cotton is expected to provide season long protection, although it is generally accepted that later in the season only the *cry2Ab* gene may be effective.

From the outset there have been reports of *Helicoverpa* larvae that often develop to the medium-large size class in some fields of Bollgard II. Sometimes larvae reach numbers which are greater than threshold levels, and are too large to effectively control using some conventional insecticides. In these situations growers are mostly concerned about potential economic damage but this situation may also interfere with resistance management.

In Australia a relatively high proportion of *H. armigera* and *H. punctigera* carry a gene that can allow them to survive Cry2Ab toxin but currently there is 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 larvae collected from Bollgard II plants. As well as this, the few larvae collected from Bollgard II plants that carry a Cry2Ab resistance gene have only one copy of it which means they should be killed by the plant.

Because young Bollgard II plants control larvae, and contain the *cry1Ac* and *cry2Ab* genes, it is logical that larvae surviving on older plants are exposed to a non-lethal dose of one or both of the toxins. This situation is a major driver of the evolution of resistance.

Addressing concerns from industry

Early reports suggested that Bollgard II fields with medium-large larvae are restricted to St George in South Western Queensland. It is also thought that the percentage of Bollgard II fields with medium-large larvae may be increasing.

To find out if these perceptions are true, and to estimate the percentage of Bollgard II fields with medium-large larvae that are treated, we conducted a survey of 46 Crop Consultant Australia (CCA) members who serviced Bollgard II in 2005–06, 2006–07 and/or 2007–08. The accredited planting of Bollgard II reduced over time with approximately 230,000, 114,000, and 61,000 hectares planted in 2005–06, 2006–07 and 2007–08 respectively. In all years, consultant responses accounted for at least 66 per cent of the Bollgard II planted, and in all seasons there was good representation from consultants in all valleys.

Reports of larvae in Bollgard II fields generally fall into one of two categories. The first is where neonates develop into larvae smaller than three mm but do not continue to develop. Technically these situations are below threshold and were not considered in our survey.

In the second situation, larvae reach three mm and often continue to develop past eight mm. The current threshold for *Helicoverpa* in Bollgard II is at least two larvae three to eight mm per metre in at least two consecutive checks or one larvae over eight mm per metre. We considered a field to carry larvae if it reached this threshold level.

The area of Bollgard II grown in a valley was not related to the percentage of Bollgard II with surviving larvae. This meant that we could use percentages to compare the incidence of Bollgard II with larvae among valleys. We did not correct our data for *Helicoverpa* pressure because the percentage of Bollgard II with larvae in different valleys was not affected by the number of sprays for *Helicoverpa* on conventional cotton – a proxy for ‘pressure’.

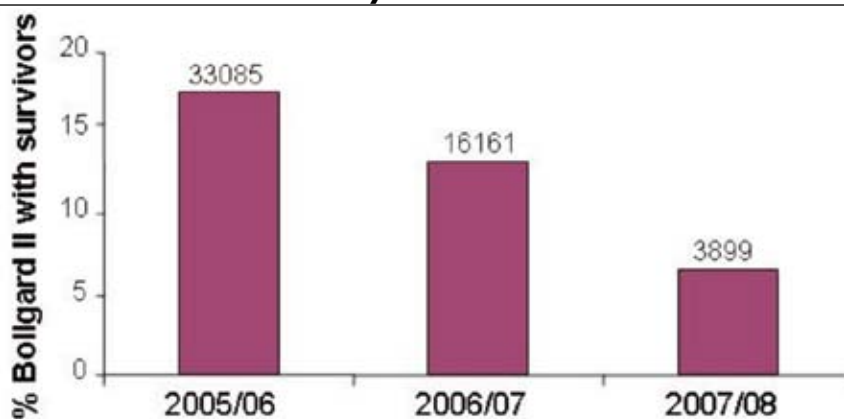
The percentage of Bollgard II with larvae has not increased

When the data were totalled across seasons and valleys, 15 per cent of the



Bt-susceptible *Helicoverpa* larvae can sometimes survive on Bollgard II plants.

FIGURE 1: The percentage of Bollgard II at threshold for each season totalled across valleys



The values at the top of each bar give the total Bollgard II hectares at threshold.

Bollgard II carried larvae that reached threshold levels at some time. As Figure 1 shows, the percentage of Bollgard II with larvae ranged between seven and 18 per cent across seasons, and declined over time. The value above each bar estimates the total area of Bollgard II in hectares that carried surviving larvae at threshold.

Clearly, these results do not support concern that the percentage of Bollgard II with larvae at threshold levels is increasing. The perceived increase may result from a

greater awareness of reports because of extension efforts, and recent articles on the issue.

No valley was more or less likely to have Bollgard II with larvae

We used the average percentage of Bollgard II with larvae across valleys (not shown here) to determine from Figure 2 which valleys showed above average percentages of larvae at threshold.

In 2005–06 the Gwydir, Lower Namoi and MacIntyre valleys had a percentage of

Bollgard II with larvae that was above the average. In 2006–07 the Lower Namoi, St George, Macquarie, and Gwydir valleys had a percentage of Bollgard II with larvae that was above the average. In 2007–08 the Upper Namoi, Macquarie, St George, and Emerald valleys had a percentage of Bollgard II with larvae that was above the average.

These data show that there is no consistent trend among seasons for any partic-
...14▷

EXCEL Agriculture
Excellent product. Excellent service.

+ the way forward



Fertiliser Rigs

- ✓ Up to 3 tonne capacity with exceptional stability and ease of lifting
- ✓ New low draft shank and clamp assembly for simplistic reliable operation
- ✓ Offering an unrivalled 770mm of underframe clearance for brilliant trash flow
- ✓ Proven stainless steel metering units for corrosion resistance
- ✓ Variable rate technology available
- ✓ Can apply gas at same time as fertilisers



Stubble Warrior

- ✓ Plant wheat back into cotton hills
- ✓ Double disc
- ✓ Single disc
- ✓ Gassing and/or fertilising



Excel Penetrator

- ✓ 4, 6, 8 row
- ✓ Side busting/centre busting capabilities
- ✓ Adaptable for gassing, dry fertiliser using Excel's new rig mounted fertiliser boxes
- ✓ 5/8" inline shear bolts
- ✓ Unrivalled trash clearance



Excel Cultivator



Cultipacker

- ✓ Machine can be pulled one way for aggressive soil breakdown and the other way for refining the soil before planting

Also available:

- ✓ Rubber Tyred Roller
- ✓ Lilleston Rig
- ✓ Parallelogram Cultivators
- ✓ Guess Row Averagers
- ✓ Listering Rigs
- ✓ Shielded Sprayer

Call now — our components fit most implements

EXCEL Agriculture

A DIVISION OF GREAT WESTERN CORPORATION PTY LTD (ABN 91 064 434 475)

Australian made and owned

74–92 Buckland Street,
PO Box 665, Toowoomba Qld 4350

PH: 07 4636 9100

FAX: 07 4636 9140 — www.excelagr.com.au

Brian Moran
0427 722 925
Graeme Easey (NSW)
0427 700 779

ular valley to be more or less likely to have Bollgard II that carries larvae. The same pattern is found if the valleys are grouped into climatic regions.

Based on the data collected from 2005–06 to 2007–08, it is not possible to predict which valley(s) will have the greatest percentage of Bollgard II that carries larvae.

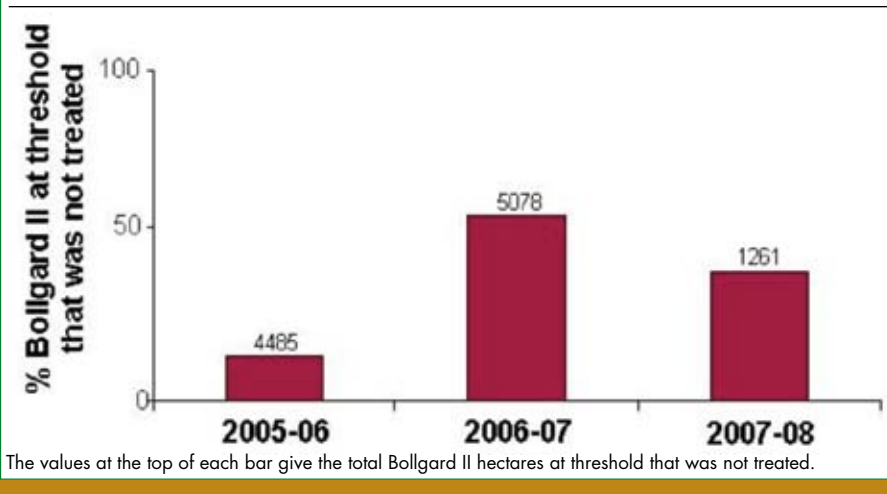
Not all of the Bollgard II with larvae was treated

To extend the life of Bollgard II, growers are obliged to follow a strict Resistance Management Plan (RMP). Despite this strategy, there are concerns that Cry2Ab resistant alleles in both *Helicoverpa* species may be increasing. We know from digs under Bollgard II fields which reached threshold that reasonable numbers of the medium-large larvae can pupate and emerge as moths.

Because larvae surviving on Bollgard II probably consume Bt toxin at a non-lethal dose, it is possible that selection for resistance occurs. For example, if Cry1Ac is expressed at a sub-lethal dose, insects that carry two copies of the Cry2Ab resistance gene may be favoured. Perhaps of greater concern is the impact of a sub-lethal dose of Cry2Ab on the evolution of Cry1Ac resistance because studies on *H. armigera* in China show that some insects can be partially resistant to this toxin even if they have only one copy of a Cry1Ac resistance gene.

This situation means that it is important for resistance management to treat Boll-

FIGURE 3: The percentage of Bollgard II at threshold in each season that was not treated



gard II that carries medium-large larvae to stop them from contributing offspring to future generations.

Because from a resistance perspective we were interested only in cases where larvae could possibly survive on Bollgard II and contribute to the next generation, we ignored the few responses in our survey where threshold was reached only with larvae less than eight mm (these larvae may have eventually died). As Figure 3 shows, in some years more than half of the area with at least one medium-large larvae per metre was not treated. This finding is a concern for managing resistance.

Expected Bollgard II performance

A secondary aim of the survey was to find out how consultants expected Bollgard II to perform and see if this expectation

changed after several years experience with the technology.

Throughout the industry 55 per cent of consultants believed that up to 2.5 of every 10 Bollgard II fields would reach threshold levels, which fits with the pre-commercialisation performance data reported in the Bollgard II cotton Technical Manual. About 27 per cent of consultants expected that Bollgard II would never reach threshold levels and 18 per cent thought that more than 2.5 of every 10 Bollgard II fields would reach threshold levels.

For most consultants (61 per cent) the expected performance of Bollgard II did not change after several years experience with the technology. With the same length of experience, about 22 per cent of consultants expected more fields to reach threshold, and about 17 per cent of consultants expected fewer fields to reach threshold.

There was no relationship between a consultant's expected performance of Bollgard II and growing region. These different responses may relate to direct experience with the product, including opportunities to work with Bollgard II during the pre-commercialisation trials.

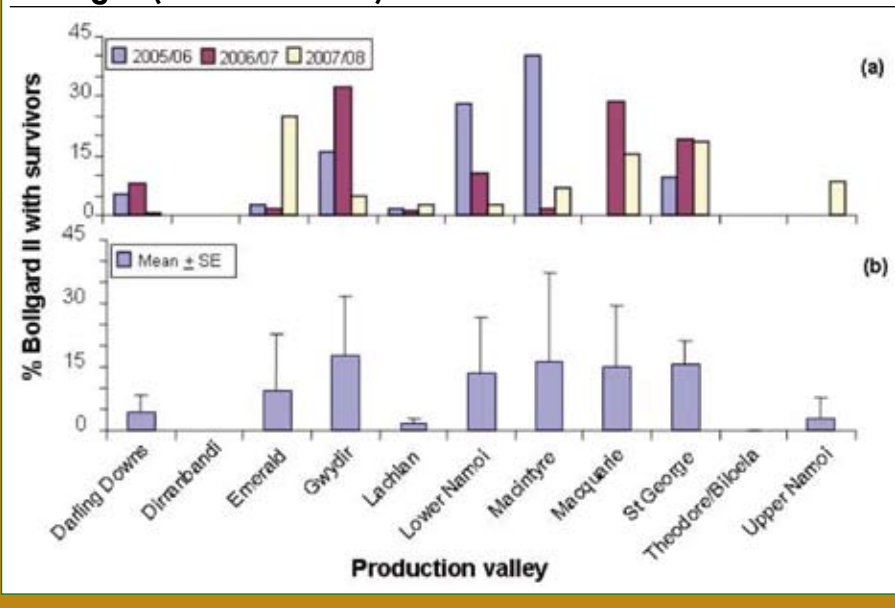
CASE STUDIES FROM 2007-08

A follow-up questionnaire to consultants that reported survivors in 2007–08 did not identify practices or conditions that led to some Bollgard II fields carrying larvae while others did not.

In 2007–08 the Bollgard II fields at threshold were planted early to mid-October except in Emerald where planting was in mid-September. Most plantings were Sicot 71BRF or Sicot 71BR but some in St George were Sicot 289BR and Sicot 80BRF. In Emerald the fields carried lar-

...16▷

FIGURE 2: The percentage of Bollgard II at threshold for (a) each valley totalled for each season and (b) each valley averaged (with error bars) across seasons



vae mostly during late flower but in other regions larvae were mostly found at peak flower.

Before infestation, about 33 per cent of consultants scouted for larvae in Bollgard II using visual checks alone, beat sheets alone, or beat sheets plus visual checks. After infestation, most consultants (64 per cent) used beat sheets plus visual checks and about 36 per cent used visual checks alone. Regardless of timing, the number of checks ranged from two to six in every 100 hectares.

Survival occurred after high pressure (more than 40 eggs per metre) but also moderate (10–40 eggs per metre) and low pressure (less than 10 eggs per metre). So, although eggs have to be present for larvae to develop, in many cases high pest pressure is not the only factor that leads to larvae developing on some Bollgard II

hosts. Even with high egg pressure the number of medium-large larvae appeared to reach a maximum of three per metre.

In most cases (64 per cent) larvae were described as being evenly distributed in the affected field, and were found on all structures of the plant (leaves, squares, flowers, boll caps, small bolls); only one field had larvae only on flowers. Most consultants thought that there would have been a loss of yield if an insecticide was not applied.

The previous crop planted in an affected field included cotton, sorghum, mungbean, chickpea, wheat, or fallow. In Bollgard II fields with larvae, the plant populations (8–13 per metre), pupae busting practices, pesticide applications, and PIX applications were similar to those in nearby fields of Bollgard II that did not carry larvae.

An obvious stressor (nutrition or water deficiencies) was identified by consultants in only a few of the Bollgard II fields with larvae. Mostly there were no obvious stres-

sors and sometimes Bollgard II that did not carry larvae was grown close to and under the same conditions as the affected fields. This means that it is not possible to identify obvious management practices or conditions that lead to some fields of Bollgard II carrying larvae while others do not.

Plants in affected fields may have a different physiology or genetic makeup that affects the rate of toxin production, neither of which can be noticed by simply observing the plant. The fact that some fields are affected while others are not suggests that the behaviour of *Helicoverpa* is not likely to lead to survival, but it is possible that larvae behave differently on plants that have a lower initial level of Bt toxin. Because it is difficult to predict if a field of Bollgard II will progress to carry larvae, it is also difficult to make accurate control decisions.

THRESHOLDS

Researching economic thresholds for Bollgard II is difficult because situations where larvae develop cannot be predicted. By artificially damaging Bollgard II plants and/or leaving some parts of affected Bollgard II fields unsprayed, it may be possible to determine if yield penalties exist when medium-large larvae are not treated. These approaches are being adopted by PhD student Baoqian Lu from the University of New England who is supported financially by a Monsanto contribution to the Cotton Australia project through the Cotton CRC. Future research may also need to consider the role thresholds can play in reducing the selection pressure for Bt resistance.

THE FUTURE OF THIS SURVEY

The main part of the survey, which gathers information on areas of Bollgard II at threshold and the proportion of this area that was treated, will be conducted annually. For the 2008–09 season, the survey will be available from June 1 as a link on the Cotton CRC website: <http://www.cotton.crc.org.au/> (Industry/Tools/Forms & Questionnaires – Industry). In future years it will be incorporated into the CCA Post-season Survey.

The survey could not have occurred without the wonderful support from many CCA consultants. We also acknowledge support from the CRDC, Cotton CRC, Monsanto, and CSIRO. Full acknowledgments can be found in the final report for the survey on the Cotton CRC website: (Industry/Publications/Pests and beneficials/ Insect Resistance Management).

Further information: Sharon.Downes@csiro.au, Ph: 02 6799 1576–0427 480 967

¹CSIRO Entomology and Cotton Catchment Communities CRC.

Supported by Crop Consultants Australia.



At Dinner Plain the pace is easy going...

Dinner Plain is the place where the family can be together by the fireside or miles apart exploring the cross-country trail network. Where you stroll the treelined streets simply for the sights or to meet friends for a restaurant dinner or drinks at the bar. The village itself helps set the community atmosphere, natural building materials and earthy tones blur the line between man made and alpine environment. Over 200 lodges and chalets with all the conveniences of a modern resort.

Dinner Plain is the place for your next holiday.

Explore our website at www.dinnerplain.com or call our info number **1300 734 365** or email to info@dinnerplain.com

Dinner Plain
visitor Information
Centre