Cotton Catchment Communities CRC

SUMMER SCHOLARSHIP
Final Report
(D*ue within 3 months on completion of project*)

Project title: **Sprays for surviving Helicoverpa larvae in Bollgard II® survey**

Aims and milestones:

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Executive Summary:

Bollgard II® has revolutionised the cotton industry in Australia by enabling production with significantly reduced insecticide application. Specifically, pesticide use on this product is reduced by an average of 7.1 sprays (80% of active ingredient) relative to non-Bt cotton. The users manual for this product states that "Bollgard II® is not *Helicoverpa* proof", and that "high pest pressure, end of season or plant stress may necessitate pesticide application". The survey reported herein was conducted in response to concerns from the industry that the situations where Bollgard II® required sprays for Helicoverpa larvae were largely restricted to the St George Area and were increasing over time. We found that the presence of larvae in occasional fields of Bollgard II® is widespread among valleys and climatic regions, and did not increase between 2005/06 and 2007/08. We also found that among-valley differences in the proportion of Bollgard II® that carried larvae is not related to the area planted to this product or the pressure imposed by Helicoverpa. Bollgard II® fields that carried medium to large larvae were not always treated with an insecticide for control, and the potential implications of this situation for managing resistance to Bt are discussed.

Background:

The lepidopteran *Helicoverpa armigera* is distributed throughout the Old World where it is the major pest on numerous crops, particularly cotton. It poses the principal threat to transgenic cotton in Australia, India and China because of its recidivist nature in evolving resistance to conventional insecticides. The capacity of this species to develop resistance to a Bt toxin (Cry1Ac) is evident from experiments in Australia, India, and China where field-collected populations challenged by selection in the laboratory developed high levels of resistance. In contrast, the Australian endemic species *H. punctigera* has not shown a propensity to evolve resistance to conventional insecticides. This may reflect the different dispersal ecology and population dynamics of the two species. Regardless of the mechanism, and despite both species being innately tolerant to Bt toxins, these different histories in developing resistance to insecticides renders *H. armigera* and *H. punctigera* a relatively high and low risk respectively at developing resistance to Bt cotton.

Bt cotton varieties known as Ingard® in the New World (Bollgard elsewhere) were released for commercial production in Australia in the 1995/96 season. With this technology growers were able to move away from a Helicoverpa control regime that often required considerable applications of insecticides. Ingard® expressed the *cry1Ac* gene produced by *Bacillus thuringiensis* which is toxic to Lepidoptera species. Whilst younger plants effectively controlled larvae, a decline in toxin expression later in the season meant that during this period insecticide use was necessary following egg lays by Helicoverpa. In 2004/05 Ingard® was superseded by Bollgard II®, which expresses Cry2Ab throughout the growing period in addition to the original Cry1Ac event which declined over time. This dual toxin technology is expected to provide growers with season long protection against Helicoverpa, although it is generally accepted that later in the season only the *cry2Ab* gene may be effective in this control.

Bollgard II® has been widely adopted within Australia with around 80% of industry growing the technology. From the outset there have been reports of Helicoverpa larvae surviving, and often developing to the medium-large size class, in some fields of Bollgard II®. In many situations larvae reach numbers which exceed the recommended threshold levels for Bollgard II®, and are too large to adequately control using some conventional insecticides. In these situations the growers are primarily concerned with the economic damage caused by the larvae. However, there may also be implications of this situation for resistance management.

A relatively high baseline frequency of alleles conferring resistance to Cry2Ab exists in *H. armigera* and *H. punctigera*. However, currently there is no evidence that Helicoverpa larvae are able to survive on Bollgard II® host plants due to Bt resistance. In particular, for both Helicoverpa species there is no difference in the proportion of individuals carrying Cry2Ab resistance genes in a random sample versus larvae collected from Bollgard II® host plants. Moreover, the Cry2Ab resistance genes isolated for populations of Australian Helicoverpa are recessive and all individuals found to carry this gene were heterozygotes (i.e., they should have been killed by any toxin in the plant). Bollgard II® host plants control the larvae early in the season and contain the *cry1Ac* and *cry2Ab*. It is therefore likely that, irrespective of the mechanism allowing survival, the larvae are being exposed to a sub-lethal dose of one or both of the toxins. This condition is a major driver of the evolution of resistance.

Aims and objectives:

Preliminary reports suggested that the occurrence of fields with survivors was restricted to the growing region of St George in south western Queensland. There is also a perception throughout the industry that the frequency of fields with this problem may be increasing over time. A major aim of our work was to investigate these perceptions by examining the frequency with which surviving larvae were found at or above threshold levels throughout the Australian cotton industry for the seasons 2005/06, 2006/07 and 2008/09. From the perspective of resistance management we were also interested in gauging the proportion of fields which carried larvae that were treated with a conventional insecticide. In a follow up questionnaire we gathered basic information on broad environmental factors that may be used to predict whether a field would carry larvae.

Methodology:

Part A: Broad overview

The survey entitled 'Sprays for surviving Helicoverpa in Bollgard II®' was launched on the Cotton Catchment Communities CRC website on 1st April 2008 with a deadline of 21st April 2008. A list of active members from the Crop Consultants Association (previously Cotton Consultants

Association; hereafter CCA) was obtained from the Chief Executive Officer. These consultants were contacted by email and requested twice to respond to the survey. The response to this initial request was poor with few consultants completing the survey.

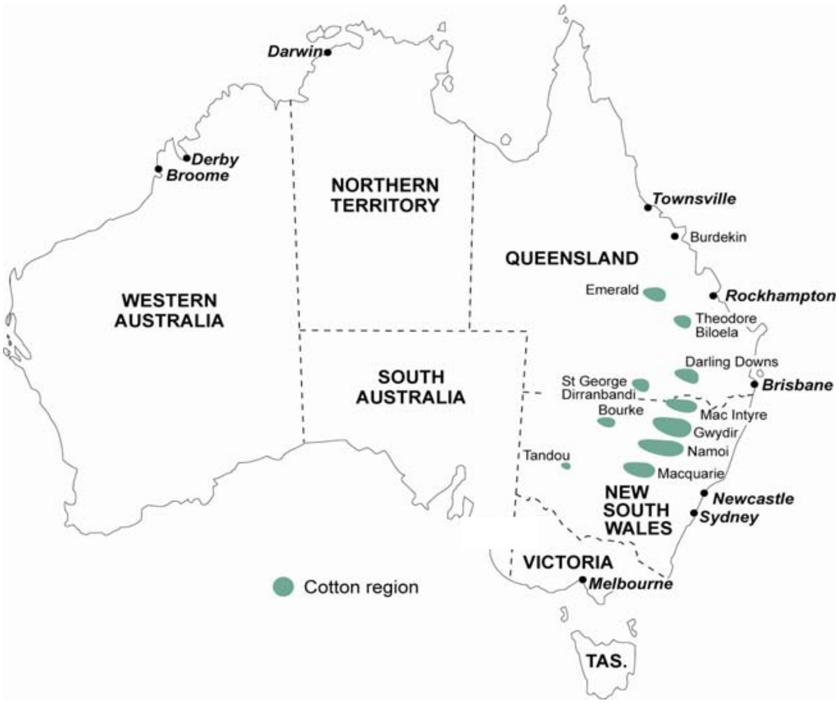

Figure 1: Map of Australia showing the main cotton regions sampled in the study.

On the 30th July 2008 we issued a further email request to consultants to complete the survey, followed by a vigorous telephone and email campaign. Although consultants were assured that responses would remain confidential, they were asked to provide their name so as to be removed from the list of members yet to complete the survey. Consultants were also asked to nominate which of the main cotton growing regions they were working within (see Figure 1) and complete separate surveys if they were working in more than one valley.

The survey covered the period 2005-2007. Sixty three consultants were asked to respond. We received 45 responses with 14 consultants failing to respond and four consultants replying that they were unable to complete the survey. Figure 2 shows for each season the total area of Bollgard II® accounted for by consultants that responded to the survey versus data supplied by Monsanto on the accredited planting of Bollgard II®.

The accredited planting of Bollgard II® reduced over time with approximately 230000, 114000, and 61000 hectares planted in 2005/06, 2006/07 and 2007/08 respectively (see Figure 2). When compared to the accredited planting the percentage of Bollgard II® accounted for by consultants summed across years and valleys was 71. When considering the data for 2005/06, 2006/07, and 2007/08 separately, the percentage of Bollgard II® accounted for by consultants summed across the valleys was 66% (range 32-100, mean = 63), 79% (range = 42-100, mean = 66) and 73% (range = 47-100, mean = 65) respectively. There was no significant difference among valleys in the percentage of Bollgard II® accounted for by consultants (ANOVA, valley as factor and year as replicate, df = 10,21, $F = 1.3$, $P = 0.27$). We therefore assume that the dataset gives an unbiased representation across seasons and valleys.

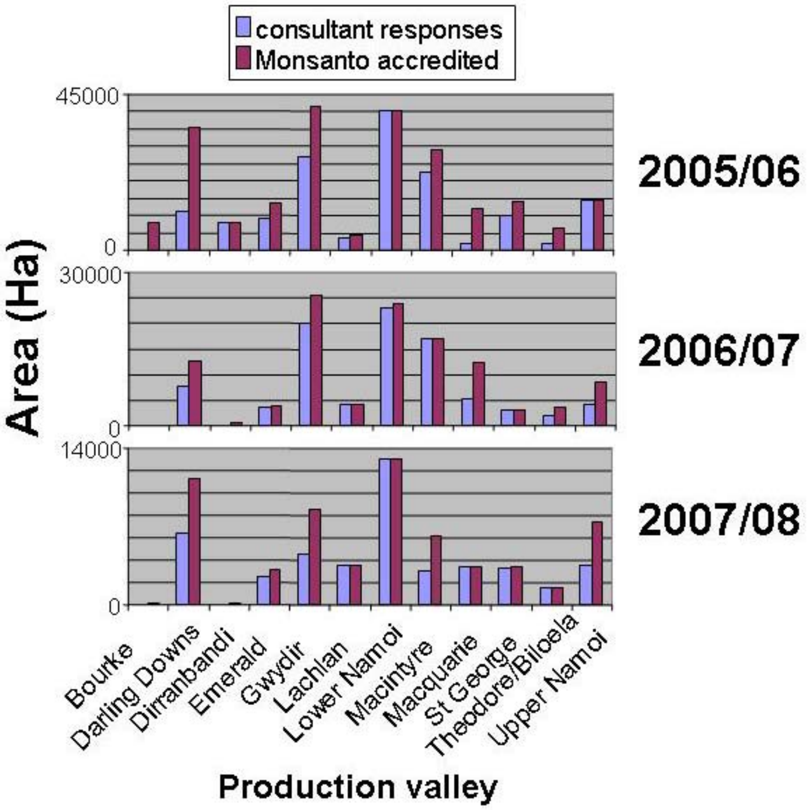

Figure 2: The total area of Bollgard II® accounted for by consultants that responded to the survey versus data supplied by Monsanto on the accredited planting of Bollgard II®.

Since we did not account for any of the Bollgard II® planted in Bourke in 2005/06 (125 ha), 2006/07 (13 ha) and 2007/08 (8000 ha), this valley was not included in subsequent data analyses. We did not account for any of the Bollgard II® planted in Dirranbandi in 2007/08 (67 ha) and 2006/07 (494 ha) respectively, therefore we included data from this valley for 2005/06 only in subsequent analyses.

(i) Extent and distribution of the problem and targeted sprays
The main aim of Part A of the survey was to ascertain the proportion of Bollgard II® with surviving larvae throughout the industry and among the different cotton regions. We defined "Bollgard II® with surviving larvae" as the area that reached the recommended threshold for applying a spray (also the threshold determined for Ingard® cotton): at least 2 larvae 3-8 mm/m in at least two consecutive checks or 1 larvae > 8mm/m. We also wanted to determine the proportion of Bollgard II® with surviving larvae throughout the industry that received at least one spray for Helicoverpa. Finally, we were interested in whether these proportions changed through time.

To obtain this information we asked the consultants to answer the following questions separately for the 2005/06, 2006/07 and 2007/08 cotton seasons:

1. How many hectares of Bollgard II® did you inspect in your role as a consultant?
2. How many hectares of Bollgard II® carried at least 2 larvae 3-8 mm/m in at least two consecutive checks or 1 larvae > 8mm/m?
3. How many hectares of Bollgard II® did you spray at least once for larvae
   (a) what area was sprayed only for Helicoverpa?
   (b) what area was sprayed for other pests (e.g., mirids) that also targeted Helicoverpa?

We calculated the percentage of Bollgard II® with surviving larvae by dividing the area of Bollgard II® that carried at least 2 larvae 3-8 mm/m in at least two consecutive checks or 1 larvae > 8mm/m by the area of Bollgard II® accounted for by consultants.

For the analyses looking at climatic region as a factor, we grouped the valleys as follows: Tropical (Emerald, Theodore), Darling Downs (Darling Downs), Middle (Lower Namoi, St George, Dirranbandi, MacIntyre, Gwydir) and Cool (Upper Namoi, Macquarie, Lachlan).

Subsequent to the survey, it became apparent that in a few cases threshold levels were reached based on small larvae only. In terms of sprays for Helicoverpa, we were interested in cases where larvae could potentially contribute to subsequent generations. Medium to large larvae in Bollgard II® fields can survive to pupation and successfully emerge in the laboratory, but this may not be the case for small larvae. Therefore, we excluded from the analyses of sprays for Helicoverpa the few cases were threshold was based entirely on the survival of small larvae. This is a conservative approach since even after applying a spray some small larvae may have developed. If the information was not available through comments taken by phone or in the box provided at the end of the survey, we contacted consultants that had larvae at threshold levels and determined whether these situations comprised only small larvae.

In the analyses incorporating information on Bollgard II® sprayed for Helicoverpa we refer to the sum of hectares provided in response to question 3a and 3b as "hectares of Bollgard II® sprayed at least once for Helicoverpa larvae".

For the analyses looking at the area of Bollgard II® with surviving larvae that was not treated with a spray, for each consultant we subtracted the area sprayed at least once for Helicoverpa from the total area at threshold. For each year, we summed this area and divided it by the total area for that year that was at threshold.

When presenting the data as “area” at threshold or sprayed, we multiplied the accredited planting area by the proportional data obtained from consultants to estimate the total production area affected (hereafter referred to as ‘weighted’ data).

Although there was variation in the area of Bollgard II® grown among valleys, this factor was not significantly related to the percentage of Bollgard II® with surviving larvae ($r^2 < 0.20$, $P > 0.21$; Figure 3). We therefore used percentage values to compare the incidence of Bollgard II® with surviving larvae among valleys.

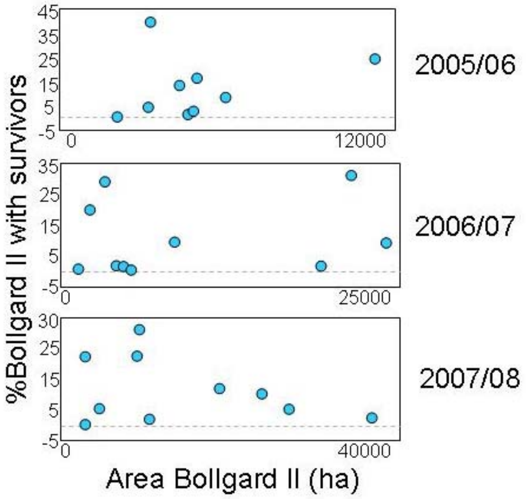

Figure 3: The percentage area of Bollgard II® accounted for by consultants that carried surviving Helicoverpa larvae at threshold levels and its relationship to the area of Bollgard II® planted. Data are presented separately for the three years of the study.

In order to determine whether it was necessary to weight our data according to Helicoverpa pressure we used the CCA Post Season Survey data (Doyle and Coleman 2005/06; Western Research Institute 2006/07) to examine the relationship between incidence of Bollgard II® with surviving larvae and the average number of sprays for Helicoverpa on conventional cotton fields in each valley. Data were available for the 2005/06 and 2006/07 seasons only. In both of these seasons a simple regression analysis determined no significant relationship ($r^2 < 0.32$, $P > 0.11$) between the percentage of Bollgard II® with surviving larvae versus sprays on conventional cotton (Figure 4), and we assumed that this was also the case for 2007/08. Consequently we deemed that it was not necessary to weight our data according to Helicoverpa pressure.

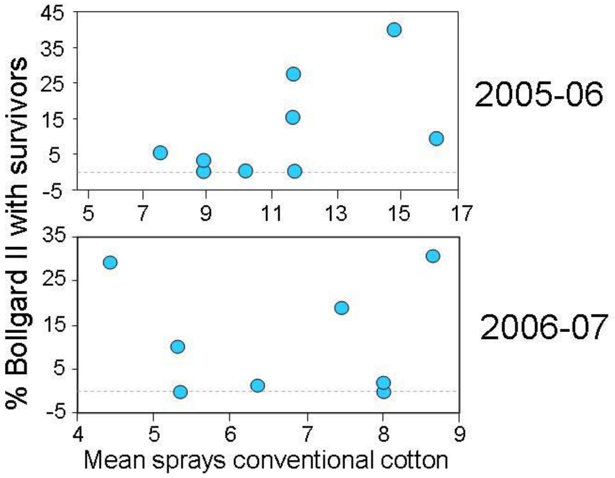

Figure 4: The relationship between percentage area of Bollgard II® accounted for by consultants that carried surviving Helicoverpa larvae versus the average number of sprays for Helicoverpa in conventional cotton fields. Each point represents one valley. Data are presented separately for 2005-06 and 2006-07.

(ii) Expectations of Bollgard II® performance

The secondary aim of Part A of the survey was to ascertain how consultants expected Bollgard II® to perform in controlling larvae and determine whether this expectation changed with several years experience of the technology. To obtain this information we asked the consultants to answer the following questions by selecting a response from multiple choices:

1. On average throughout the industry what do you currently expect the number of Bollgard II® fields that reach threshold levels of Helicoverpa larvae to be?: 0, 0-0.25, 0.25-0.50, 0.50-0.75, 0.75-1.00.
2. Has this expectation changed since 2005/06?: Increased, Decreased, Not changed

In asking the first question we assumed that consultants would select a response that closely matched their experience with the technology during the previous three seasons. The second question therefore was designed to gauge their expectation at the outset of the products availability.

Part B: Detailed case studies on fields reaching threshold in 2007/08

Following Part A of the survey, a second questionnaire was forwarded to consultants who had Bollgard II® with surviving larvae in 2007/08. The questionnaire was designed to gather data on factors that may influence the occurrence of Helicoverpa survival in Bollgard II®, and was based on a survey compiled by CSIRO and Monsanto prior to the 2007/08 season.

The questions were designed to obtain information on checking methods, crop stage, extent of damage by larvae, and the location of damage within the plant. The second part of the questionnaire investigated the environmental conditions the crop was subject to, including previous crop planted in that field, tillage operations (pupae busting), plant population density, herbicide and insecticide application leading up to surviving larvae, as well as soil and nutritional conditions. If relevant, the consultants answered the same questions on environmental conditions for Bollgard II® fields that did not carry larvae but were at the same crop stage as the field(s) with survivors. These results are presented mainly in a descriptive format. The complete questionnaire is included as an appendix to this report.

Results:

Part A: Broad overview

Extent and distribution of Bollgard II® fields with surviving larvae

When the data are summed across years and valleys 15% of the Bollgard II® accounted for by the consultants carried surviving larvae at threshold levels. In 2005/06, 2006/07, and 2007/08 the percentage of Bollgard II® with surviving larvae summed across valleys was 18, 13, and 7 respectively (Figure 5). The interaction between season and the total area of Bollgard II® with and without surviving larvae is statistically significant (Fishers Exact Test, df=2, X^2 = 3500, P = 0.001).

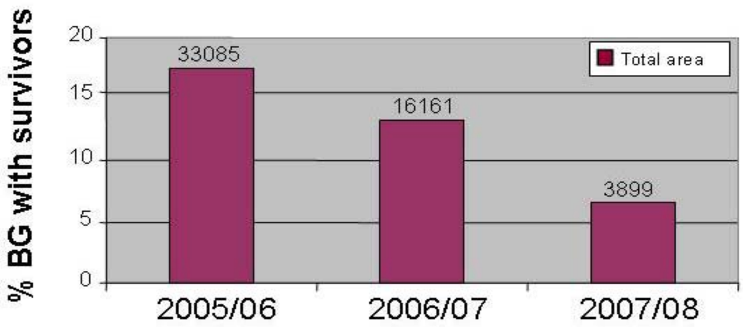

Figure 5: The percentage area of Bollgard II® accounted for by consultants that carried surviving Helicoverpa larvae at threshold levels. Data are presented for each year as a total across all valleys and the values at the top of each bar is the weighted total are of Bollgard II® that was at threshold.

In 2005/06, 2006/07, and 2007/08 the percentage of Bollgard II® with surviving larvae averaged across valleys was 10.3 $\pm$ 4.4 (range 0-40), 10.2 $\pm$ 3.9 (0-32) and 8.6 $\pm$ 2.7 (range 0-25) respectively (Figure 6). There is no statistically significant difference among years in the average percentage of Bollgard II® with surviving larvae in each valley (ANOVA, df=2,27, F = 0.07, P = 0.93). This result largely reflects relatively high percentage levels of Bollgard II® with surviving larvae in a few valleys with relatively small plantings in 2007/08. Although for each study year there is no significant relationship between area planted to Bollgard II® and the percentage of area with surviving larvae (in all cases, R <

0.42), the relationship is negative in 2007/08 versus positive in 2006/07 and 2005/06.

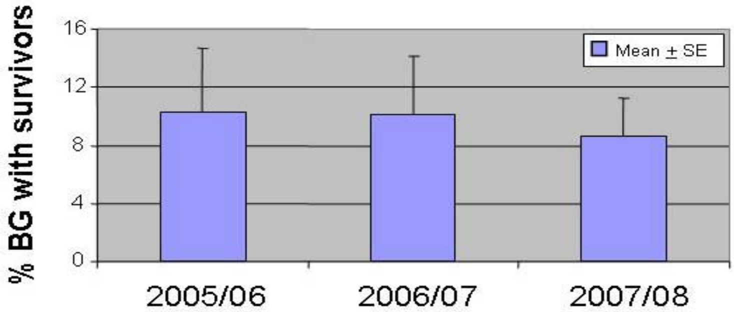

Figure 6: The percentage area of Bollgard II® accounted for by consultants that carried surviving Helicoverpa larvae at threshold levels. Data are presented for each year as an average + SE across valleys.

In 2005/06 the Gwydir (15.9), Lower Namoi (27.8) and MacIntyre (40.4) valleys achieved a percentage of Bollgard II® with surviving larvae that was above the average for that year (Figure 7a). In 2006/07 the Lower Namoi (10.4), St George (19.0), Macquarie (28.9), and Gwydir (32.3) valleys achieved a percentage of Bollgard II® with surviving larvae that was above the average for that year (Figure 7a). In 2007/08 the Upper Namoi (8.7), Macquarie (15.9), St George (18.5), and Emerald (24.8) valleys achieved a percentage of Bollgard II® with surviving larvae that was above the average for that year (Figure 7a). There is no significant variation among valleys in percentage of Bollgard II® with surviving larvae over the three study years (ANOVA with valley as the factor and year as the replicate, df=10,22, F = 1.2, P = 0.33; Figure 7b).

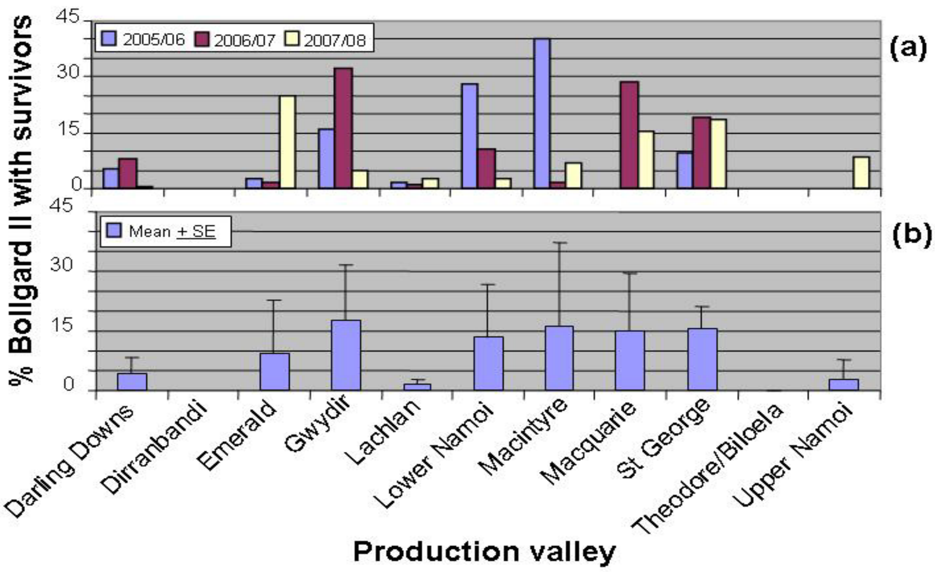

Figure 7: The percentage area of Bollgard II® accounted for by consultants that carried surviving Helicoverpa larvae at threshold levels. (a) Data are presented for each valley separately for the three years of the study. (b) Data are presented for each valley as an average + SE across the three years of the study.

In 2005/06, 2006/07 and 2007/08 the percentage of Bollgard II® with surviving larvae averaged across the four climatic regions (Tropical, Darling Downs, Middle, Cool) was 9.8 + 4.1 (range 2-26), 8.9 + 3.6 (1-15) and 8.0 + 2.6 (range 1-15) respectively. There is no statistically significant difference among years in the average percentage of Bollgard II® with surviving larvae in each region (ANOVA, df=2,27, F = 0.04, P = 0.96).

In 2005/06 the Middle (25.6) region achieved a percentage of Bollgard II® with surviving larvae that was above the average for that year (Figure 8a). In 2006/07 the Middle (15.4) and Cool (11.5) regions achieved a percentage of Bollgard II® with surviving larvae that was above the average for that year (Figure 8a). In 2007/08 the Tropical (15.4) and Cool (9.0) regions achieved a percentage of Bollgard II® with surviving larvae that was above the average for that year (Figure 8a). There is no significant variation among regions in percentage of Bollgard II® with surviving larvae over the three study years (ANOVA with region as the factor and year as the replicate, df=3,29, F = 1.0, P = 0.41; Figure 8b).

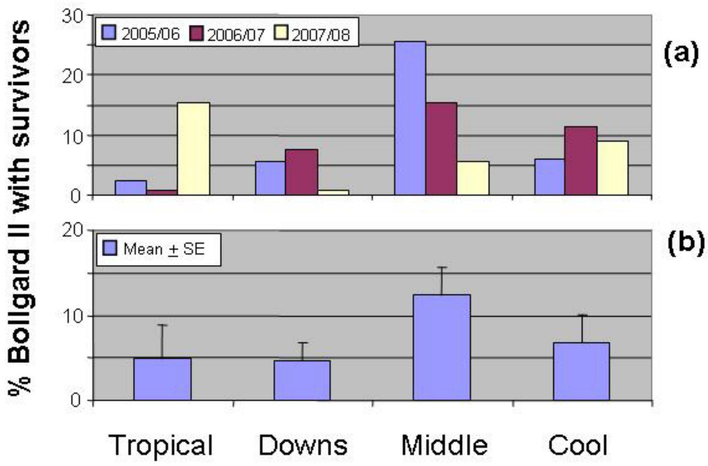

Figure 8: The percentage area of Bollgard II® accounted for by consultants that carried surviving Helicoverpa larve at threshold levels. (a) Data are presented for each climatic region separately for the three years of the study. (b) Data are presented for each climatic region as an average ± SE across the three years of the study.

Targeted sprays

In 2005/06, 2006/07 and 2007/08, the area of Bollgard II® with surviving medium to large larvae that were not treated with a spray was 13.7, 56.2 and 38.9% respectively (Figure 9). When these data are weighted to represent total production area, this equates to 4485, 5078 and 1261 Ha respectively.

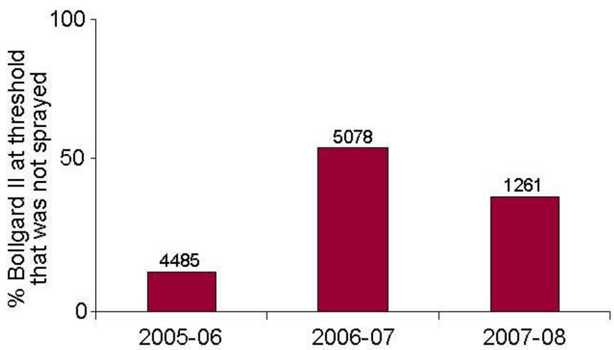

Figure 9: The percentage area of Bollgard II® accounted for by consultants that carried surviving Helicoverpa larvae at threshold levels and was not treated with a spray for Helicoverpa. Data are presented separately for the three years of the study.

Across the three year study, Bollgard II® without surviving medium to large larvae was treated with a spray only for Helicoverpa on two occasions (<0.1% of the total area). In 2005/06, 2006/07 and 2007/08, the area of Bollgard II® without surviving medium to large larvae that was treated with a spray for other pests (e.g., mirids) that also targeted Helicoverpa was 23.1, 15.4 and 13.6% respectively (Figure 10). When these data are weighted to represent total production area, this equates to 52093, 17217 and 8555 Ha respectively.

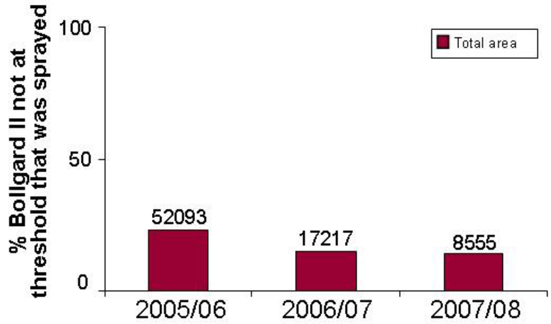

Figure 10: The percentage area of Bollgard II® accounted for by consultants that carried surviving Helicoverpa larvae at threshold levels and was not treated with a spray for Helicoverpa. Data are presented separately for the three years of the study.

(ii) Expectations of Bollgard II® performance

Throughout the industry 55% of consultants believed that up to two and a half fields in every 10 reached threshold levels of Helicoverpa (Figure 11), which is in accordance with the pre-commercialisation data reported in the Bollgard II® cotton Technical Manual (Monsanto Australia Ltd. 2005). Around 27% of consultants expected that Bollgard II® would never reach threshold levels and 18% believed that more than two and a half fields in every 10 would reach threshold levels. The consultants that selected a response that fell outside of the values indicated by pre-commercialisation data were distributed across all of the main valleys.

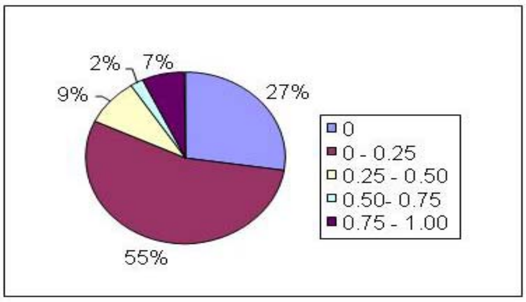

Figure 11: The responses of consultants when asked to select the number of fields that they expected to reach threshold levels of Helicoverpa throughout the industry.

The percentage of consultants whose expectation of the number of fields of Bollgard II® that carried threshold levels of Helicoverpa was increased, decreased or not changed since 2005/06 was 22, 17 and 61% (Figure 12).

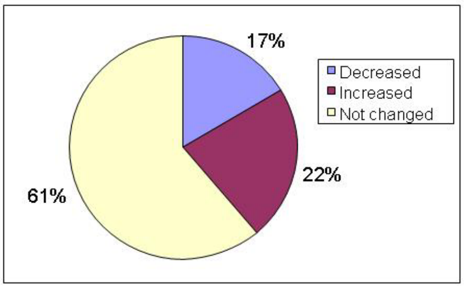

Figure 12: The number of consultants whose expectation of the number of fields with surviving larvae throughout the industry was increased, decreased or not changed since 2005/06.

Part B: Detailed case studies on fields reaching threshold in 2007/08

Of the 15 consultants that recorded threshold levels of larvae in Bollgard II® during 2007/08, 11 contributed to the subsequent questionnaire. These consultants were from five production valleys: Macquarie, St George, Lower Namoi, Upper Namoi and Emerald.

The Bollgard II® crops with surviving larvae were planted in early to mid-October except in Emerald where planting took place in mid-September. The majority of plantings were of the variety Sicot 71BRF or Sicot 71BR, although some of the plantings in St George were of the variety Sicot 289BR and Sicot 80BRF. In Emerald the crops carried most surviving larvae during late flower whilst in other regions the largest numbers of surviving larvae occurred at peak flower.

The methods used by consultants to check for *Helicoverpa* spp. in Bollgard II® varied considerably both prior and post infestation. Prior to infestation, the percentage of consultants that used visual checking only, beat sheet checking only, or a combination of beats and visual checking was 33 for each method. After infestation, the percentage of consultants that used visual checking only, beat sheet checking only, or a combination of beats and visual checking was 36, 0, and 64 for each method. Irrespective of timing, the number of checks per area ranged from 2 to 6 in every 100 ha. Survival followed high pressure but also moderate and low pressure (Figure 13). Irrespectively of egg densities, the number of medium to large larvae was capped at between 1-3/m.

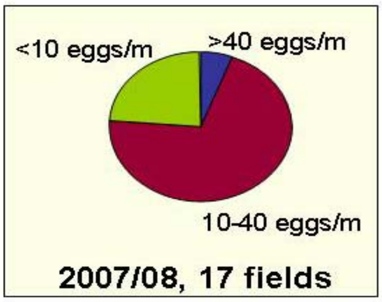

Figure 13: The number of Bollgard® II fields with surviving larvae in 2007-08 that carried larvae after low (<10 eggs/m), medium (10-40 eggs/m) or high (>40 eggs/m) egg pressure from Helicoverpa.

In all but one case the larvae were not restricted to the edge of the field. In 64% of cases larvae were described as being evenly distributed within the affected field. In 9 cases larvae were found on all structures of the plant (leaves, squares, flowers, boll caps, small bolls). Of the remaining fields only one sustained larvae exclusively on flowers. The distribution of damage within a plant closely matched the distribution of larvae on the plant. Most consultants thought that there would have been a loss of yield if an insecticide was not applied.

In 2006/07 the Bollgard II® fields that carried larvae in 2007/08 were fallow or planted to conventional cotton or Bollgard II® cotton or other typical rotation crops (i.e., sorghum, mungbean, chickpea, wheat). Plant populations in Bollgard II® fields that carried larvae ranged 8-13 plants/m. During the 2007 winter all Bollgard II® fields carrying larvae in 2007/08 were tilled to kill pupae in the soil that were overwintering from the previous season. Nine of the 11 consultants applied an insecticide to the crop two weeks prior to the problem occurring, and all of these sprays were for mirids. Most crops had an application of PIX which was applied between pre-flowering and peak flowering. None of these features were unique to fields of Bollgard II® fields that carried larvae.

Of the 16 fields that were included in the questionnaire, 2 were considered by consultants to be stressed due to water limitations or nutrient deficiencies.

Conclusion:

Bollgard II® has revolutionised the cotton industry in Australia by enabling production with significantly reduced insecticide application. Specifically, pesticide use on this product is reduced by an average of 7.1 sprays (80% of active ingredient) relative to non-Bt cotton. However, the users manual for this product states that “Bollgard II® is not *Helicoverpa* proof”, and that “high pest pressure, end of season or plant stress may necessitate pesticide application”.

The survey reported herein was conducted in response to concerns from the industry that the situations where Bollgard II® required sprays for Helicoverpa larvae were largely restricted to the St George Area and were increasing over time. We found that the presence of larvae in occasional fields of Bollgard II® is widespread among valleys and climatic regions, and did not increase between 2005/06 and 2007/08. We also found that among-valley differences in the proportion of Bollgard II® that carried larvae is not related to the area planted to Bt-cotton or the pressure imposed by Helicoverpa. Bollgard II® fields that carried larvae were not always treated with an insecticide for control.

Care must be taken when interpreting the results from data sets compiled exclusively from surveys. In particular, an insufficient response from the population may skew the results. We are confident that we obtained a robust data set that gives an unbiased representation across seasons and valleys. Forty-six consultants contributed to the survey, in each season the total area of Bollgard II® that they accounted for was at least 66% of the accredited planting of Bollgard II® provided by Monsanto, and there is no significant difference among valleys in the percentage of Bollgard II® accounted for by consultants across the three seasons.

When consultants and growers report surviving larvae in Bollgard II® fields generally they describe one of two scenarios. The first situation is where larvae develop beyond neonates into larvae that are less than 3mm in length and damage small fruit but do not develop further. Technically these situations are below threshold and hence they were not considered in this study. The second situation is where larvae reach 3mm and often continue developing beyond 8mm and damage small to medium bolls. The current threshold for Helicoverpa in Bollgard II® considers situations with several larvae between 3-8mm/m on subsequent checks to require treatment; few of the threshold levels reported in our survey were based entirely on this "small" size class. Rather, most cases of threshold levels reported herein were based on one larvae/m than was at least 8mm or a combination of several larvae between 3-8mm/m and larvae that were at least 8mm.

Our data do not support the concern from industry that the proportion of Bollgard II® that carries larvae at the current recommended threshold level is increasing over time. Indeed, from 2005/06 to 2007/08 there was a decrease in the proportion of Bollgard II® throughout the industry that carried survivors. The perceived increase in the proportion of Bollgard II® with larvae may result from a greater awareness of reports due to extension efforts, and recent articles on the issue in relevant popular science magazines. The decline in the proportion of Bollgard II® that carried larvae over time does not reflect an increase over time in "incidental" sprays (i.e., sprays for secondary pests that also target Helicoverpa). However, these sprays may effectively prevent situations where larvae may develop to threshold levels.

Our data also does not support the notion that the proportion of Bollgard II® fields that carry larvae is greater in the St George Area relative to other production regions. The proportion of Bollgard II® fields that carry larvae can vary significantly among valleys but these differences are not consistent among years. That is, valleys that experience a high proportion of Bollgard II® with larvae in one year may not experience a high proportion of Bollgard II® with larvae in other seasons. This finding holds when valleys are grouped into different climatic zones. Therefore, the incidence of Bollgard II® fields that occasionally carry larvae is widespread throughout the industry. Moreover, based on the data collected from 2005/06 to 2007/08, it is not possible to predict which valley(s) will carry the greatest proportion of larvae.

The finding that not all Bollgard II® fields with medium to large larvae are being treated is a concern for managing the development of resistance to Bt-cotton by Helicoverpa. To prolong the longevity of Bollgard II® growers must adhere to a strict resistance management program (RMP). Despite this strategy, in 2007/08 the frequency of Cry2Ab resistant alleles in *H. armigera* was significantly higher than in the previous 3 seasons, and there has been a gradual increase in Cry2Ab resistance alleles in *H. punctigera* since 2002/03.

Qualitative ELISA shows that proteins are present in the host plants of larvae that can survive on Bollgard II®, these same plants control larvae early in the season, and the development of larvae on Bollgard II® is slower than on a conventional plant. It therefore is likely that larvae surviving on Bollgard II® ingest Bt toxin at a sublethal dose. Work during 2007/08 and 2008/09 on five Bollgard II® fields that carried at least one larvae >8mm/m showed that pupation and moth emergence can occur. In theory, treating Bollgard II® crops that carry larvae will retard the evolution of Bt resistance by decreasing the number of larvae exposed to sub-lethal doses of Bt. However, even after treating Bollgard II® fields for medium to large larvae, some individuals can survive to contribute to subsequent generations.

The expectations of about half of the consultants surveyed throughout the industry matched the pre-commercialisation data reported in the Bollgard II® technical manual on the proportion of fields that may require treatment with insecticide to control Helicoverpa. Of those that did not match, around 30% expected the technology to perform better than reported. These different responses may reflect variation in direct experience with the product, including opportunities to work with Bollgard II® during the pre-commercialisation trials.

The case studies component of this survey revealed that Bollgard II® fields can carry larvae during peak to late flower after relatively high Helicoverpa egg pressure (>40 eggs/m) but this situation also can occur after relatively low Helicoverpa egg pressure (< 10 egg/m). In addition, it is common for Bollgard II® fields that sustain heavy pest pressure to provide excellent control of neonates. Clearly, while eggs must be present for larvae to develop, in many cases high pest pressure is not the only factor which results in larvae developing on some Bollgard II® hosts. Variation among valleys in Helicoverpa pressure is also not significantly related to the incidence of Bollgard II® with surviving larvae (at least in the two seasons for which data).

An obvious stressor was operating in only a few of the Bollgard II® fields that carried larvae; these stresses were nutrition or water deficiencies. In the majority of cases there were no obvious stressors and in some cases Bollgard II® that did not carry larvae was grown nearby and apparently under the same conditions as the affected fields. That is, it was not possible to indentify obvious management practices or external environmental conditions that resulted in some fields of Bollgard II® carrying larvae while others did not. This variation may reflect inherent plant physiology that affects the rate of protein production, and/or inherent genetic variation in plants, neither of which may be noticed by examining external morphology. The localised nature of the variation does not support behavioural responses of Helicoverpa to the Bt proteins as the likely mechanism, though it is possible that larvae behave differently on plants that have a lower initial level of Bt toxin. Unfortunately, because it is difficult to predict if a field of Bollgard II® will progress to carry surviving larvae, it is also difficult to make accurate Helicoverpa control decisions.

The issue of surviving larvae on Bollgard II® is a high priority for the cotton industry. This is particularly the case for crop consultants that are responsible for recommending pest control measures for this product. Current work aims to refine threshold levels in Bollgard II® with particular reference to situations where larvae potentially develop to pupae (Baoqian Lu, CCC CRC, supported by Monsanto). It is difficult to predict situations where larvae will develop on Bollgard II® in order to adequately perform the research. Nevertheless, through artificially stocking Bollgard II® plants with larvae, this work may at least identify whether a yield penalty exists at the current threshold levels.

Acknowledgments

We would like to acknowledge the significant contribution of the CCA in completing this survey. In particular, Amber Diamond assisted with distributing the survey and providing current contact details for CCA members, and John Barber and Jamie Street assisted with compiling the survey questions. We are grateful to the following CCA members who responded to our request to complete the survey: Murray Boshammer, Bernie Caffery, Geoff Rudd, Graham Boulton, Matthew Holding, Johnathon Burrell, Belinda Chase, David Parlarto, Duane Evans, Robert Holmes, Michael Stone, Sean Boland, Brad Cogan, Malcolm McNiven, Bill Cowell, Scott Rogers, Chris Maunder, Todd Peach, Brian Baird, Steve Madden, Gary Coulton, Geoff Brown, Steve Windress, Bernie Bierhoff, Alison Young, Henry Taylor, Iain Macpherson, Fred Ghirardello, Dallas King, Jim O'Connor, Michael Brosnan, Campbell Muldoon, Matthew Ward, Chris Berry, John Barber, Jamie Street, John Mulholland, Peter Haslem, Simon Struss, Rachael Brimblecombe, Warren Lang, Steve Warden, Rick Thomas, Laurie Kaelin, Hugo Weissen, Pat McGuiness, Gary Chesterfield, Matt Mitchell, Kylie Fuelling, John Fuelling, and Bill Tyrwhitt. We thank Bruce Pyke (Cotton Research and Development Corporation; CRDC), Letitia Cross and Phillip Armytage (Cotton Catchment Communities CRC; CCC CRC) for their advice on compiling the survey questions. David Larsen (CCC CRC) invested considerable time to generate a web version of the survey and forwarded results. Angus Crosson (University of Sydney) provided advice and, with permission from the CRDC, the database from the 2005/06 and 2007/08 CCA Audit data. The CCA, CCC CRC, and CSIRO financially supported the research, and the CCA provided significant in-kind contributions.

Presentations and public relations:

References:

Sprays for surviving Helicoverpa larvae in Bollgard II® survey

Gavin Whitburn (University of Queensland, CCC CRC) and Sharon Downes (CSIRO, CCC CRC)

Summary

Bollgard II® has revolutionised the cotton industry in Australia by enabling production with significantly reduced insecticide application. Specifically, pesticide use on this product is reduced by an average of 7.1 sprays (80% of active ingredient) relative to non-Bt cotton. The users manual for this product states that "Bollgard II® is not *Helicoverpa* proof", and that "high pest pressure, end of season or plant stress may necessitate pesticide application". The survey reported herein was conducted in response to concerns from the industry that the situations where Bollgard II® required sprays for Helicoverpa larvae were largely restricted to the St George Area and were increasing over time. We found that the presence of larvae in occasional fields of Bollgard II® is widespread among valleys and climatic regions, and did not increase between 2005/06 and 2007/08. We also found that among-valley differences in the proportion of Bollgard II® that carried larvae is not related to the area planted to this product or the pressure imposed by Helicoverpa. Bollgard II® fields that carried medium to large larvae were not always treated with an insecticide for control, and the potential implications of this situation for managing resistance to Bt are discussed.

Introduction

The lepidopteran *Helicoverpa armigera* is distributed throughout the Old World where it is the major pest on numerous crops, particularly cotton. It poses the principal threat to transgenic cotton in Australia, India and China because of its recidivist nature in evolving resistance to conventional insecticides. The capacity of this species to develop resistance to a Bt toxin (Cry1Ac) is evident from experiments in Australia, India, and China where field-collected populations challenged by selection in the laboratory developed high levels of resistance. In contrast, the Australian endemic species *H. punctigera* has not shown a propensity to evolve resistance to conventional insecticides. This may reflect the different dispersal ecology and population dynamics of the two species. Regardless of the mechanism, and despite both species being innately tolerant to Bt toxins, these different histories in developing resistance to insecticides renders *H. armigera* and *H. punctigera* a relatively high and low risk respectively at developing resistance to Bt cotton.

Bt cotton varieties known as Ingard® in the New World (Bollgard elsewhere) were released for commercial production in Australia in the 1995/96 season. With this technology growers were able to move away from a Helicoverpa control regime that often required considerable applications of insecticides. Ingard® expressed the *cry1Ac* gene produced by *Bacillus thuringiensis* which is toxic to Lepidoptera species. Whilst younger plants effectively controlled larvae, a decline in toxin expression later in the season meant that during this period insecticide use was necessary following egg lays by Helicoverpa. In 2004/05 Ingard® was superseded by Bollgard II®, which expresses Cry2Ab throughout the growing period in addition to the original Cry1Ac event which declined over time. This dual toxin technology is expected to provide growers with season long protection against Helicoverpa, although it is generally accepted that later in the season only the *cry2Ab* gene may be effective in this control.

Bollgard II® has been widely adopted within Australia with around 80% of industry growing the technology. From the outset there have been reports of Helicoverpa larvae surviving, and often developing to the medium-large size class, in some fields of Bollgard II®. In many situations larvae reach numbers which exceed the recommended threshold levels for Bollgard II®, and are too large to adequately control using some conventional insecticides. In these situations the growers are primarily concerned with the economic damage caused by the larvae. However, there may also be implications of this situation for resistance management.

A relatively high baseline frequency of alleles conferring resistance to Cry2Ab exists in *H. armigera* and *H. punctigera*. However, currently there is no evidence that Helicoverpa larvae are able to survive on Bollgard II® host plants due to Bt resistance. In particular, for both Helicoverpa species there is no difference in the proportion of individuals carrying Cry2Ab resistance genes in a random sample versus larvae collected from Bollgard II® host plants. Moreover, the Cry2Ab resistance genes isolated for populations of Australian Helicoverpa are recessive and all individuals found to carry this gene were heterozygotes (i.e., they should have been killed by any toxin in the plant). Bollgard II® host plants control the larvae early in the season and contain the *cry1Ac* and *cry2Ab*. It is therefore likely that, irrespective of the mechanism allowing survival, the larvae are being exposed to a sub-lethal dose of one or both of the toxins. This condition is a major driver of the evolution of resistance.

Preliminary reports suggested that the occurrence of fields with survivors was restricted to the growing region of St George in south western Queensland. There is also a perception throughout the industry that the frequency of fields with this problem may be increasing over time. A major aim of our work was to investigate these perceptions by examining the frequency with which surviving larvae were found at or above threshold levels throughout the Australian cotton industry for the seasons 2005/06, 2006/07 and 2008/09. From the perspective of resistance management we were also interested in gauging the proportion of fields which carried larvae that were treated with a conventional insecticide. In a follow up questionnaire we gathered basic information on broad environmental factors that may be used to predict whether a field would carry larvae.

Methods

Part A: Broad overview

The survey entitled 'Sprays for surviving Helicoverpa in Bollgard II®' was launched on the Cotton Catchment Communities CRC website on 1st April 2008 with a deadline of 21st April 2008. A list of active members from the Crop Consultants Association (previously Cotton Consultants Association; hereafter CCA) was obtained from the Chief Executive Officer. These consultants were contacted by email and requested twice to respond to the survey. The response to this initial request was poor with few consultants completing the survey.

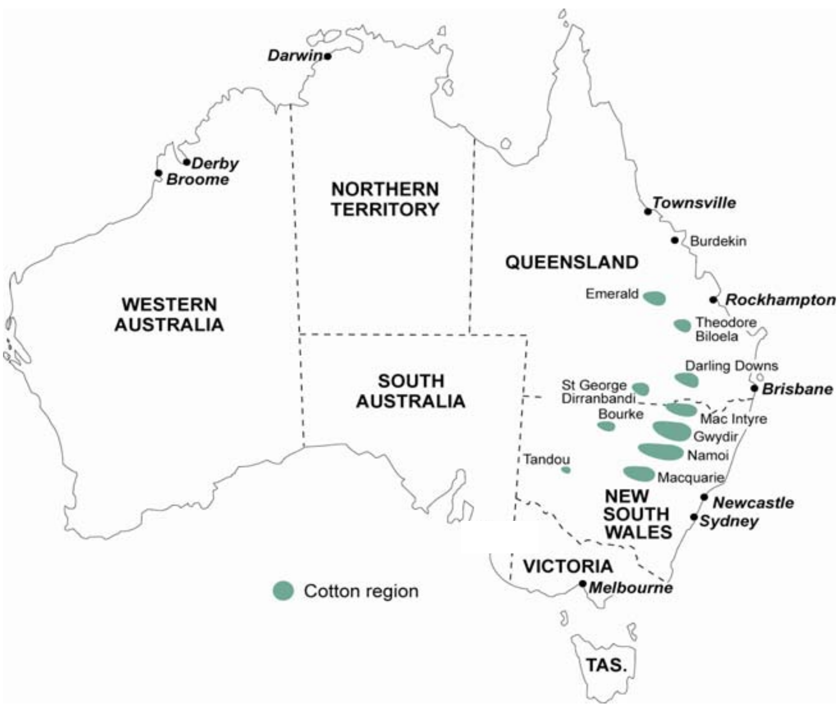

Figure 1: Map of Australia showing the main cotton regions sampled in the study.

On the 30th July 2008 we issued a further email request to consultants to complete the survey, followed by a vigorous telephone and email campaign. Although consultants were assured that responses would remain confidential, they were asked to provide their name so as to be removed from the list of members yet to complete the survey. Consultants were also asked to nominate which of the main cotton growing regions they were working within (see Figure 1) and complete separate surveys if they were working in more than one valley.

The survey covered the period 2005-2007. Sixty three consultants were asked to respond. We received 45 responses with 14 consultants failing to respond and four consultants replying that they were unable to complete the survey. Figure 2 shows for each season the total area of Bollgard II® accounted for by consultants that responded to the survey versus data supplied by Monsanto on the accredited planting of Bollgard II®.

The accredited planting of Bollgard II® reduced over time with approximately 230000, 114000, and 61000 hectares planted in 2005/06, 2006/07 and 2007/08 respectively (see Figure 2). When compared to the accredited planting the percentage of Bollgard II® accounted for by consultants summed across years and valleys was 71. When considering the data for 2005/06, 2006/07, and 2007/08 separately, the percentage of Bollgard II® accounted for by consultants summed across the valleys was 66% (range 32-100, mean = 63), 79% (range = 42-100, mean = 66) and 73% (range = 47-100, mean = 65) respectively. There was no significant difference among valleys in the percentage of Bollgard II® accounted for by consultants (ANOVA, valley as factor and year as replicate, df = 10,21, F = 1.3, P = 0.27). We therefore assume that the dataset gives an unbiased representation across seasons and valleys.

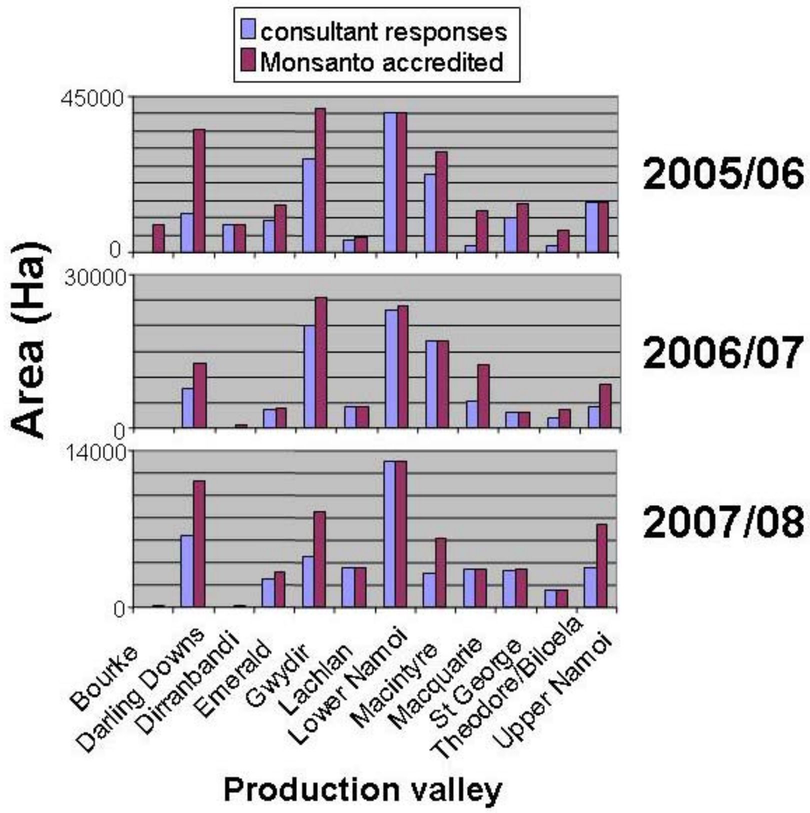

Figure 2: The total area of Bollgard II® accounted for by consultants that responded to the survey versus data supplied by Monsanto on the accredited planting of Bollgard II®.

Since we did not account for any of the Bollgard II® planted in Bourke in 2005/06 (125 ha), 2006/07 (13 ha) and 2007/08 (8000 ha), this valley was not included in subsequent data analyses. We did not account for any of the Bollgard II® planted in Dirranbandi in 2007/08 (67 ha) and 2006/07 (494 ha) respectively, therefore we included data from this valley for 2005/06 only in subsequent analyses.

(i) Extent and distribution of the problem and targeted sprays

The main aim of Part A of the survey was to ascertain the proportion of Bollgard II® with surviving larvae throughout the industry and among the different cotton regions. We defined "Bollgard II® with surviving larvae" as the area that reached the recommended threshold for applying a spray (also the threshold determined for Ingard® cotton): at least 2 larvae 3-8 mm/m in at least two consecutive checks or 1 larvae > 8mm/m. We also wanted to determine the proportion of Bollgard II® with surviving larvae throughout the industry that received at least one spray for Helicoverpa. Finally, we were interested in whether these proportions changed through time.

To obtain this information we asked the consultants to answer the following questions separately for the 2005/06, 2006/07 and 2007/08 cotton seasons:

1. How many hectares of Bollgard II® did you inspect in your role as a consultant?
2. How many hectares of Bollgard II® carried at least 2 larvae 3-8 mm/m in at least two consecutive checks or 1 larvae > 8mm/m?
3. How many hectares of Bollgard II® did you spray at least once for larvae
   (a) what area was sprayed only for Helicoverpa?
   (b) what area was sprayed for other pests (e.g., mirids) that also targeted Helicoverpa?

We calculated the percentage of Bollgard II® with surviving larvae by dividing the area of Bollgard II® that carried at least 2 larvae 3-8 mm/m in at least two consecutive checks or 1 larvae > 8mm/m by the area of Bollgard II® accounted for by consultants.

For the analyses looking at climatic region as a factor, we grouped the valleys as follows: Tropical (Emerald, Theodore), Darling Downs (Darling Downs), Middle (Lower Namoi, St George, Dirranbandi, MacIntyre, Gwydir) and Cool (Upper Namoi, Macquarie, Lachlan).

Subsequent to the survey, it became apparent that in a few cases threshold levels were reached based on small larvae only. In terms of sprays for Helicoverpa, we were interested in cases where larvae could potentially contribute to subsequent generations. Medium to large larvae in Bollgard II® fields can survive to pupation and successfully emerge in the laboratory, but this may not be the case for small larvae. Therefore, we excluded from the analyses of sprays for Helicoverpa the few cases were threshold was based entirely on the survival of small larvae. This is a conservative approach since even after applying a spray some small larvae may have developed. If the information was not available through comments taken by phone or in the box provided at the end of the survey, we contacted consultants that had larvae at threshold levels and determined whether these situations comprised only small larvae.

In the analyses incorporating information on Bollgard II® sprayed for Helicoverpa we refer to the sum of hectares provided in response to question 3a and 3b as "hectares of Bollgard II® sprayed at least once for Helicoverpa larvae".

For the analyses looking at the area of Bollgard II® with surviving larvae that was not treated with a spray, for each consultant we subtracted the area sprayed at least once for Helicoverpa from the total area at threshold. For each year, we summed this area and divided it by the total area for that year that was at threshold.

When presenting the data as "area" at threshold or sprayed, we multiplied the accredited planting area by the proportional data obtained from consultants to estimate the total production area affected (hereafter referred to as 'weighted' data).

Although there was variation in the area of Bollgard II® grown among valleys, this factor was not significantly related to the percentage of Bollgard II® with surviving larvae ($r^2 < 0.20$, $P > 0.21$; Figure 3). We therefore used percentage values to compare the incidence of Bollgard II® with surviving larvae among valleys.

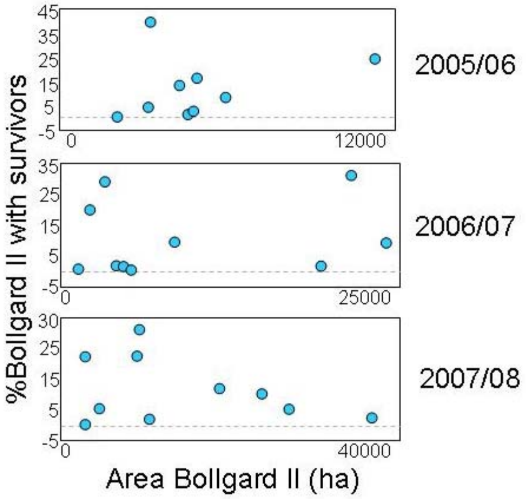

Figure 3: The percentage area of Bollgard II® accounted for by consultants that carried surviving Helicoverpa larvae at threshold levels and its relationship to the area of Bollgard II® planted. Data are presented separately for the three years of the study.

In order to determine whether it was necessary to weight our data according to Helicoverpa pressure we used the CCA Post Season Survey data (Doyle and Coleman 2005/06; Western Research Institute 2006/07) to examine the relationship between incidence of Bollgard II® with surviving larvae and the average number of sprays for Helicoverpa on conventional cotton fields in each valley. Data were available for the 2005/06 and 2006/07 seasons only. In both of these seasons a simple regression analysis determined no significant relationship ($r^2 < 0.32$, $P > 0.11$) between the percentage of Bollgard II® with surviving larvae versus sprays on conventional cotton (Figure 4), and we assumed that this was also the case for 2007/08. Consequently we deemed that it was not necessary to weight our data according to Helicoverpa pressure.

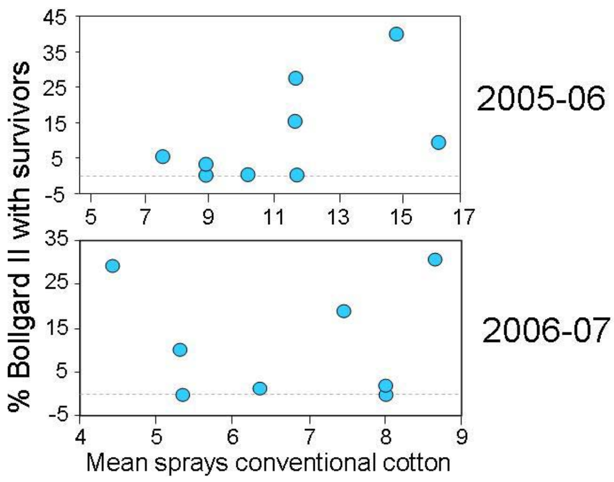

Figure 4: The relationship between percentage area of Bollgard II® accounted for by consultants that carried surviving Helicoverpa larvae versus the average number of sprays for Helicoverpa in conventional cotton fields. Each point represents one valley. Data are presented separately for 2005-06 and 2006-07.

(ii) Expectations of Bollgard II® performance

The secondary aim of Part A of the survey was to ascertain how consultants expected Bollgard II® to perform in controlling larvae and determine whether this expectation changed with several years experience of the technology. To obtain this information we asked the consultants to answer the following questions by selecting a response from multiple choices:

1. On average throughout the industry what do you currently expect the number of Bollgard II® fields that reach threshold levels of Helicoverpa larvae to be?: 0, 0-0.25, 0.25-0.50, 0.50-0.75, 0.75-1.00.
2. Has this expectation changed since 2005/06?: Increased, Decreased, Not changed

In asking the first question we assumed that consultants would select a response that closely matched their experience with the technology during the previous three seasons. The second question therefore was designed to gauge their expectation at the outset of the products availability.

Part B: Detailed case studies on fields reaching threshold in 2007/08

Following Part A of the survey, a second questionnaire was forwarded to consultants who had Bollgard II® with surviving larvae in 2007/08. The questionnaire was designed to gather data on factors that may influence the occurrence of Helicoverpa survival in Bollgard II®, and was based on a survey compiled by CSIRO and Monsanto prior to the 2007/08 season.

The questions were designed to obtain information on checking methods, crop stage, extent of damage by larvae, and the location of damage within the plant. The second part of the questionnaire investigated the environmental conditions the crop was subject to, including previous crop planted in that field, tillage operations (pupae busting), plant population density, herbicide and insecticide application leading up to surviving larvae, as well as soil and nutritional conditions. If relevant, the consultants answered the same questions on environmental conditions for Bollgard II® fields that did not carry larvae but were at the same crop stage as the field(s) with survivors. These results are presented mainly in a descriptive format. The complete questionnaire is included as an appendix to this report.

Results

Part A: Broad overview

Extent and distribution of Bollgard II® fields with surviving larvae

When the data are summed across years and valleys 15% of the Bollgard II® accounted for by the consultants carried surviving larvae at threshold levels. In 2005/06, 2006/07, and 2007/08 the percentage of Bollgard II® with surviving larvae summed across valleys was 18, 13, and 7 respectively (Figure 5). The interaction between season and the total area of Bollgard II® with and without surviving larvae is statistically significant (Fishers Exact Test, df=2, X^2 = 3500, P = 0.001).

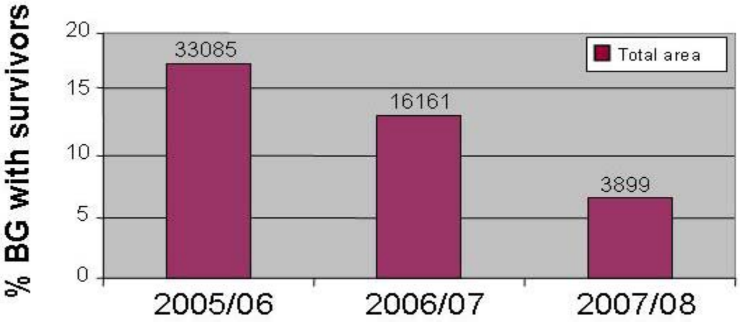

Figure 5: The percentage area of Bollgard II® accounted for by consultants that carried surviving Helicoverpa larvae at threshold levels. Data are presented for each year as a total across all valleys and the values at the top of each bar is the weighted total are of Bollgard II® that was at threshold.

In 2005/06, 2006/07, and 2007/08 the percentage of Bollgard II® with surviving larvae averaged across valleys was 10.3 ± 4.4 (range 0-40), 10.2 ± 3.9 (0-32) and 8.6 ± 2.7 (range 0-25) respectively (Figure 6). There is no statistically significant difference among years in the average percentage of Bollgard II® with surviving larvae in each valley (ANOVA, df=2,27, F = 0.07, P = 0.93). This result largely reflects relatively high percentage levels of Bollgard II® with surviving larvae in a few valleys with relatively small plantings in 2007/08. Although for each study year there is no significant relationship between area planted to Bollgard II® and the percentage of area with surviving larvae (in all cases, $R < 0.42$), the relationship is negative in 2007/08 versus positive in 2006/07 and 2005/06.

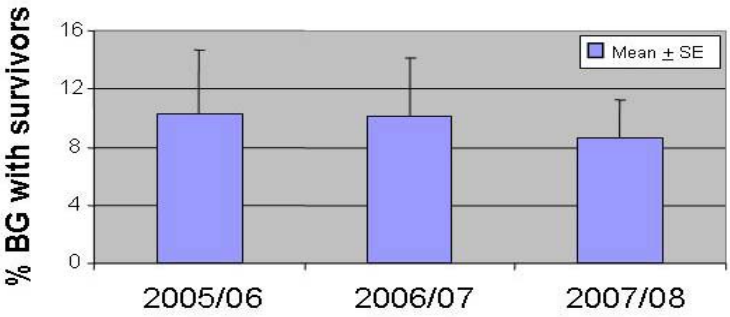

Figure 6: The percentage area of Bollgard II® accounted for by consultants that carried surviving Helicoverpa larvae at threshold levels. Data are presented for each year as an average ± SE across valleys.

In 2005/06 the Gwydir (15.9), Lower Namoi (27.8) and MacIntyre (40.4) valleys achieved a percentage of Bollgard II® with surviving larvae that was above the average for that year (Figure 7a). In 2006/07 the Lower Namoi (10.4), St George (19.0), Macquarie (28.9), and Gwydir (32.3) valleys achieved a percentage of Bollgard II® with surviving larvae that was above the average for that year (Figure 7a). In 2007/08 the Upper Namoi (8.7), Macquarie (15.9), St George (18.5), and Emerald (24.8) valleys achieved a percentage of Bollgard II® with surviving larvae that was above the average for that year (Figure 7a). There is no significant variation among valleys in percentage of Bollgard II® with surviving larvae over the three study years (ANOVA with valley as the factor and year as the replicate, df=10,22, F = 1.2, P = 0.33; Figure 7b).

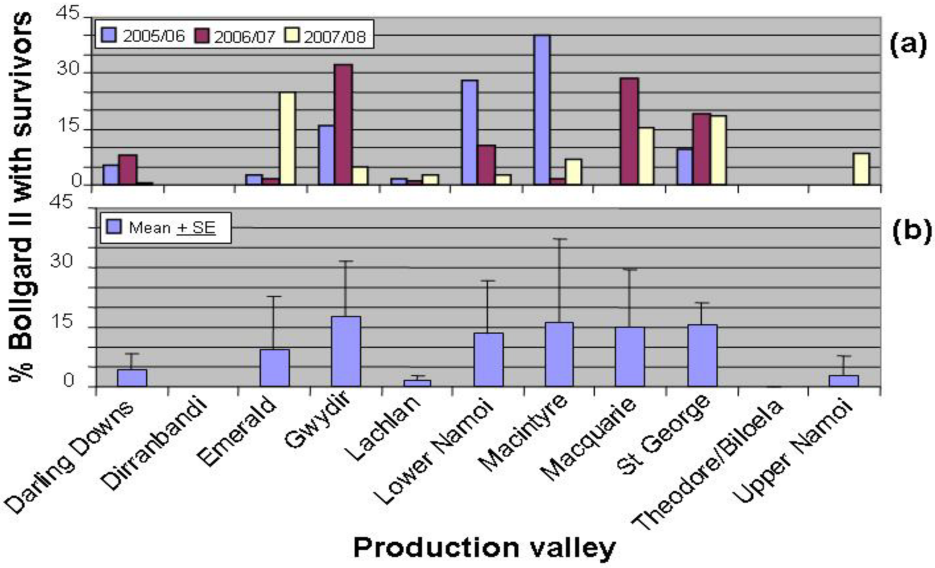

Figure 7: The percentage area of Bollgard II® accounted for by consultants that carried surviving Helicoverpa larvae at threshold levels. (a) Data are presented for each valley separately for the three years of the study. (b) Data are presented for each valley as an average ± SE across the three years of the study.

In 2005/06, 2006/07 and 2007/08 the percentage of Bollgard II® with surviving larvae averaged across the four climatic regions (Tropical, Darling Downs, Middle, Cool) was 9.8 ± 4.1 (range 2-26), 8.9 ± 3.6 (1-15) and 8.0 ± 2.6 (range 1-15) respectively. There is no statistically significant difference among years in the average percentage of Bollgard II® with surviving larvae in each region (ANOVA, df=2,27, F = 0.04, P = 0.96).

In 2005/06 the Middle (25.6) region achieved a percentage of Bollgard II® with surviving larvae that was above the average for that year (Figure 8a). In 2006/07 the Middle (15.4) and Cool (11.5) regions achieved a percentage of Bollgard II® with surviving larvae that was above the average for that year (Figure 8a). In 2007/08 the Tropical (15.4) and Cool (9.0) regions achieved a percentage of Bollgard II® with surviving larvae that was above the average for that year (Figure 8a). There is no significant variation among regions in percentage of Bollgard II® with surviving larvae over the three study years (ANOVA with region as the factor and year as the replicate, df=3,29, F = 1.0, P = 0.41; Figure 8b).

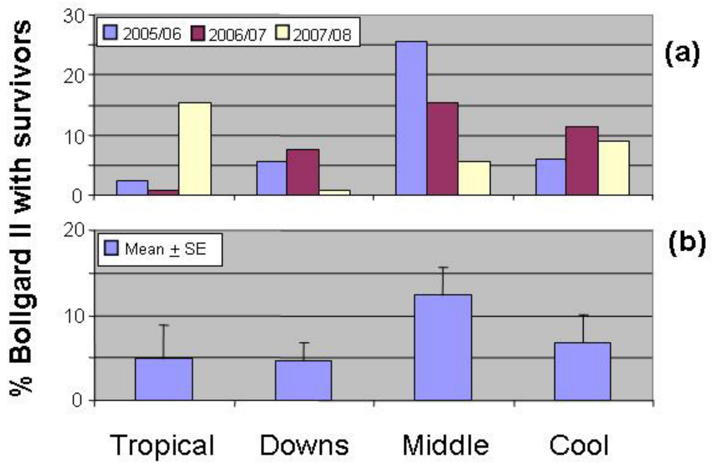

Figure 8: The percentage area of Bollgard II® accounted for by consultants that carried surviving Helicoverpa larve at threshold levels. (a) Data are presented for each climatic region separately for the three years of the study. (b) Data are presented for each climatic region as an average ± SE across the three years of the study.

Targeted sprays

In 2005/06, 2006/07 and 2007/08, the area of Bollgard II® with surviving medium to large larvae that were not treated with a spray was 13.7, 56.2 and 38.9% respectively (Figure 9). When these data are weighted to represent total production area, this equates to 4485, 5078 and 1261 Ha respectively.

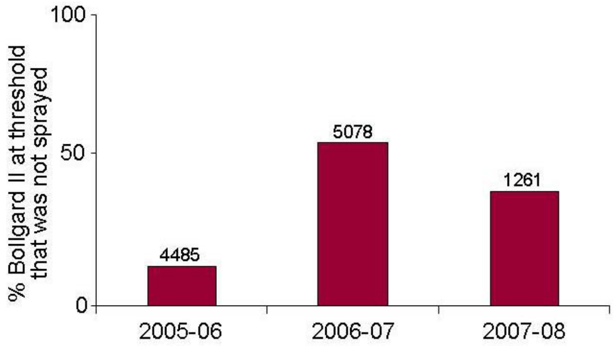

Figure 9: The percentage area of Bollgard II® accounted for by consultants that carried surviving Helicoverpa larvae at threshold levels and was not treated with a spray for Helicoverpa. Data are presented separately for the three years of the study.

Across the three year study, Bollgard II® without surviving medium to large larvae was treated with a spray only for Helicoverpa on two occasions (<0.1% of the total area). In 2005/06, 2006/07 and 2007/08, the area of Bollgard II® without surviving medium to large larvae that was treated with a spray for other pests (e.g., mirids) that also targeted Helicoverpa was 23.1, 15.4 and 13.6% respectively (Figure 10). When these data are weighted to represent total production area, this equates to 52093, 17217 and 8555 Ha respectively.

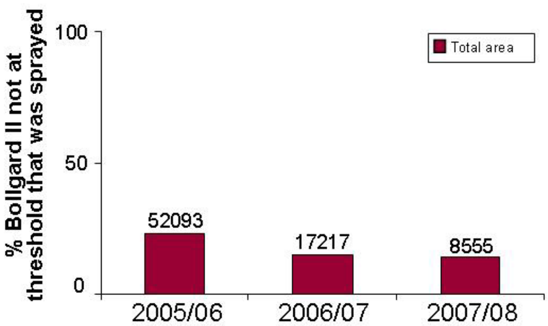

Figure 10: The percentage area of Bollgard II® accounted for by consultants that carried surviving Helicoverpa larvae at threshold levels and was not treated with a spray for Helicoverpa. Data are presented separately for the three years of the study.

(ii) Expectations of Bollgard II® performance

Throughout the industry 55% of consultants believed that up to two and a half fields in every 10 reached threshold levels of Helicoverpa (Figure 11), which is in accordance with the pre-commercialisation data reported in the Bollgard II® cotton Technical Manual (Monsanto Australia Ltd. 2005). Around 27% of consultants expected that Bollgard II® would never reach threshold levels and 18% believed that more than two and a half fields in every 10 would reach threshold levels. The consultants that selected a response that fell outside of the values indicated by pre-commercialisation data were distributed across all of the main valleys.

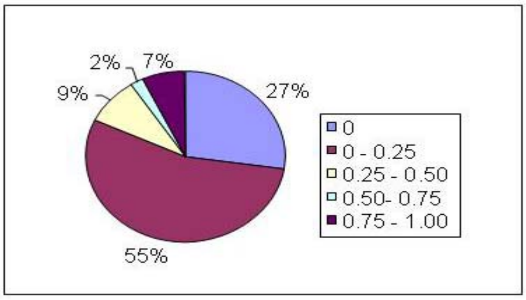

Figure 11: The responses of consultants when asked to select the number of fields that they expected to reach threshold levels of Helicoverpa throughout the industry.

The percentage of consultants whose expectation of the number of fields of Bollgard II® that carried threshold levels of Helicoverpa was increased, decreased or not changed since 2005/06 was 22, 17 and 61% (Figure 12).

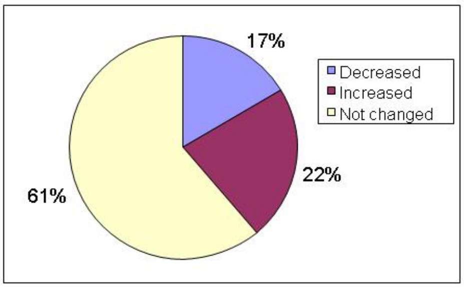

Figure 12: The number of consultants whose expectation of the number of fields with surviving larvae throughout the industry was increased, decreased or not changed since 2005/06.

Part B: Detailed case studies on fields reaching threshold in 2007/08

Of the 15 consultants that recorded threshold levels of larvae in Bollgard II® during 2007/08, 11 contributed to the subsequent questionnaire. These consultants were from five production valleys: Macquarie, St George, Lower Namoi, Upper Namoi and Emerald.

The Bollgard II® crops with surviving larvae were planted in early to mid-October except in Emerald where planting took place in mid-September. The majority of plantings were of the variety Sicot 71BRF or Sicot 71BR, although some of the plantings in St George were of the variety Sicot 289BR and Sicot 80BRF. In Emerald the crops carried most surviving larvae during late flower whilst in other regions the largest numbers of surviving larvae occurred at peak flower.

The methods used by consultants to check for *Helicoverpa* spp. in Bollgard II® varied considerably both prior and post infestation. Prior to infestation, the percentage of consultants that used visual checking only, beat sheet checking only, or a combination of beats and visual checking was 33 for each method. After infestation, the percentage of consultants that used visual checking only, beat sheet checking only, or a combination of beats and visual checking was 36, 0, and 64 for each method. Irrespective of timing, the number of checks per area ranged from 2 to 6 in every 100 ha. Survival followed high pressure but also moderate and low pressure (Figure 13). Irrespectively of egg densities, the number of medium to large larvae was capped at between 1-3/m.

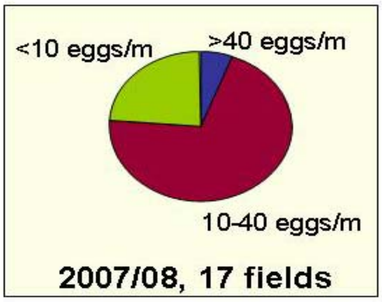

Figure 13: The number of Bollgard® II fields with surviving larvae in 2007-08 that carried larvae after low (<10 eggs/m), medium (10-40 eggs/m) or high (>40 eggs/m) egg pressure from Helicoverpa.

In all but one case the larvae were not restricted to the edge of the field. In 64% of cases larvae were described as being evenly distributed within the affected field. In 9 cases larvae were found on all structures of the plant (leaves, squares, flowers, boll caps, small bolls). Of the remaining fields only one sustained larvae exclusively on flowers. The distribution of damage within a plant closely matched the distribution of larvae on the plant. Most consultants thought that there would have been a loss of yield if an insecticide was not applied.

In 2006/07 the Bollgard II® fields that carried larvae in 2007/08 were fallow or planted to conventional cotton or Bollgard II® cotton or other typical rotation crops (i.e., sorghum, mungbean, chickpea, wheat). Plant populations in Bollgard II® fields that carried larvae ranged 8-13 plants/m. During the 2007 winter all Bollgard II® fields carrying larvae in 2007/08 were tilled to kill pupae in the soil that were overwintering from the previous season. Nine of the 11 consultants applied an insecticide to the crop two weeks prior to the problem occurring, and all of these sprays were for mirids. Most crops had an application of PIX which was applied between pre-flowering and peak flowering. None of these features were unique to fields of Bollgard II® fields that carried larvae.

Of the 16 fields that were included in the questionnaire, 2 were considered by consultants to be stressed due to water limitations or nutrient deficiencies.

Discussion

Bollgard II® has revolutionised the cotton industry in Australia by enabling production with significantly reduced insecticide application. Specifically, pesticide use on this product is reduced by an average of 7.1 sprays (80% of active ingredient) relative to non-Bt cotton. However, the users manual for this product states that “Bollgard II® is not *Helicoverpa* proof”, and that “high pest pressure, end of season or plant stress may necessitate pesticide application”.

The survey reported herein was conducted in response to concerns from the industry that the situations where Bollgard II® required sprays for Helicoverpa larvae were largely restricted to the St George Area and were increasing over time. We found that the presence of larvae in occasional fields of Bollgard II® is widespread among valleys and climatic regions, and did not increase between 2005/06 and 2007/08. We also found that among-valley differences in the proportion of Bollgard II® that carried larvae is not related to the area planted to Bt-cotton or the pressure imposed by Helicoverpa. Bollgard II® fields that carried larvae were not always treated with an insecticide for control.

Care must be taken when interpreting the results from data sets compiled exclusively from surveys. In particular, an insufficient response from the population may skew the results. We are confident that we obtained a robust data set that gives an unbiased representation across seasons and valleys. Forty-six consultants contributed to the survey, in each season the total area of Bollgard II® that they accounted for was at least 66% of the accredited planting of Bollgard II® provided by Monsanto, and there is no significant difference among valleys in the percentage of Bollgard II® accounted for by consultants across the three seasons.

When consultants and growers report surviving larvae in Bollgard II® fields generally they describe one of two scenarios. The first situation is where larvae develop beyond neonates into larvae that are less than 3mm in length and damage small fruit but do not develop further. Technically these situations are below threshold and hence they were not considered in this study. The second situation is where larvae reach 3mm and often continue developing beyond 8mm and damage small to medium bolls. The current threshold for Helicoverpa in Bollgard II® considers situations with several larvae between 3-8mm/m on subsequent checks to require treatment; few of the threshold levels reported in our survey were based entirely on this “small” size class. Rather, most cases of threshold levels reported herein were based on one larvae/m than was at least 8mm or a combination of several larvae between 3-8mm/m and larvae that were at least 8mm.

Our data do not support the concern from industry that the proportion of Bollgard II® that carries larvae at the current recommended threshold level is increasing over time. Indeed, from 2005/06 to 2007/08 there was a decrease in the proportion of Bollgard II® throughout the industry that carried survivors. The perceived increase in the proportion of Bollgard II® with larvae may result from a greater awareness of reports due to extension efforts, and recent articles on the issue in relevant popular science magazines. The decline in the proportion of Bollgard II® that carried larvae over time does not reflect an increase over time in “incidental” sprays (i.e., sprays for secondary pests that also target Helicoverpa). However, these sprays may effectively prevent situations where larvae may develop to threshold levels.

Our data also does not support the notion that the proportion of Bollgard II® fields that carry larvae is greater in the St George Area relative to other production regions. The proportion of Bollgard II® fields that carry larvae can vary significantly among valleys but these differences are not consistent among years. That is, valleys that experience a high proportion of Bollgard II® with larvae in one year may not experience a high proportion of Bollgard II® with larvae in other seasons. This finding holds when valleys are grouped into different climatic zones. Therefore, the incidence of Bollgard II® fields that occasionally carry larvae is widespread throughout the industry. Moreover, based on the data collected from 2005/06 to 2007/08, it is not possible to predict which valley(s) will carry the greatest proportion of larvae.

The finding that not all Bollgard II® fields with medium to large larvae are being treated is a concern for managing the development of resistance to Bt-cotton by Helicoverpa. To prolong the longevity of Bollgard II® growers must adhere to a strict resistance management program (RMP). Despite this strategy, in 2007/08 the frequency of Cry2Ab resistant alleles in *H. armigera* was significantly higher than in the previous 3 seasons, and there has been a gradual increase in Cry2Ab resistance alleles in *H. punctigera* since 2002/03.

Qualitative ELISA shows that proteins are present in the host plants of larvae that can survive on Bollgard II®, these same plants control larvae early in the season, and the development of larvae on Bollgard II® is slower than on a conventional plant. It therefore is likely that larvae surviving on Bollgard II® ingest Bt toxin at a sublethal dose. Work during 2007/08 and 2008/09 on five Bollgard II® fields that carried at least one larvae >8mm/m showed that pupation and moth emergence can occur. In theory, treating Bollgard II® crops that carry larvae will retard the evolution of Bt resistance by decreasing the number of larvae exposed to sub-lethal doses of Bt. However, even after treating Bollgard II® fields for medium to large larvae, some individuals can survive to contribute to subsequent generations.

The expectations of about half of the consultants surveyed throughout the industry matched the pre-commercialisation data reported in the Bollgard II® technical manual on the proportion of fields that may require treatment with insecticide to control Helicoverpa. Of those that did not match, around 30% expected the technology to perform better than reported. These different responses may reflect variation in direct experience with the product, including opportunities to work with Bollgard II® during the pre-commercialisation trials.

The case studies component of this survey revealed that Bollgard II® fields can carry larvae during peak to late flower after relatively high Helicoverpa egg pressure (>40 eggs/m) but this situation also can occur after relatively low Helicoverpa egg pressure (< 10 egg/m). In addition, it is common for Bollgard II® fields that sustain heavy pest pressure to provide excellent control of neonates. Clearly, while eggs must be present for larvae to develop, in many cases high pest pressure is not the only factor which results in larvae developing on some Bollgard II® hosts. Variation among valleys in Helicoverpa pressure is also not significantly related to the incidence of Bollgard II® with surviving larvae (at least in the two seasons for which data).

An obvious stressor was operating in only a few of the Bollgard II® fields that carried larvae; these stresses were nutrition or water deficiencies. In the majority of cases there were no obvious stressors and in some cases Bollgard II® that did not carry larvae was grown nearby and apparently under the same conditions as the affected fields. That is, it was not possible to indentify obvious management practices or external environmental conditions that resulted in some fields of Bollgard II® carrying larvae while others did not. This variation may reflect inherent plant physiology that affects the rate of protein production, and/or inherent genetic variation in plants, neither of which may be noticed by examining external morphology. The localised nature of the variation does not support behavioural responses of Helicoverpa to the Bt proteins as the likely mechanism, though it is possible that larvae behave differently on plants that have a lower initial level of Bt toxin. Unfortunately, because it is difficult to predict if a field of Bollgard II® will progress to carry surviving larvae, it is also difficult to make accurate Helicoverpa control decisions.

The issue of surviving larvae on Bollgard II® is a high priority for the cotton industry. This is particularly the case for crop consultants that are responsible for recommending pest control measures for this product. Current work aims to refine threshold levels in Bollgard II® with particular reference to situations where larvae potentially develop to pupae (Baoqian Lu, CCC CRC, supported by Monsanto). It is difficult to predict situations where larvae will develop on Bollgard II® in order to adequately perform the research. Nevertheless, through artificially stocking Bollgard II® plants with larvae, this work may at least identify whether a yield penalty exists at the current threshold levels.

Acknowledgments

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Larvae on Bollgard II® Post Season Survey

CCA
CSIRO
MONSANTO imagine®

Date: **Consultant*:** **Phone no:**

Property: **Field No:** **Variety:**

Planting Date: **Crop Stage:** **Crop height:**

This survey is confidential but we would like the opportunity to clarify answers if necessary.

Section 1: Larvae and damage **How do you "check" your Bollgard II prior to a problem?**	Beats only Visuals only Combination Other No samples/ha
How do you "check" your Bollgard II during a problem?	Beats only Visuals only Combination Other No samples/ha
Score the egg pressure leading up to the problem. If you conduct egg counts please provide details.	Low (<10/m) Medium (10-40/m) High (>40/m) Egg count:
If visuals, where on the plants did you find larvae?	leaves/squares/flowers/boll caps/small bolls/large bolls
Were most (>80%) larvae within 50 m of the field edge?	Yes No
Were the larvae evenly distributed throughout the field?	Yes No
Was there any damage? **If yes, where did you see damage?**	Yes No leaves/squares/flowers/boll caps/small bolls/large bolls
Did you spray for Helicoverpa?	Yes No
If yes, do you think there would have been yield loss due to damage if you did not spray? If no, do you think there was yield loss due to damage?	Yes No
Section 2: Environmental conditions	
When and what was the previous crop in this field? If it was cotton please note the trait/conventional.	Crop: Planted (month/year): BGII/ Stack/ Conventional
Was the field pupae busted to 10 cm in winter 2007?	Yes No Yes but < 10 cm
What was the plant population density?	___plants/m of row
Was herbicide applied in the 2 weeks prior to larvae?	Yes (active:) No
Was an insecticide applied in the 2 weeks prior to larvae?	Yes (active:) No
Due to soil type is the field prone to water-logging?	Yes No
Due to slope is the field prone to water-logging?	Yes No
Was the irrigation interval increased at any time to stretch water supply?	Yes No
Was PIX applied? **If so, what was the rate and timing?**	Yes No Rate: Date: Crop stage:
Were there any fields at the same crop stage that were unaffected?	Yes No If yes please fill in survey for that field.

Comments:_e.g. # beneficials/m____

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**************** Please fax to Gavin Whitburn and Sharon Downes CSIRO 02 67931186****************