

End of Season results from CSIRO Bt resistance monitoring 2008/09

Hatching and species composition

Across all sampled valleys there were 34248 eggs or larvae submitted to the main program in 2008/09. The majority of collections were eggs, although there were some early season larvae from chick pea and late season larvae from various crops. Of the eggs submitted, 57% successfully hatched. Of the eggs from cotton and pigeon pea that successfully hatched and the larvae from chick pea, melons, and pigeon pea, 66% were *H. armigera*.

The following table shows the data on total number of samples, and average hatching, and species composition separately for each valley. The % *H. armigera* values do not include hosts that are known to be dominated by this species (i.e., maize and sorghum). Unless otherwise indicated, the total number of samples refers to eggs only.

The levels of hatching and species composition presented in the table are averages for each valley and the actual levels vary greatly among properties. The values in brackets to the right of % hatch and % *H. armigera* indicate the range among collections. For instance, for the period between 9 September and 25 November, in Emerald the % *H. armigera* is 82 and ranges from 16 to 100 among properties sampled. The periods across the top of the table represent the dates at which the samples were identified to species, which is about 12 days after they were received as eggs or up to 7 days after larvae collections were received.

Valley	Trait	9 Sep-25 Nov	26 Nov-23 Dec	24 Dec-21 Jan	22 Jan-25 Feb	26 Feb-31 Mar	Total
Lower Namoi	No. of samples	1458	5315	1470	2289	1455	11987
	% hatch	67 (46-83)	64 (26-96)	62 (35-95)	49 (30-73)	63 (14-100)	60 (14-100)
	% <i>H. armigera</i>	all Ha hosts ^c	27 (7-45)	57 (27-90)	71 (45-94)	64 (19-100)	51 (7-100)
Upper Namoi	No. of samples	784	3429	225	90	590	5118
	% hatch	67 (46-76)	60 (6-78)	3 ^d	45 ^d	49 (6-73)	52 (6-78)
	% <i>H. armigera</i>	all Ha hosts ^c	64 (45-98)	all Ha hosts ^c	39 ^d	47 (35-65)	61 (35-98)
Emerald	No. of samples	988 ^a	352	465	109	446	2360
	% hatch	46 (15-89)	63 (33-91)	53 (52-53)	63 (60-66)	83 (50-100)	65 (15-100)
	% <i>H. armigera</i>	82 (16-100)	59 (44-76)	93 (90-97)	66 (61-72)	79 (38-100)	82 (16-100)
North Qld	No. of samples	38 ^b	-	-	-	-	38 ^b
	% hatch	NA	-	-	-	-	NA
	% <i>H. armigera</i>	100 ^d	-	-	-	-	100 ^d
St George	No. of samples	-	1463	1971	643	384	4461
	% hatch	-	61 (33-86)	61 (40-83)	38 (22-41)	30 (6-46)	51 (6-86)
	% <i>H. armigera</i>	-	49 (27-67)	87 (66-94)	52 (39-61)	63 (35-100)	63 (27-100)
Gwydir	No. of samples	538	605	1303	122	833	3401
	% hatch	77 (68-83)	70 (52-88)	76 (20-89)	61 (48-73)	70 (58-83)	70 (20-89)
	% <i>H. armigera</i>	16 (11-19)	all Ha hosts ^c	81 (63-97)	77 ^d	94 (89-100)	82 (11-100)
Macintyre	No. of samples	-	871	1994	844	1277	4986
	% hatch	-	17 (1-34)	58 (43-56)	33 (7-94)	41 (24-55)	37 (1-94)
	% <i>H. armigera</i>	-	15 (0-52)	80 (72-88)	56 (0-81)	77 (47-100)	66 (0-100)
Macquarie	No. of samples	-	175	515	68	-	758
	% hatch	-	63 (8-91)	68 (58-100)	69 (58-80)	-	67 (8-100)
	% <i>H. armigera</i>	-	23 (0-25)	13 (1-80)	60 (20-100)	-	42 (0-100)
Darling Downs	No. of samples	-	-	987	107	-	1094
	% hatch	-	-	26 (1-53)	21 ^d	-	24 (1-53)
	% <i>H. armigera</i>	-	-	84 (33-91)	82 ^d	-	83 (33-91)
Lachlan	No. of samples	-	-	-	45	-	45
	% hatch	-	-	-	27 ^d	-	27 ^d
	% <i>H. armigera</i>	-	-	-	33 ^d	-	33 ^d

^a 792 individuals from this sample were collected as larvae

^b 38 individuals from this sample were collected as larvae

^c all samples were collected off hosts that are known to be dominated by *H. armigera*

^d this sample is from a single collection

F₂ screens for Cry1Ac and Cry2Ab resistance

F₂ screens can detect heterozygote individuals (RS). They involve testing the grandchildren of pairs of moths raised from eggs collected from field populations, and therefore take about 10 weeks to run. Our screens test for genes that confer high level resistance that is likely to be of threat to the industry. **The CSIRO F₂ screens for Bt resistance in the 2008/09 season are complete.**

In 2008/09 we screened 804 and 812 alleles from *H. armigera* against Cry1Ac and Cry2Ab respectively. **We isolated no cases in *H. armigera* of alleles conferring resistance to Cry1Ac** (see Table below). For *H. armigera* the cumulative frequency of alleles conferring resistance to Cry1Ac since the program began (2002/03) is 0/3778. **We isolated 8 cases in *H. armigera* of alleles conferring resistance to Cry2Ab** (see the following Table). For *H. armigera* the cumulative frequency of alleles conferring resistance to Cry2Ab since the program began is 24/3790 (0.006). We have tested 15 of these 24 cases and all are of the same kind of Cry2Ab resistance. **The frequency obtained in 2008/09 is 0.009 which is similar to the frequency obtained at the end of the season in 2007/08, i.e., 0.005.** Based on the F₂ screen data there has been no statistically significant change in Cry2Ab resistance alleles in *H. armigera* since 2002/03.

In 2008/09 for *H. punctigera* we screened 1088 alleles against Cry1Ac and Cry2Ab. **We isolated two cases in *H. punctigera* of an allele conferring resistance to Cry1Ac** (see Table below). These are the first alleles conferring resistance to Cry1Ac that have been isolated in *H. punctigera* since the program began in 2002/03; thus the cumulative frequency is 2/4490 (0.0005). **We isolated 13 cases in *H. punctigera* of alleles conferring resistance to Cry2Ab** (see the following Table). For *H. punctigera* the cumulative frequency of alleles conferring resistance to Cry2Ab since the program began is 21/4502 (0.005). We have tested 3 of these 21 cases and all are of the same kind of Cry2Ab resistance. **The frequency obtained in 2008/09 is 0.012 which is about 3 times higher than at the end of the 2007/08 season, i.e., 0.004.**

Statistical tests which account for differences in sample sizes among seasons using the F₂ screens show **an increase in Cry2Ab resistance alleles in *H. punctigera* that has been significant for the past two seasons.** Specifically, the frequency of Cry2Ab alleles detected in the F₂ screens has increased by a factor of around 2 and 3 in the last two seasons. We used Regression analysis to look at the type of increase in frequency; an R² value of 1 indicates a perfect fit of the data. **The data fit a linear increase well (R² = 0.78) but an exponential growth curve provides an even better fit (R² = 0.93).**

Species	Year	Cry1Ac F ₂ screen		Cry2Ab F ₂ screen	
		alleles tested	positive	alleles tested	positive
<i>Helicoverpa punctigera</i>	2002/03	8	0	8	0
	2003/04	60	0	60	0
	2004/05	1012	0	1024	1
	2005/06	468	0	468	0
	2006/07	712	0	712	2
	2007/08	1142	0	1142	5
	2008/09	1088	2	1088	13
	Total	4490	2	4502	21
<i>Helicoverpa armigera</i>	2002/03	136	0	132	1
	2003/04	280	0	284	2
	2004/05	364	0	368	0
	2005/06	900	0	900	4
	2006/07	522	0	522	5
	2007/08	772	0	772	4
	2008/09	804	0	812	8
	Total	3778	0	3790	24

F₁ screens for Cry2Ab resistance

To increase the number of insects that could be processed during the season, CSIRO developed protocols for testing the frequency of the common Cry2Ab resistance detected with F₂ screens using a shorter method called an F₁ test. F₁ screens can detect heterozygote (RS) individuals. They involve testing the offspring of single-pair matings between moths from Cry2Ab resistant strains maintained in the laboratory (SP15 for *H. armigera* and Hp4-13 for *H. punctigera*) and moths raised from eggs collected from field populations. They take around 5 weeks to conduct. **The CSIRO F₁ screens for Bt resistance in the 2008/09 season are complete.**

The F₁ method assumes that the various isolates of Cry2Ab resistance alleles detected so far are of the same kind. These protocols were immediately adopted by Monsanto for *H. armigera*. During the following two years CSIRO determined that the same mechanism appeared to confer resistance in all of the isolates of Cry2Ab detected in *H. armigera* to date. Late in 2006 CSIRO began F₁ tests to determine the frequency of this SP15-type of Cry2Ab resistance for *H. armigera* and in 2007/08 continued these tests at a larger scale in two laboratories.

In both laboratories the results from 2007/08 confirmed previous findings from Monsanto that **the frequency of Cry2Ab resistance alleles in *H. armigera* using F₁ screens is up to 6 times higher than that determined with the F₂ tests:** 2007/08 CSIRO data, F₁ screen = 22/686 alleles (0.03), F₂ screen = 4/772 alleles (0.005). Currently, we believe that **the frequencies obtained from the F₁ screens are likely to most accurately reflect the situation in the field.** Both CSIRO and Monsanto are working to better understand the differences between the F₁ and F₂ screens.

In 2008/09 CSIRO screened 3104 alleles from *H. armigera* and **isolated 69 cases of alleles conferring resistance to Cry2Ab.** For *H. armigera* the cumulative frequency of alleles conferring resistance to Cry2Ab since the CSIRO program began (2007/08) is 91/3798. **The frequency obtained in 2008/09 is 0.022 which is not significantly different to that obtained at the end of the season in 2007/08.**

We began F₁ screens with *H. punctigera* in 2007/08. In 2008/09 we screened 640 alleles from *H. punctigera* and **isolated 30 cases of alleles conferring resistance to Cry2Ab.** For *H. punctigera* the cumulative frequency of alleles conferring resistance to Cry2Ab since the CSIRO program began (2007/08) is 32/836. **The frequency obtained in 2008/09 is 0.047 which is about 5 times higher than the frequency obtained at the end of the season in 2007/08, and this difference is statistically significant.**

Species	Year	Cry2Ab F ₁ screen		
		alleles tested	scored positive	frequency
<i>Helicoverpa punctigera</i>	2007/08	196	2	0.010
	2008/09	640	30	0.047
	Total	836	32	0.038
<i>Helicoverpa armigera</i>	2007/08	694	22	0.032
	2008/09	3104	69	0.022
	Total	3798	91	0.026

Screens of larvae from non-cotton regions

To gain insight into the potential role of Bollgard II in selecting for resistance, in May 2009 larvae of *H. punctigera* were collected from several locations in Central Australia. These sites were at least 1,000km from the nearest cotton growing regions. The larvae were transported to the CSIRO Narrabri laboratories and used in F₁ screens with Hp4-13. **Of the 944 alleles of *H. punctigera* screened 5 proved positive for Cry2Ab resistance. This frequency of 0.005 is significantly lower than the frequency of 0.047 obtained in 2008/09 in the cropping regions.**

In both years for which we have frequencies for *H. punctigera* from the cropping region using F₂ screens and F₁ screens the later is 4 times greater than the former. Using this factor we can extrapolate from the F₂ screen data that the estimated frequency of Cry2Ab resistance genes in *H. punctigera* at the time Bollgard II[®] was introduced (2004) was 0.008. Although this information should be used only as a guide, it suggests that **the Cry2Ab frequencies obtained from inland *H. punctigera* in 2009 are similar to those obtained in cropping areas prior to the widespread availability of Bollgard II.**

‘Survivors’ from Bollgard II® plants

There were reports of at least 1 medium-large larvae/m and/or Helicoverpa sprays in Bollgard II fields on some properties in Emerald, Theodore, Byee, Gwydir, Goondiwindi, Darling Downs, and the Namoi valleys. Most affected fields were at mid-flowering to late-flowering though some carried larvae until first open boll. In 2008/09 we received 889 Helicoverpa larvae from Bollgard II plants for resistance testing.

To date we have no evidence that the survival is due to Bt resistance.

Because Cry1Ac resistance genes are not common in field populations, and Cry2Ab resistant insects are killed by Cry1Ac, the most plausible field resistance scenario is that larvae collected from Bollgard II carry two Cry2Ab genes and can survive due to a decline in efficacy of Cry1Ac.

Nevertheless, we submit our larvae to an F₂ screen so that we can detect any resistance to Cry1Ac and Cry2Ab resistance that is similar or different to the form already detected. If the single pairs of moths set up for the F₂ screen fail to mate after 7 days, we match the male with a female from the Cry2Ab resistant strains maintained in the laboratory and perform an F₁ screen. So most of the Bollgard II survivors are screened with an F₂ test but some are screened with F₁ tests.

In 2008/09 we used F₁ screens to test 35 *H. armigera* that were collected as survivors on Bollgard II and 3 scored positive for carrying one copy of the Cry2Ab gene (3/70 alleles, 0.043). We used F₁ screens to test 8 *H. punctigera* that were collected as survivors on Bollgard II and none scored positive for carrying the Cry2Ab gene (0/16 alleles). We used F₂ screens to test 6 *H. armigera* that were collected as survivors on Bollgard II and none scored positive for carrying a gene conferring resistance to Cry2Ab or Cry1Ac (0/12 alleles). We used F₂ screens to test 10 *H. punctigera* that were collected as survivors on Bollgard II and none scored positive for carrying a gene conferring resistance to Cry2Ab or Cry1Ac (0/20 alleles).

Our data to date suggest that Bollgard II survivors occasionally carry Cry2Ab resistance genes but there is no marked difference in the frequencies of genes in this sample compared to insects that are collected randomly. As with the random sample, the Bollgard II survivors that scored positive for Cry2Ab resistance have only one copy of the gene which means they should be killed by the toxin. Virtually all of the larvae that survive on Bollgard II do not carry resistance genes for Cry1Ac or Cry2Ab.

2005–2007: Surviving Helicoverpa in Bollgard II® survey results

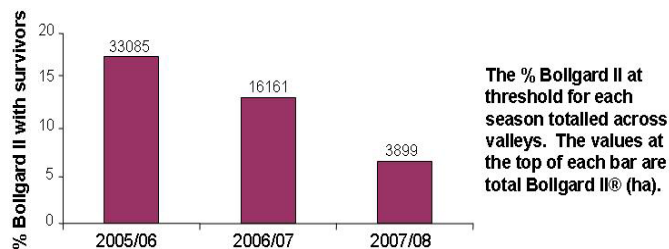
Helicoverpa larvae can reach the medium-large size in some fields of Bollgard II®. Sometimes larvae reach numbers which are greater than threshold levels. In these situations consultants and growers are mostly concerned about potential economic damage but this situation may also affect resistance management.

By the end of 2007/08 there was growing concern from the industry that Bollgard II® with survivors was largely restricted to St George, and more larvae were surviving in Bollgard II® fields than in previous seasons. To find out if these perceptions are true, and estimate the % Bollgard II® at threshold that is treated, we surveyed 46 CCA members on data from 2005/06, 2006/07 and 2007/08. In these seasons the total licensed Bollgard II® area was 230,000, 114,000, and 61,000 ha. In each season the survey covered >66% of the Bollgard II® area and all valleys were well represented.

We considered Bollgard II® to have survivors if it was at threshold: at least 2 larvae 3-8 mm/m in at least 2 consecutive checks or 1 larvae > 8mm/m.

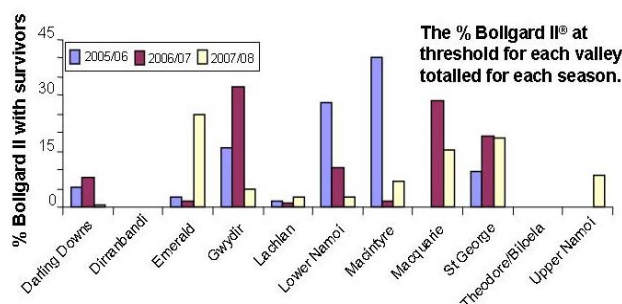
The % Bollgard II® with survivors is not increasing

When the data were totalled for each season across valleys 15% of the Bollgard II® area reached threshold. In 2005/06 18% of the Bollgard II® area reached threshold, while only 7% reached threshold in 2007/08. The perceived increase in survivors may reflect greater awareness of the issue via extension efforts.



There is no trend among seasons for one valley to be more or less likely to have Bollgard II® with survivors

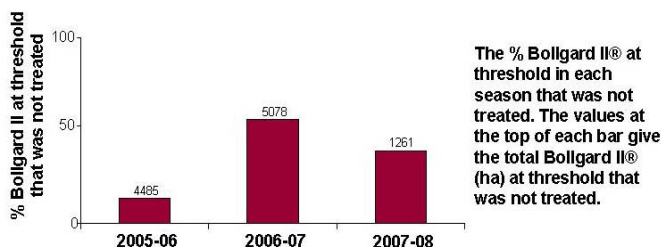
In 2005/06 the Gwydir, Lower Namoi and MacIntyre valleys had a percentage of Bollgard II® with larvae that was above the average. In 2006/07 the Lower Namoi, St George, Macquarie, and Gwydir valleys had a percentage of Bollgard II® with larvae that was above the average. In 2007/08 the Upper Namoi, Macquarie, St George, and Emerald valleys had a percentage of Bollgard II® with larvae that was above the average.



Not all of the Bollgard II® with survivors was treated

To extend the life of Bollgard II® growers follow a strict Resistance Management Plan. Despite this strategy, there are concerns that Cry2Ab resistant alleles in both *Helicoverpa* species may be increasing.

In some years more than half of the area that reached threshold (of at least 1 medium-large larvae/m) was not treated. *This finding is a concern for managing resistance.* Because larvae surviving on Bollgard II® probably consume Bt toxin at a non-lethal dose, it is possible that there is greater selection for resistance. This makes it important to treat Bollgard II® at threshold to stop larvae from contributing to future generations. Follow-up pupae digs in fields that carried threshold numbers of larvae have confirmed that survivors are able to successfully pupate and emerge as moths.



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. 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 not all fields are affected 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.

Thresholds

Researching economic thresholds for Bollgard II® has 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 research whether yield penalties exist when medium-large larvae are not treated. Future research may also need to consider the role thresholds can play in reducing the selection pressure for Bt resistance.

The presence of medium-large larvae in Bollgard II® is:

- not due to Bt resistance
- not increasing (until 2007/08)
- widespread among valleys and climatic regions
- not always controlled (but it should be)

The full report from this survey can be found at:

<http://www.cotton.crc.org.au/> (Industry – Publications – Pests & Beneficials – Insect Resistance Management).

We acknowledge the significant support of the Crop Consultants Australia, Cotton CRC, CSIRO and CRDC.

2008-09 Surviving Helicoverpa in Bollgard II[®] survey NOW OPEN

It is important that we continue to add to the 2005/06 – 2007/08 data set to monitor potential changes in surviving Helicoverpa in Bollgard II. To do this we will again be relying on CCA members to contribute their data.

In future this brief (5 question) survey will be incorporated into the CCA end of season survey. However, for 2008/09 the survey will be conducted online. We will contact by telephone those consultants that do not complete the online survey by 31st October 2009. If you would like to complete the survey over the phone please call Sharon Downes on 0267991576/0427480967.

The survey can be accessed by:

1. Clicking on the following link:

http://www.cottoncrc.org.au/content/Industry/Tools/Forms_Questionnaires_-_Industry/Sprays_for_surviving_Helicoverpa_in_Bollgard_II_Survey_2009.aspx OR

2. Go to the Cotton CRC Home Page (http://www.cottoncrc.org.au/content/Industry/CRC_home.aspx)
Click on “Industry”
Click on “Tools”
Click on “Current Questionnaires_Industry”
Click on “Sprays for surviving Helicoverpa in Bollgard II Survey 2009”

Take home messages

- Cry2Ab resistance alleles were present at detectable levels before Bollgard II was widespread.
- In *H. punctigera* these levels have steadily increased since Bollgard II was adopted.
- This increase in Cry2Ab resistance alleles in *H. punctigera* is supported by F₁ and F₂ screen data.
- The rate of increase in the longer-term F₂ data best fits an exponential growth.
- The frequencies of Cry2Ab resistance alleles are significantly lower in *H. punctigera* populations from central Australia that are not exposed to Bollgard II.
- A proportion of migrating *H. punctigera* from central Australia will carry Cry2Ab resistance genes.
- In *H. armigera* the frequency of Cry2Ab resistance alleles remains high at 5% of the population.
- In *H. punctigera* the frequency of Cry2Ab resistance alleles is at 10% of the population.
- There have been no reported field failures of Bollgard II and the occasional occurrence of threshold levels of Helicoverpa in some Bollgard II fields is not due to Bt resistance.
- In any year around 15% of the area grown to Bollgard II[®] will carry surviving larvae.
- This proportion did not increase from 2005/06 to 2007/08.
- Among seasons no one valley is more or less likely to have Bollgard II[®] with survivors.
- Not all of the Bollgard II[®] with survivors is treated.
- Although survivors on Bollgard II are not currently resistant, it would be useful to control them so that they are not exposed to low doses of toxin which can select for resistance in the future.

Many thanks to CCA members Matthew Holding, Murray Boshammer, Iain Macpherson, David Parlarto, Matt Ceeney, and Jamie Street, and Dave Murray and his team, for assisting with collections. Special thanks to David Parlarto, Duane Evans, Susan Maas, John Barber, Jamie Street, John Mulholland, Elle MacPherson, Steve Madden, Rick Thomas, Peter Gregg, Bec Kirkby, Leah Austin, Brett Enkleman, Alice Del Socorro and Dallas King for their help with the survivors on Bollgard II work. We appreciate the excellent assistance provide by Sally Ceeney, Lauryn Hanna, Susan Maas, Dallas King, Ingrid Renkin, and James Hill in co-ordinating the collections of eggs and larvae as part of their RCEO role. The Bt resistance monitoring program is funded by the Cotton Research and Development Corporation. We appreciate the support from the Cotton CRC.