

## End of January results from Bt resistance monitoring 2007/08

### ***Hatching, parasitism and species composition***

Across all sampled valleys there were 19339 eggs submitted to the program until 16 January 2008. Of those eggs, 49% successfully hatched, 20% were parasitised (by anything, not just *Trichogramma*), and 31% did not successfully hatch presumably due to infertility, desiccation, damage or unsuccessful parasitism. Of the eggs that successfully hatched on cotton and pigeon pea, 46% were *H. armigera*.

**The following table shows the data on egg numbers, hatching, parasitism 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). The levels of egg parasitism 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 % parasitised indicate the range among collections. For instance, for the period between 5 November and 17 December, in the Darling Downs the % parasitism is 27 and ranges from 0 to 84% 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 10-14 days after they were received as eggs.

Valley	Trait	5 Nov to 17 Dec	18 Dec to 16 Jan
Lower Namoi	number of eggs	716	3190
	% hatch	73	58
	% parasitised	6 (2-17)	23 (0-72)
	% <i>H. armigera</i>	70	62
Upper Namoi	number of eggs	369	4853
	% hatch	66	43
	% parasitised	18 (8-26)	38 (0-67)
	% <i>H. armigera</i>	70	59
Emerald	number of eggs	900	693
	% hatch	64	47
	% parasitised	26 (13-37)	30 (10-65)
	% <i>H. armigera</i>	11	29
Darling Downs	number of eggs	88	984
	% hatch	71	32
	% parasitised	27 (0-84)	63 (29-89)
	% <i>H. armigera</i>	50	51
Lachlan Valley	number of eggs	20	193
	% hatch	70	64
	% parasitised	0 (0)	0 (0)
	% <i>H. armigera</i>	20	28
St George	number of eggs	1808	2512
	% hatch	54	44
	% parasitised	10 (0-49)	26 (0-56)
	% <i>H. armigera</i>	57	49
Gwydir	number of eggs	809	1331
	% hatch	59	59
	% parasitised	16 (0-30)	16 (0-36)
	% <i>H. armigera</i>	7	39
Macintyre	number of eggs	0	472
	% hatch	-	41
	% parasitised	-	22 (8-31)
	% <i>H. armigera</i>	-	57

*Note: It takes approximately 3 weeks to test material sent as eggs, including scoring for parasites and identifying all hatched material to species. Some collections submitted after 16 January have yet to be scored for all of the characteristics that we measure in the program. These data will be included in the end of month report for February 2008.*

### ***F<sub>0</sub> screens for Cry1Ac and Cry2Ab resistance***

F<sub>0</sub> screens are likely to pick up only individuals that are homozygous resistant (RR) to Bt. Around 2% survival is expected as a baseline for the doses of toxins used in the F<sub>0</sub> screens. It is critical to consider sample sizes when assessing the significance of survival estimates greater than 2%.

**The table below shows the percentage of larvae surviving the F<sub>0</sub> screens for Bt resistance.** The number of larvae tested is in the parentheses to the right of survivorship. Data are provided separately for different regions, for Cry1Ac and Cry2Ab, and for *H. armigera* and *H. punctigera*.

In all sampled regions the total survival of larvae tested in our program (i.e., the total number of survivors divided by the total number of individuals tested) is not substantially greater than 2%, and is not markedly higher than the total survival detected in previous years. So our **data from the F<sub>0</sub> screens do not indicate any major changes from previous seasons in survival rates to discriminating doses of Cry1Ac or Cry2Ab.**

*Note that the 3.57% of H. punctigera surviving the Cry1Ac screens from 18 Dec – 16 Jan in the Lachlan Valley is based on a small sample of 37 tested individuals. The value represents survival by one individual that did not successfully pupate. Similarly, the 4.08% of H. armigera surviving the Cry1Ac screens from 18 Dec – 16 Jan in the Darling Downs is based on a small sample of 49 tested individuals. The value represents survival by two individuals that did not successfully pupate.*

		<b>% F<sub>0</sub> individuals surviving discriminating dose (no. individuals tested)</b>							
		<b><i>Helicoverpa armigera</i></b>				<b><i>Helicoverpa punctigera</i></b>			
<b>Toxin</b>	<b>Valley</b>	<b>5 Nov - 17 Dec</b>		<b>18 Dec - 16 Jan</b>		<b>5 Nov - 17 Dec</b>		<b>18 Dec - 16 Jan</b>	
Cry1Ac	Gwydir	0.00	(165)	0.93	(108)	0.36	(18)	2.09	(239)
	Upper Namoi	0.00	(68)	1.47	(546)	not tested		1.23	(163)
	Lachlan Valley	0.00	(1)	0.00	(7)	0.00	(4)	3.57	(37)
	St George	0.00	(149)	1.73	(231)	0.00	(93)	1.46	(205)
	Darling Downs	0.00	(8)	4.08	(49)	0.00	(5)	0.00	(39)
	Emerald	0.00	(20)	0.00	(16)	0.00	(241)	1.69	(118)
	Lower Namoi	1.40	(143)	1.64	(487)	1.67	(60)	0.00	(128)
	Macintyre	not tested		0.00	(42)	not tested		0.00	(23)
Cry2Ab	Gwydir	0.00	(66)	0.00	(31)	not tested		0.00	(61)
	Upper Namoi	0.00	(13)	0.00	(184)	0.00	(6)	0.00	(24)
	Lachlan Valley	not tested		not tested		not tested		not tested	
	St George	0.00	(29)	0.00	(42)	0.00	(26)	0.00	(26)
	Darling Downs	not tested		0.00	(16)	not tested		0.00	(5)
	Emerald	0.00	(5)	0.00	(3)	0.00	(33)	0.00	(51)
	Lower Namoi	0.00	(8)	0.62	(162)	0.00	(17)	0.00	(33)
	Macintyre	not tested		not tested		not tested		not tested	

### ***F<sub>2</sub> screens for Cry1Ac and Cry2Ab resistance***

F<sub>2</sub> 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 F<sub>2</sub> screens for Bt resistance in the 2007/08 season are underway.** Due to the early season predominance of *H. punctigera*, our tests thus far have focussed on this species.

Until 16 January we screened 612 alleles from *H. punctigera* against Cry1Ac and Cry2Ab. **We isolated no cases in *H. punctigera* of alleles conferring resistance to Cry1Ac** (see Table below). The cumulative frequency of alleles conferring resistance to Cry1Ac since the program began (2002/03) is 0/2872 for *H. punctigera*. **We isolated 2 cases in *H. punctigera* of alleles conferring resistance to Cry2Ab** (see the following Table). The cumulative frequency of alleles conferring resistance to Cry2Ab since the program began is 5/2884 (0.0017) for *H. punctigera*.

Until 16 January we screened 24 alleles from *H. armigera* against Cry1Ac and Cry2Ab. **We isolated no cases in *H. armigera* of alleles conferring resistance to Cry1Ac or Cry2Ab.** For *H. armigera* the cumulative frequency of alleles conferring resistance since the program began (2002/03) is 0/2226 for Cry1Ac and 12/2230 (0.005) for Cry2Ab (see Table below).

Species	Year	Cry1Ac F <sub>2</sub> screen		Cry2Ab F <sub>2</sub> screen	
		alleles tested	scored positive	alleles tested	scored 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
	<b>2007/08</b>	<b>612</b>	<b>0</b>	<b>612</b>	<b>2</b>
	Total	2872	0	2884	5
<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
	<b>2007/08</b>	<b>24</b>	<b>0</b>	<b>24</b>	<b>0</b>
	Total	2226	0	2230	12

### ***Screens with ‘survivors’ from Bollgard II® plants***

**There were reports of surviving larvae in Bollgard II fields on 4 properties in Emerald early in January.** One property had been previously damaged with hail. No collections were submitted to the CSIRO program but Monsanto have some larvae and plant material from the affected properties that they will screen for resistance and Bt expression, respectively.

**There was one report of surviving larvae in a Bollgard II field in the Lower Namoi Valley in mid-January** but our two comprehensive searches, performed several days apart, revealed little to no damage and we found only one larvae.

**There were two reports of surviving larvae in Bollgard II fields in the Upper Namoi Valley in late-January.** In the first field, the extent of damage, abundance of larvae, and distribution of larvae suggest that the damaged section of the field may be conventional cotton. We collected 115 larvae from the field (67% *H. armigera*; not included in the table below) and took plant samples to test for the presence of the Cry genes. The second field was sprayed below threshold before a collection could be obtained.

**In late-January there were reports of surviving larvae in Bollgard II fields in St George.** The problem appeared to be widespread across numerous properties, and the earliest planted fields were the first to be affected at around 3-5 nodes above white flower. We have collections of larvae from 7 affected properties.

Valley	No. Farms	No. larvae			% <i>H. armigera</i>
		□ small	□ medium	total	
Lower Namoi	4	3	3	6	60
Lachlan	1	1	0	1	0
St George	7	51	169	207	82

□ small = very small larvae and small larvae (neonate and 2<sup>nd</sup> instar)  
 □ medium = medium larvae and large larvae (3<sup>rd</sup> instar, 4<sup>th</sup> instar and 5<sup>th</sup> instar)  
 Ha = *Helicoverpa armigera*, Hp = *Helicoverpa punctigera*

Unlike last season where 70% of the collections comprised *H. punctigera*, the majority of larvae from the 2007/08 season collected thus far at St George are *H. armigera* (see above table). This value is higher than the 49% of *H. armigera* in the sample of eggs identified during 18 Dec – 16 Jan, though the egg collections that are likely to be from the same cohort as the survivors are due to be processed in the next 2 weeks.

From 25-27 January we studied a number of fields and properties in St George and recorded up to 2 medium-large larvae in each metre of row. We will continue our studies of affected properties which include collecting larvae for tests of Bt resistance and increased Bt tolerance, quantifying within-plant and among-plant *Helicoverpa* damage, and collecting plant material for ELISA tests of presence/absence of Cry genes and levels of Bt expression.

The first collections of larvae are pupae. **We have not yet performed any tests for Bt resistance or increased tolerance to Bt with the survivors collected from Bollgard II.** We will process the plant material for ELISA tests at the end of the season.

### ***Larvae on Bollgard II in season survey***

In an effort to identify the possible factors leading to the presence of larvae in some locations and not others, Kristen Knight (Monsanto) and I are conducting a survey (see page 6 of this report) that has the support of the Cotton Consultants Association. We urge all growers and consultants to contribute to our knowledge of this important issue by completing the brief survey.

The survey is divided into two sections. The first section gathers information for situations where larvae are present, and the second section enquires about environmental conditions and is relevant to situations where larvae are present or absent.

Throughout the season we ask that a survey be completed for each Bollgard II<sup>®</sup> field that has been scouted and contains at least one surviving larvae. It is also important that we gather information on situations where there are no larvae surviving on Bollgard II<sup>®</sup> so that we can discount factors that are common to problem and non-problem locations as potential drivers of the survival. We ask that a survey be completed for one randomly selected scouted Bollgard II<sup>®</sup> field per week during early January until the end of February, which is the period during which survivors have been recorded in the past.

**Many thanks to the volunteer and dedicated collectors that contributed material to the program, and especially to John Barber, Jamie Street and Dallas King for their help with the survivors on Bollgard II work.**

## How to collect *Helicoverpa* for resistance testing

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Conventional insecticides and Bollgard II<sup>®</sup> play a vital role in the effective management of *Helicoverpa armigera* and *H. punctigera*. *H. armigera* has an exceptional record of evolving resistance to insecticides, with transgenic crops not immune to this threat. To ensure that these important management tools are available for cotton protection in the long term, the Cotton Research and Development Corporation (CRDC) supports programs that monitor insecticide resistance in field populations.

The program for conventional insecticides monitors resistance to all Heliocides, especially the softer IPM-compatible chemistries which are widely used while the program for transgenic cotton screens larvae for susceptibility to the two toxins (Cry1Ac and Cry2Ab) produced by Bollgard II<sup>®</sup>. The Transgenic and Insect Management Strategy (TIMS) Committee uses these results to formulate the Insecticide Resistance Management Strategy and, if required recommends changes to the Bollgard II<sup>®</sup> Resistance Management Plan. The quality and integrity of our results, and thus the recommendations from TIMS, improves with an increase in the number of eggs and larvae tested.

This season the CRDC will support dedicated *Helicoverpa* collectors in the St George, Emerald, MacIntyre, Darling Downs, and Lachlan valleys. The support will be administered by the Cotton Consultants Association of Australia whose key members will supervise the collections and work with Regional Cotton Extension Officers to post the material.

Even with this assistance, it remains important that volunteer collectors, growers, and consultants gather and submit material. Small egg collections from many people require minimal effort and provide vital information when accumulated across a large area. We especially rely on voluntary collections for sporadic events like suspected spray failures and the presence of live larvae on Bollgard II<sup>®</sup> plants. This article outlines the protocols for collecting and dispatching *Helicoverpa* material for resistance testing.

### Collecting eggs

Field collected eggs are the most significant component of the monitoring programs.

**Step 1.** From any host plant collect the parts that carry eggs: e.g. cotton (leaves, flowers), sorghum (heads), maize (silks, leaf cuttings), pigeon pea (leaves, flowers), etc. Select from plants located throughout fields that have not been sprayed with heliocides for 7-10 days.

**Step 2.** Ideally, collect at least 100 eggs/field, but all collections regardless of size are valued.

**Step 3.** Material from each field should be placed into separate paper bags (available from your RCEO). Complete the details on the label: (a) Date Collected; (b) Location (farm, field, area); (c) Crop (distinguish Bollgard II<sup>®</sup> and conventional cotton); (d) Collector; (e) Last Spray (date and insecticide, if known); and (f) Approximate number of leaves, heads or silks.

### Collecting larvae from suspected spray failures

Larvae that survive a spray can be tested to determine if resistance is the cause of the failure.

**Step 1.** Collect as many larvae as possible—the more the better.

**Step 2.** Place larvae in a plastic container (food tub, ice cream container) with leaves of any host except Bollgard II<sup>®</sup> for food. If larvae are maintained in high densities they may eat each other. Therefore, be sure to divide samples between bags and provide adequate food.

## **Collecting large larvae found on Bollgard II®**

We are interested in larvae of *H. armigera* and *H. punctigera* that are at least 6mm. Ideally, the larvae would be accompanied by associated leaf samples that can be tested for the presence of Bt but even if you cannot complete step 2 below, your contribution of larvae will be valuable.

If you have numerous larvae in a field, it is more useful to collect many larvae with just the leaf from the host plant than few larvae with leaves from host plants and surrounding plants. If you have numerous fields with larvae, it is more useful to spread the collections across a number of fields rather than have more larvae from one field. There is no minimum number of larvae per site or region – even one larva is useful to us!

We have modified our 2006/07 collection kit to include larger bags for plant leaves and a labelled collection bag per sample for easy completion of details. Contact Trudy Staines on 02 6799 1500 if you would like some posted to you or your group.

**Step 1:** Collect the larva and place it in the small container with air holes and some food other than Bollgard II® (e.g. conventional cotton). If you have numerous larvae contact your RCEO immediately and they will provide collecting trays filled with artificial diet.

**Step 2:** If possible, collect a leaf from the plant that the larva was feeding on (indicated by the rectangle in the figure) and place it into the paper bag labelled ‘HOST PLANT’. Collect a leaf from each of the four plants that surround the host plant (i.e., the two plants in the same row, and the two plants in the adjacent row, as indicated by the circles in the figure) and place them all into the paper bag labelled ‘SURROUNDING PLANTS’. Please remove the 3<sup>rd</sup> unfurled leaf from the top of the plant as the sample. It is critical that the leaf material is fresh.



**Step 3:** Place the host leaf bag and surrounding leaf bag, and/or the pot containing the larvae, into the main collection bag and fill in the following details on the label: date, farm, field, plant variety, and collector. Please ensure that the two leaf bags, larvae pot, and main collection bag have the same sample numbers. If you did not collect leaves from host plants, it is fine to place multiple larvae (pots) from the same field in the same main collection bag.

## **Dispatching collected material**

All tests are conducted at the Australian Cotton Research Institute in Narrabri, NSW by Dr Sharon Downes (Bt resistance), and Dr Louise Rossiter (conventional chemistry).

**Step 1:** Keep material alive and fresh by storing it in a refrigerator or esky with a freezer brick that has been wrapped in paper.

**Step 2:** Within 24-48 hours, arrange to deliver the material to your RCEO or arrange to have the larvae or the larvae plus associated plant parts delivered directly to Australian Cotton Research Institute, 21888 Kamilaroi Highway, Narrabri NSW 2390. We can pay for the postage (i.e. tick the ‘receiver pays’ option on the freight form collected from your RCEO). If your property is in the Namoi valley, deliver the material to the ACRI or call Tracey Parker or Fiona McKenzie on 02 6799 1500 to arrange a pick up.

**For further information contact your RCEO or call Louise or Sharon on 02 6799 1500.**

<h2 style="margin: 0;"><u>Larvae on Bollgard II® In Season Survey</u></h2>			
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**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. Could you please fill out and return survey as close to when finding larvae as possible.*

<b><u>Section 1: Larvae and damage</u></b>	
Are you finding larvae? If no, move to section 2.	Yes No
Where on the plant are you finding larvae?	leaves/squares/flowers/boll caps/small bolls/large bolls
On which fruiting branches are the larvae?	1 <sup>st</sup> 2 <sup>nd</sup> 3 <sup>rd</sup> 4 <sup>th</sup> 5 <sup>th</sup> 6 <sup>th</sup> 7 <sup>th</sup> 8 <sup>th</sup> 9 <sup>th</sup> 10 <sup>th</sup> 11 <sup>th</sup> 12 <sup>th</sup> from bottom
Are most (>80%) larvae within 50 m of the field edge?	Yes No
How many larvae per metre (average of 3 checks)?	_____ larvae/m
What size are the majority (> 80%) of larvae?	VS, Small, SM, Medium, ML, Large, mixed
If this is a follow up check, describe the rate of development for most (>80%) larvae compared to what you would expect on conventional cotton?	much slower          slower          same          faster
Is there any damage?	Yes No
If yes, where are you seeing damage?	leaves/squares/flowers/boll caps/small bolls/large bolls
<b><u>Section 2: Environmental conditions</u></b>	
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 is the plant population density?	_____ plants/m of row
Has herbicide been applied in the past 2 weeks?	Yes (active: _____) No
Has an insecticide been applied in the past 2 weeks?	Yes (active: _____) No
What were the environmental conditions at the time of application?	Temp: _____ RH: _____ Windspeed: _____
How many fruiting branches on a typical plant? If relevant, how many NAWF?	_____ fruiting branches ____ NAWF
Describe the cover of broadleaf weeds in the field?	Weed free    0-5%    5-10%    >10%
Is the field neighboring broadleaf weeds (fallow field, fence, tail drain, head ditch, remnant vegetation)?	Yes No
Is the soil type prone to water-logging?	Yes No
Has the irrigation interval been increased at any time to stretch water supply?	Yes No
Has PIX been applied?	Yes No
If so, what was the rate and timing?	Rate: _____ Date: _____ Crop stage: _____

Comments: \_e.g. egg counts, no beneficials/m \_\_\_\_\_

Can larvae be collected?  If so, you will be contacted by Kristen Knight Monsanto or Sharon Downes CSIRO.

\*\*\*\*\* Please fax to Kristen Knight 07 46348500 and Sharon Downes CSIRO 02 67931186\*\*\*\*\*