ATTENTION ALL CCA MEMBERS WHO HAVE NOT YET COMPLETED THE SPRAYS FOR SURVIVING HELICOVERPA IN BOLLGARD II SURVEY

It is critical that **all** consultants respond to this survey!

By providing your name on the form you will be removed from our follow-up list of members that did not complete the online survey.

To access the survey form:

1. Click on the following link:

   http://www.cottoncrc.org.au/content/Industry/Tools/CurrentQuestionairres_Industry/Sprays_for_surviving_Helicoverpa_in_Bollgard_II.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 Questionairres_Industry”
   Click on “Sprays for Surviving Helicoverpa in Bollgard II”

End of April results from Bt resistance monitoring 2007/08

Hatching, parasitism and species composition

Across all sampled valleys there were 36485 eggs submitted to the program from mid-November 2007 until mid-March 2008. Of those eggs, 50% successfully hatched, 24% were parasitised (by anything, not just Trichogramma), and 26% did not successfully hatch presumably due to infertility, desiccation, damage or unsuccessful parasitism. Of the eggs that successfully hatched on cotton and pigeon pea, 52% 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-17 Dec**	**18 Dec-16 Jan**	**17 Jan-13 Feb**	**14 Feb-19 Mar**	**Season total**
Lower Namoi	No. of eggs	716	3190	1536	1255	6697
	% hatch	73	58	72	33	54
	% parasitised	6 (2-17)	23 (0-72)	29 (0-36)	31 (0-66)	24 (0-72)
	% *H. armigera*	70	62	33	56	56
Upper Namoi	No. of eggs	369	4853	3999	1954	11175
	% hatch	66	43	48	37	50
	% parasitised	18 (8-26)	38 (0-67)	30 (0-56)	55 (6-69)	35 (0-69)
	% *H. armigera*	70	59	47	62	57
Emerald	No. of eggs	900	693	0	0	1593
	% hatch	64	47	-	-	59
	% parasitised	26 (13-37)	30 (10-65)	-	-	29 (10-65)
	% *H. armigera*	11	29	-	-	21
Darling Downs	No. of eggs	88	984	495	440	2007
	% hatch	71	32	20	57	51
	% parasitised	27 (0-84)	63 (29-89)	51 (34-89)	21 (7-52)	33 (0-89)
	% *H. armigera*	50	51	63	31	49
Lachlan Valley	No. of eggs	20	193	424	0	637
	% hatch	70	64	51	-	63
	% parasitised	0 (0)	0 (0)	5 (0-9)	-	3.5 (0-9)
	% *H. armigera*	20	28	30	-	26
St George	No. of eggs	1808	2512	2084	632	7036
	% hatch	54	44	56	32	47
	% parasitised	10 (0-49)	26 (0-56)	17 (0-53)	47 (28-61)	25 (0-61)
	% *H. armigera*	57	49	30	31	43
Gwydir	No. of eggs	809	1331	545	3184	5869
	% hatch	59	59	60	35	53
	% parasitised	16 (0-30)	16 (0-36)	20 (3-29)	44 (8-80)	22 (3-80)
	% *H. armigera*	7	39	47	45	34
Macintyre	No. of eggs	0	472	338	0	810
	% hatch	-	41	47	-	44
	% parasitised	-	22 (8-31)	16 (9-87)	-	19 (8-87)
	% *H. armigera*	-	57	53	-	55
Macquarie	No. of eggs	0	0	461	285	746
	% hatch	-	-	61	53	58
	% parasitised	-	-	36 (8-89)	10 (0-48)	26 (0-89)
	% *H. armigera*	-	-	20	42	35

A similar total number of eggs were collected in 2007/08 (36485) compared to 2006/07 (34851). However, a greater proportion of eggs from 2007/08 were contributed from collectors outside of the Namoi and Gwydir (35%) compared to 2006/07 (13%). These differences largely reflect the availability of dedicated egg collectors in the different valleys between years.

The Lower Namoi, Upper Namoi, Macintyre, St George, Gwydir and Darling Downs valleys were sampled in 2006/07 and 2007/08. In 2007/08 the season totals for % eggs hatched for these valleys were lower than those obtained in 2006/07 (range = 3-31% lower, average = 18% lower). In 2007/08 the season totals for % eggs parasitised for these valleys were higher than those obtained in 2006/07 (range = 6-26% higher, average = 15% higher). For each valley the differences in season totals for % *H. armigera* in 2007/08 compared to 2006/07 were: Lower Namoi = 17% higher, Upper Namoi = 35% higher, Macintyre = 1% higher, St George = 15% higher, Darling Downs = 6% higher and Gwydir = 8% lower.

F_0 screens for Cry1Ac and Cry2Ab resistance

F_0 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_0 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_0 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*.

Toxin	Valley	% F_0 individuals surviving discriminating dose (no. individuals tested)									
		5 Nov-17 Dec		18 Dec-16 Jan		17 Jan-13 Feb		14 Feb-19 Mar		Season total	
Helicoverpa armigera											
Cry1Ac	Gwydir	0.00	(165)	0.93	(108)	0.00	(37)	0.85	(117)	0.47	(427)
	Upper Namoi	0.00	(68)	1.47	(546)	0.41	(242)	0.45	(224)	0.93	(1080)
	Lachlan Valley	0.00	(1)	0.00	(7)	0.00	(7)	not tested		0.00	(15)
	St George	0.00	(149)	1.73	(231)	0.00	(40)	0.00	(41)	0.87	(461)
	Darling Downs	0.00	(8)	4.08	(49)	5.88	(17)	0.00	(8)	3.66	(82)
	Emerald	0.00	(20)	0.00	(16)	not tested		not tested		0.00	(36)
	Lower Namoi	1.40	(143)	1.64	(487)	0.00	(69)	2.50	(80)	1.54	(779)
	Macintyre	not tested		0.00	(42)	0.00	(38)	not tested		0.00	(80)
Cry2Ab	Gwydir	0.00	(66)	0.00	(31)	0.00	(30)	1.37	(73)	0.50	(200)
	Upper Namoi	0.00	(13)	0.00	(184)	0.85	(117)	2.60	(77)	0.77	(391)
	Lachlan Valley	not tested		not tested		not tested		not tested		not tested	
	St George	0.00	(29)	0.00	(42)	0.00	(5)	not tested		0.00	(76)
	Darling Downs	not tested		0.00	(16)	not tested		0.00	(2)	0.00	(18)
	Emerald	0.00	(5)	0.00	(3)	not tested		not tested		0.00	(8)
	Lower Namoi	0.00	(8)	0.62	(162)	0.00	(22)	0.00	(9)	0.50	(201)
	Macintyre	not tested		not tested		not tested		not tested		not tested	
Helicoverpa punctigera											
Cry1Ac	Gwydir	0.46	(218)	2.09	(239)	2.63	(38)	0.33	(307)	1.00	(802)
	Upper Namoi	not tested		1.23	(163)	2.15	(325)	2.70	(74)	1.96	(562)
	Lachlan Valley	0.00	(4)	2.70	(37)	1.12	(89)	not tested		1.54	(130)
	St George	0.00	(93)	1.46	(205)	1.44	(278)	0.00	(14)	1.19	(590)
	Darling Downs	0.00	(5)	0.00	(39)	0.00	(13)	0.00	(95)	0.00	(152)
	Emerald	1.24	(242)	1.69	(118)	not tested		not tested		1.39	(360)
	Lower Namoi	1.67	(60)	0.00	(128)	0.38	(261)	0.00	(60)	0.39	(509)
	Macintyre	not tested		0.00	(23)	0.00	(14)	not tested		0.00	(37)
Cry2Ab	Gwydir	not tested		0.00	(61)	0.00	(8)	0.00	(58)	0.00	(127)
	Upper Namoi	0.00	(6)	0.00	(24)	0.00	(107)	not tested		0.00	(137)
	Lachlan Valley	not tested		none tested		none tested		not tested		not tested	
	St George	0.00	(26)	0.00	(26)	0.00	(76)	not tested		0.00	(128)
	Darling Downs	not tested		0.00	(5)	not tested		0.00	(18)	0.00	(23)
	Emerald	0.00	(33)	0.00	(51)	not tested		not tested		0.00	(84)
	Lower Namoi	0.00	(17)	0.00	(33)	0.00	(38)	0.00	(1)	0.00	(89)
	Macintyre	not tested		none tested		not tested		not tested		not tested	

In all sampled regions the season 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_0 screens do not indicate any major changes from previous seasons in survival rates to discriminating doses of Cry1Ac or Cry2Ab**.

F_2 screens for Cry1Ac and Cry2Ab resistance

F_2 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_2 screens for Bt resistance in the 2007/08 season are in progress.

Until 30 April we screened 1060 alleles from *H. punctigera* against Cry1Ac and Cry2Ab. **We isolated no cases in *H. punctigera* of alleles conferring resistance to Cry1Ac** (see Table below). For *H. punctigera* the cumulative frequency of alleles conferring resistance to Cry1Ac since the program began (2002/03) is 0/3320. **We isolated 3 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 6/3332 (0.0018).

Until 30 April we screened 488 alleles from *H. armigera* against Cry1Ac and Cry2Ab. **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 0/2690. **We isolated 3 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 15/2694 (0.0056).

Species	Year	Cry1Ac F_2 screen		Cry2Ab F_2 screen	
		alleles tested	scored positive	alleles tested	scored positive
Helicoverpa punctigera	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	**1060**	**0**	**1060**	**3**
	Total	3320	0	3332	6
Helicoverpa armigera	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	**488**	**0**	**488**	**3**
	Total	2690	0	2694	15

'Survivors' from Bollgard II® plants

There were reports from early-January until late-February of surviving larvae at threshold in Bollgard II fields on some properties in Emerald, Lower Namoi, Upper Namoi, Gwydir, Macquarie, Macintyre and St George. All affected fields were at mid-flowering to late-flowering. In most reported cases, sprays for Helicoverpa were applied.

During 2007/08 we received 764 Helicoverpa larvae from Bollgard II plants. Our work focused on St George and 739 of the 764 larvae are from 10 properties in this area. Unlike last season where 70% of the collections comprised *H. punctigera*, the majority of larvae from the 2007/08 season collected at St George are *H. armigera* (see below table). This value is higher than the % of *H. armigera* in the sample of eggs collected during January and February in St George, and suggests that *H. armigera* may be differentially surviving on Bollgard II.

Valley	No. Farms	No. larvae			% *H. armigera*
		small	medium	total	
Namoi	4	8	15	23	85
Lachlan	2	1	1	2	50
St George	10	77	662	739	89

small = very small and small larvae (neonate and 2nd instar)

medium = medium and large larvae (3rd, 4th and 5th instar)

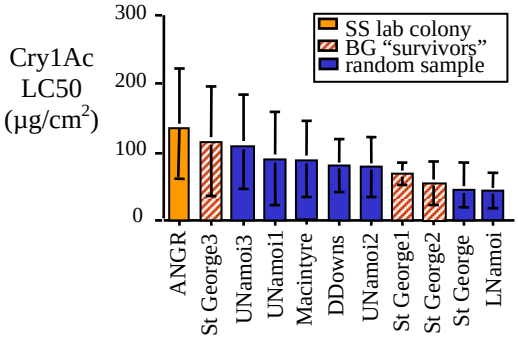
Our work at St George was focused on three fields, each on different properties (Farm A, Farm B and Farm C). The following table lists several attributes for each of the sites. **The consultant, variety, soil type, PIX application, and previous crop differed among sites.** Farm A had not received a spray for Helicoverpa at the time that we sampled but Farms B and C were sprayed 7-14 days previously with Affirm.

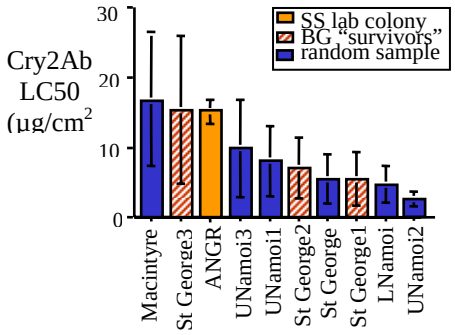
We estimated the number of larvae by sampling 1 meter of whole plants at 5 equally spaced positions along three equally spaced rows of cotton and taking an average of the 15 sampled plots for each field. **Larvae ranged between 0.9 and 2.1/m and were evenly distributed throughout the field.** We collected around 200 larvae from each site and reared them in the laboratory. **The percentage of larvae that emerged as healthy moths in the laboratory ranged between 54 and 80.** Several weeks after our initial survey, when all fields had received at least one spray for Helicoverpa, **we dug 15-20m of soil for pupae and detected between 0.4 and 0.6/m**. In the laboratory **between 42 and 58% of each collection of pupae emerged as healthy moths.**

	Farm A	**Farm B**	**Farm C**
Consultant	A	B	C
Variety	289BR	71BR	80BRF
Soil type	Sandy	Med-Clay	Med-Clay
Early PIX application?	Yes	No	No
Previous crop	Sorghum	Bollgard II	Fallow
Sprayed at time of survey?	No	Yes	Yes
No. larvae/m	2.1	1.6	0.9
Distribution of larvae in field	Even	Even	Even
% larvae to emerge as moths in laboratory	68	54	80
No. pupae/m (15-20m sample)	0.4	0.6	0.6
% pupae to emerge as moths in laboratory	50	58	42

We allocated a majority of larvae collected from each site to create three *H. armigera* colonies that were comprised entirely of survivors on Bollgard II from the three separate properties. We scored the responses of these colonies to Cry1Ac and Cry2Ab across a range of doses and compared this to our Bt-susceptible laboratory strain as well as several other strains that we created from egg collections from cotton in a number of valleys, including St George. The highest dose was similar to the discriminating dose used in our screens, and the subsequent 6 doses were each half the strength of the previous dose. Our assay therefore covered a large range of doses that should detect survival of the toxin ranging from a high level resistance to some tolerance.

The vertical axis on the graphs below shows, for Cry1Ac and Cry2Ab respectively, the dose that killed 50% of insects from each tested strain (known as the LD50). The LD50 values have been ordered from highest to lowest. **Against both toxins the responses of the survivor colonies are not significantly different from the response of our Bt susceptible laboratory strain or strains made up of randomly collected eggs from cotton in a number of different valleys.**





We assigned larvae from St George and elsewhere, and the pupae dug from under Bollgard II fields in St George, to the F_2 screens. This work is in progress but to date **we have screened 128 alleles from *H. armigera* survivors and 36 alleles from *H. punctigera* survivors and none proved positive for conferring resistance to Cry1Ac or Cry2Ab**.

For a subset of survivor larvae we have leaf samples from the host and surrounding plants. We used **qualitative** ELISA tests to analyze this material for the **presence or absence** of Cry1Ac and Cry2Ab. Note that our analyses were performed on a biased sample of Bollgard II® because only plants supporting larvae were selected and tested.

The following table summarises the results of the ELISA tests according to valley and separately for Cry1Ac and Cry2Ab. The total leaf samples include those that were the host of the larvae at the time of collection and those that immediately surrounded the host plant. We scored a sample as negative only if duplicate samples from that leaf proved negative.

A total of 7 samples of the 168 tested scored negative for Cry1Ac or Cry2Ab. In one sample from the Lachlan valley the same leaf tested negative for Cry1Ac and Cry2Ab. In one sample from the Lower Namoi the leaf tested negative for Cry1Ac and positive for Cry2Ab. In 5 samples from St George the leaf tested positive for Cry1Ac and negative for Cry2Ab.

Our data suggest that **of the samples taken from Bollgard II® plants that were hosts, or nearby hosts, of the collected surviving Helicoverpa larvae, at least one of the two Bt proteins was present in 99.4% of cases, Cry1Ac was absent and Cry2Ab was present in < 1% of cases, and Cry1Ac was present and Cry2Ab was absent in 2.9% of cases.**

Valley	Total leaf samples	No. positive samples	
		Cry1Ac	Cry2Ab
Lower Namoi	4	3	4
St George	159	159	154
Lachlan	5	4	4
Total	**168**	**166**	**162**

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