

End of December results from Bt resistance monitoring 2008/09

Changes to the monitoring program for 2008/09

The main changes at a glance

- We have increased the intensity and length of our sampling for F₁ screens and F₂ screens.
- We no longer conduct F₀ screens for Bt resistance.
- We no longer score eggs that do not successfully hatch for parasitism.

Since 2002 we used F₂ tests as the primary method for screening for Bt resistance genes in *Helicoverpa*. F₂ screens can detect heterozygote individuals (RS). They involve testing the grandchildren of pairs of moths raised from eggs collected from field populations. No alleles allowing insects to survive Cry1Ac have been detected but for *H. armigera* the baseline frequency of alleles conferring resistance to Cry2Ab is approximately 4 in 1000. The different cases of Cry2Ab resistance detected so far are all of the same kind.

To increase the number of insects that could be processed during the season CSIRO developed protocols for testing the frequency of this common Cry2Ab resistance using a shorter version of the F₂ method called an F₁ test. The methods are the same as those for the F₂ screens except that we test the F₁ generation produced from a cross between a Cry2Ab resistant moth and a field collected moth.

We expected to get the same resistance frequencies using the two methods but results from tests with *H. armigera* indicated that the Cry2Ab resistance frequency for F₁ screens is around 6 times higher than that determined with the F₂ tests. In particular, the frequency of Cry2Ab alleles in *H. armigera* at the end of the 2007/08 season was approximately 3 in 100 individuals.

Unfortunately, the frequencies obtained from the F₁ screens are likely to most accurately reflect the situation in the field. This is because, compared to F₂ screens, the F₁ screens involve one less mating cycle in the laboratory and do not involve mating among siblings, which in nature is not likely to occur often. This is a concern but even more critical for resistance evolution is whether the results recorded in 2007/08 represent a shift in the number resistant individuals being detected in the field.

For the last four years Monsanto have used the F₁ protocol developed by CSIRO to screen for resistance in *H. armigera* at a field level. In the three years from 2004/05 to 2006/07 the frequency of resistance detected in the field had remained fairly consistent with no significant differences between years. The 2007/08 combined data set from CSIRO and Monsanto however indicates that Cry2Ab resistance alleles in *H. armigera* are significantly higher than previous years. CSIRO have also detected a significant increase over time in Cry2Ab resistance alleles in *H. punctigera* using the F₂ screen method.

Consequently from 2008/09 we will prioritise increasing the intensity and length of our sampling for resistance alleles using F₁ screens and F₂ screens. In addition to providing robust estimates, this change will enable us to examine if the frequencies of Cry2Ab resistance alleles shift throughout time, for example, late in the season when the efficacy of Cry1Ac is expected to decline in Bollgard II and there may be opportunities for selection for Cry2Ab resistance.

We are no longer conducting F₀ screens. This method is efficient but is only likely to detect individuals that are homozygous resistant (RR) to Bt which are expected to be exceptionally rare. Our focus is on detecting the more common heterozygous (RS) insects that are killed by Bt toxins even though they carry a resistance allele. The detection of changes in the frequencies of these individuals would in theory allow us to adapt our resistance management strategy before homozygous resistant insects became common and field failures are apparent. In addition to detecting any RS insects, the F₁ and F₂ screens will also detect RR insects.

We are no longer scoring eggs that did not successfully hatch for parasitism as gathering this information required considerable resources and is considered to be of lower importance than robust estimates of resistance frequencies.

Hatching and species composition

Across all sampled valleys there were 15176 samples submitted to the program until 23 December 2008. The majority of samples were eggs, although there were some early season collections of larvae from chick pea. Of the eggs submitted, 58% successfully hatched. Of the eggs from cotton and pigeon pea that successfully hatched and the larvae from chick pea, 48% 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	Season total
Lower Namoi	No. of samples	1458	5315	6773
	% hatch	67 (46-83)	64 (26-96)	0.65 (26-96)
	% <i>H. armigera</i>	all Ha hosts ^c	27 (7-45)	0.27 (7-45)
Upper Namoi	No. of samples	784	3429	4213
	% hatch	67 (46-76)	60 (6-78)	0.61 (6-78)
	% <i>H. armigera</i>	all Ha hosts ^c	64 (45-98)	0.64 (45-98)
Emerald	No. of samples	988 ^a	352	1340
	% hatch	46 (15-89)	63 (33-91)	0.53 (15-91)
	% <i>H. armigera</i>	82 (16-100)	59 (44-76)	0.75 (16-100)
North Queensland	No. of samples	38 ^b	-	38
	% hatch	NA	-	NA
	% <i>H. armigera</i>	100 ^d	-	100 ^d
St George	No. of samples	-	1463	1463
	% hatch	-	61 (33-86)	0.61 (33-86)
	% <i>H. armigera</i>	-	49 (27-67)	0.49 (27-67)
Gwydir	No. of samples	538	605	1143
	% hatch	77 (68-83)	70 (52-88)	0.74 (52-88)
	% <i>H. armigera</i>	16 (11-19)	all Ha hosts ^c	0.16 (11-19)
Macintyre	No. of samples	-	871	871
	% hatch	-	17 (1-34)	0.17 (1-34)
	% <i>H. armigera</i>	-	15 (0-52)	0.15 (0-52)
Macquarie	No. of samples	-	175	175
	% hatch	-	63 (8-91)	0.63 (8-91)
	% <i>H. armigera</i>	-	23 (0-25)	0.23 (0-25)

^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.

So far in 2008/09 we screened 156 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 0/3558. **We isolated no 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 8/3570 (0.0022).

So far in 2008/09 we screened 32 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 0/3006. **We isolated 1 case 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 17/3010 (0.0056) which is similar to that in previous years. We have tested 10 of these 17 cases and all are of the same kind of Cry2Ab resistance.

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	156	0	156	0
	Total	3558	0	3562	8
<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	32	0	32	1
	Total	3006	0	3010	17

F₁ screens for SP15-like Cry2Ab resistance in H. armigera

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 a Cry2Ab resistant strain maintained in the laboratory (SP15) and moths raised from eggs collected from field populations. They take around 5 weeks to complete.

So far in 2008/09 we screened 136 alleles from *H. punctigera* and **isolated 1 case of alleles conferring resistance to Cry2Ab** (see Table below). For *H. punctigera* the cumulative frequency of alleles conferring resistance to Cry2Ab since the CSIRO program began (2007/08) is 3/332. This frequency is similar to that obtained in 2007/08, i.e., 0.01.

So far in 2008/09 we screened 300 alleles from *H. armigera* and **isolated 8 cases of alleles conferring resistance to Cry2Ab** (see Table below). For *H. armigera* the cumulative frequency of alleles conferring resistance to Cry2Ab since the CSIRO program began (2007/08) is 17/578. This frequency is similar to that obtained in 2007/08, i.e., 0.03.

Species	Year	Cry2Ab F ₁ screen		
		alleles tested	scored positive	frequency
<i>Helicoverpa punctigera</i>	2007/08	194	2	0.010
	2008/09	136	1	0.007
	Total	332	3	0.009
<i>Helicoverpa armigera</i>	2002/03	278	9	0.032
	2008/09	300	8	0.027
	Total	578	17	0.029

'Survivors' from Bollgard II® plants

There were reports from late-December of up to 1.5 medium-large larvae/m in Bollgard II fields on some properties in Emerald. All affected fields were at mid-flowering to late-flowering. Of the 85 *Helicoverpa* larvae received from Bollgard II plants in Emerald, **31 were *H. punctigera* and 54 were *H. armigera*.** This value is similar to the % of *H. armigera* in the sample of eggs collected during December in Emerald, and does not suggest that either species may be differentially surviving on Bollgard II. As in previous seasons, we will submit healthy moths from these collections to the F₂ screening component of the Bt resistance monitoring program.

Our results since the program began (2005/06) suggests that in both species there is **no significant difference among Bollgard II survivors vs. a random sample** in the cumulative frequency of alleles conferring Cry2Ab resistance. In order to keep a check on resistance we need to continue to sample larvae collected as survivors on Bollgard II.

If your crop reaches or is close to threshold, please let myself, Trudy Staines and/or your RCEO know ASAP so that we can try and arrange for samples to be collected for further testing.

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