Helicoverpa: Species mix, parasitism and resistance monitoring

By Louise Rossiter[1], Sharon Downes and Rod Mahon

The development of resistance by *Helicoverpa armigera* and *H. punctigera* to insecticides or the Bt (*Bacillus thuringiensis*) toxins in Bollgard II is a major threat to their successful control. Monitoring insect field populations enables early warning of the onset of resistance and thereby permits an opportunity to refine our resistance management strategies to maintain their effectiveness.

Two research programs, based at the Australian Cotton Research Institute in Narrabri, are currently monitoring resistance in all the major cotton growing areas of Australia. CSIRO manage a project that measures resistance frequencies to the Bt toxins in Bollgard II, (Cry 1Ac and Cry 2Ab). The NSW DPI runs a project that monitors resistance levels to over 10 different insecticides used against Helicoverpa species, encompassing both the IPM compatible soft chemistries and the broad spectrum, 'harder' chemistries.

In addition to information on resistance frequencies in the field, other ecological data are gained including the species mix (*H. armigera* or *H. punctigera*), levels of parasitism of eggs by Trichogramma wasp and the viability of Helicoverpa eggs. The major findings from both projects during the past three years, and their implications for pest and resistance management, are presented in this article.

Technical Officer Fiona Rayner (NSW DPI) applies a calibrated dose of insecticide to a *H. armigera* larva during a resistance screen.

Species mix

For our work, it is important to know which species of Helicoverpa is tested for susceptibility. This is because *H. armigera* has a history of evolving resistance to a range of insecticides and therefore presents a major resistance risk, whereas *H. punctigera* has never developed field resistance. For the same reason, *H. armigera* is also likely to pose a major resistance threat to Bt toxins.

The 2003–04 and 2004–05 seasons were dominated by high *H. punctigera* pressure, which began at the start of the season and continued into January and February in some areas. Very low *H. armigera* pressure was observed in most areas across both seasons, particularly in 2004–05, except in the Darling Downs (Table 1). Insecticides and Bollgard II both provided good control of populations dominated by *H. punctigera*. But the high numbers present last season meant that control was required through to mid season in some areas.

While egg collections available for resistance testing in the 2002–03 season yielded reasonable numbers of *H. armigera*, this species was poorly represented in samples during the past two seasons. Consequently, measures of resistance levels in these years to the Bt toxins or insecticides have not been as robust as in 2002–03. Where egg collections were limited, the newer IPM compatible insecticides of greatest interest and use were preferentially tested at the expense of 'older' broad-spectrum chemistries.

Over the past three seasons, the Bt monitoring program has focussed on refining the techniques used to detect resistance genes present in Helicoverpa populations as well as investigating the frequency of resistance and identifying possible trends over time.

BT RESISTANCE MONITORING RESULTS: 2002–05

During the past three seasons, resistance screens were conducted using grubs collected directly from the field as eggs. These are known as F_0 screens (F_0 are the field collected grubs; F_1 are their offspring; and, F_2 are the offspring of the F_1 — the grandchildren of the F_0).

Approximately 1600 *H. armigera* and 2500 *H. punctigera* were challenged with various Bt products. F_2 screens were also done with approximately 400 *H. armigera* and 500 *H. punctigera*, and scored for the presence of genes conferring resistance to Cry1Ac and Cry2Ab (Table 2).

Data from both the F_0 and F_2 tests suggest that there is no immediate cause for concern for the longevity of the extremely valuable transgenic technology currently available to Australian cotton growers. Furthermore, there have not been any reported field failures of Bollgard II due to resistance, despite the occasional presence of larvae on transgenic plants. During the 2004–05 season, a total of 78 medium/large grubs found in Bollgard II fields were sent to the ACRI for testing but none proved to be resistant to either Cry1Ac or Cry2Ab.

Importantly, a gene that confers high level resistance to Cry2Ab is present in field populations of *H. armigera*. This gene does not confer resistance to Cry1Ac. CSIRO data (Table 2) indicate the

TABLE 1: Collection information for 2004–05 (percentage *H. armigera* determined only from crops attracting both species)

Valley	Trait	November	December	January	February	March	Season total
Gwydir	number of eggs	0	4200	6963	509	0	**11,672**
	% hatch	–	62	48	27	–	**52**
	% *H. armigera*	–	2	30	7	–	**17**
	% parasitism	–	1	24	40	–	**17**
Darling Downs	number of eggs	329	4033	2497	1879	711	**9449**
	% hatch	18	60	53	42	33	**50**
	% *H. armigera*	9	6	38	73	92	**28**
	% parasitism	–	7	24	33	49	**24**
Macintyre	number of eggs	0	5318	5703	1399	96	**12,516**
	% hatch	–	63	40	54	39	**52**
	% *H. armigera*	–	6	18	5	65	**10**
	% parasitism	–	5	39	31	–	**22**
Emerald	number of eggs	761	318	219	–	–	**1298**
	% hatch	37	30	25	–	–	**33**
	% *H. armigera*	21	21	29	–	–	**22**
	% parasitism	–	–	26	–	–	**26**
St George/ Dirrinbandi	number of eggs	1609	–	299	77	–	**1985**
	% hatch	76	–	42	35	–	**69**
	% *H. armigera*	0	–	24	14	–	**2**
	% parasitism	1	–	21	27	–	**5**
Lower Namoi	number of eggs	6371	7415	9959	713	3112	**27,570**
	% hatch	52	73	44	58	41	**54**
	% *H. armigera*	0.5	4	14	56	69	**10**
	% parasitism	2	15	26	5	38	**21**
Riverina	number of eggs	312	192	129	95	–	**728**
	% hatch	70	70	38	67	–	**64**
	% *H. armigera*	0	0	2	5	–	**1**
	% parasitism	–	18	0	48	–	**35**
Macquarie	number of eggs	179	5555	224	44	–	**6198**
	% hatch	38	65	66	44	–	**63**
	% *H. armigera*	6	7	10	14	–	**7**
	% parasitism	–	1	19	58	–	**5**
Upper Namoi	number of eggs	1045	5543	4100	5131	–	**15,819**
	% hatch	89	65	67	44	–	**60**
	% *H. armigera*	0	6	3	21	–	**7**
	% parasitism	1	7	10	39	–	**18**
Walgett	number of eggs	0	0	2273	2583	0	**4856**
	% hatch	–	–	61	57	–	**59**
	% *H. armigera*	–	–	20	15	–	**17**
	% parasitism	–	–	24	31	–	**26**

form of the gene that confers resistance was detected on three occasions out of the 780 genes examined.

There is also a gene present in field populations of *H. armigera* that confers high-level resistance to Cry1Ac. But this gene is rare and has not yet been detected among the 780 F_2 screens performed (Table 2). This gene does not give cross-resistance to Cry2Ab.

Cry2Ab resistance genes were far more common in natural populations than expected, especially as to date there has been little opportunity for selection in cotton fields. Indeed, the first case of Cry2Ab resistance was isolated before Bollgard II was released commercially.

Despite the unexpectedly high frequency of genes that confer resistance to Cry2Ab, our current knowledge of the ecology and resistance profiles of Australian *H. armigera* populations suggest the Resistance Management Plan (RMP) is adequate for preventing a rapid increase in the frequency of this form of resistance.

Computer models that incorporate all our current knowledge of resistance frequencies for both Cry1Ac and Cry2Ab, fitness costs, degree of dominance and refuge size, suggest that Bollgard II should prove effective at managing Helicoverpa species in the medium to long term.

INSECTICIDE RESISTANCE MONITORING RESULTS: 2002–05

The following is a summary of the resistance situation for the insecticides used against Helicoverpa species.

H. armigera:

Spinosad (Tracer): The window for Tracer use was restricted in the 2002–03 IRMS due to a low but increasing resistance trend. Fortunately, monitoring over the past three seasons indicates this trend has reversed. In 2004–05, occasional resistant individuals were detected, indicating that resistance genes persist in the

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populations but at such a low frequency that field efficacy would not be compromised.

Indoxacarb (Steward) and **Emamectin Benzoate (Affirm):** Insects resistant to Steward or Affirm have been detected in the past three seasons across most cotton valleys but there is no indication of resistance 'hotspots' or of an increasing frequency of resistance to either chemical. Again, efficacy would not be threatened.

Chlorfenapyr (Intrepid): Varying frequencies of resistance to Intrepid have been detected since 1997–98. Use of this chemical has declined significantly in the past two seasons, with resistance frequencies mirroring the decline. Intrepid use is expected to be low in 2005–06.

Methoxyfenozide (Prodigy): No resistance has been detected to Prodigy since its introduction in the 2003–04 season, but limited testing has been conducted because of the low numbers of *H. armigera* available.

Endosulfan and pyrethroids: Resistance to both chemicals has been present for over 20 years, and while pyrethroid resistance is firmly established, endosulfan resistance frequencies vary and strongly reflect recent use patterns in different areas.

Carbamates (for example, methomyl): Limited carbamate monitoring has been conducted over the past three years as resistance was known to be widespread. Testing during 2003–04 confirmed that resistance remains prevalent across most cotton growing regions.

Chlorpyrifos and profenofos: Monitoring conducted prior to the 2002–03 season indicated that profenofos resistance occurred at low to moderate frequencies across all cotton growing regions. The limited tests performed during the past three seasons indicate that there had been little change. Conversely, while chlorpyrifos resistant individuals have been detected since 2001–02, the frequency of resistance has remained very low. There is no cross resistance between these two organophosphates.

Ovicides (Amitraz and Methomyl): No resistance has been detected to either ovicide.

Resistance management tactics for *H. armigera*, incorporating restrictions on chemical use backed up by non-chemical control measures appear to have successfully delayed resistance to the newer IPM-compatible chemistries. They have also ensured that those insecticides used in the industry for many years are still available for use.

There are multiple options available to control this pest that incorporate both chemical and non chemical tactics, allowing for an integrated approach to pest control that confers significant benefits for resistance management. It is important all of these tools are used including adherence to the annual IRMS to ensure the continued efficacy of the available insecticides.

H. punctigera:

Unlike *H. armigera*, *H. punctigera* has not developed field resistance to insecticides despite more than 20 years of selection with some insecticides. Consequently, if both species are readily available, the monitoring programs focus their attention on *H. armigera*. But some *H. punctigera* are always tested.

For this species, endosulfan and pyrethroids are monitored due to their long term use (greater than 20 years). Abamectin is also tested as it specifically targets *H. punctigera*. There has been an abundance of *H. punctigera* available for testing during the past three seasons, but as expected, there is no evidence of the development of resistance to any of the tested chemicals.

Trichogramma parasitism and egg mortality

Records of Trichogramma parasitism and egg mortality are kept for all egg collections used in the resistance monitoring programs. In 2004–05, Trichogramma parasitism was detected in all the cotton valleys at some time during the season (Table 1). There was a general trend toward increasing parasitism levels through the season, with some late season individual collections showing high rates of parasitism.

But Trichogramma parasitism did not account for total egg mortality (Table 1). For example in February in the Gwydir Valley only 27 per cent of eggs hatched. Of the remaining 73 per cent, 40 per cent were parasitised and 33 per cent failed to hatch for unknown reasons.

In the field, egg mortality may result from numerous factors, in addition to parasitism, including desiccation, dislodgment from the plant by wind or rain, and predation. High levels of egg mortality have obvious implications for pest pressure and control in the field. For the monitoring programs it means increased requirement for larger egg collections.

OUTLOOK FOR 2005–06

Weather patterns during winter/spring have ensured an abundance of non-crop hosts in inland Australia and the cotton growing regions of NSW and Queensland. Conditions have been ideal for pest populations to increase in numbers prior to the cotton season.

Pheromone trap monitoring and winter/spring sampling of crop and non-crop hosts by Colin Tann (CSIRO Entomology) indicate a typical early season abundance of *H. punctigera*. *H. armigera* was also detected in September/October samples. At the time of writing (early November) moderate-heavy pressure from *H. punctigera* is anticipated early season, with potential for significant numbers of *H. armigera* later in the year.

TABLE 2: Summary information for the F_2 screens according to species (*H. armigera* versus *H. punctigera*), season and Bt toxin

Toxin	Species	Season	No. tests	No. resistant	Frequency
Cry1Ac	*H. armigera*	2002–03	136	0	0
		2003–04	280	0	0
		2004–05	364	0	0
		TOTAL	**780**	**0**	**0**
	H. punctigera	2002–03	8	0	0
		2003–04	60	0	0
		2004–05	1012	0	0
		TOTAL	**1080**	**0**	**0**
Cry2Ab	*H. armigera*	2002–03	132	1	0.008
		2003–04	284	2	0.007
		2004–05	368	0	0
		TOTAL	**784**	**3**	**0.004**
	H. punctigera	2002–03	8	0	0
		2003–04	60	0	0
		2004–05	1024	1	0.009
		TOTAL	**1092**	**1**	**0.009**

For the long term effectiveness of both insecticides and Bollgard II, it is important that resistance management strategies are adhered to and become an essential part of an integrated approach to pest management. While it may be tempting, it is important that growers do not spray early season susceptible *H. punctigera* with a pyrethroid or similarly hard chemistry.

This can select for resistant *H. armigera* that may be present at a low frequency, and also has adverse effects on beneficial insect populations that are important in avoiding subsequent flaring of secondary pests such as aphid, mite or whitefly populations.

Likewise, the RMP for transgenic cotton is important. Appropriate refuges should be provided when growing Bollgard II and they should be managed to remain attractive to egg-laying moths and suitable for larval survival in order to effectively dilute resistant individuals that may survive within a Bollgard II crop.

How can you help?

The most important component of the monitoring programs that are essential for resistance management are field-collected eggs. Collections of eggs on leaves from growers, consultants or other interested parties are encouraged.

In the event of a suspected spray failure, eggs should be collected and sent to the ACRI to determine if the poor control was due to resistance or other causes. Likewise larvae found in a Bollgard II crop should also be sent to ACRI, preferably along with a sample of the plant they were consuming to determine if they are resistant to either Bollgard II toxin and also to determine if the host plant was expressing toxins.

Information on species mix, parasitism and egg mortality can be provided for specific collections approximately 10 days after they are received. Relevant data are also distributed on a valley wide basis during the season through the local IDO or DA.

For more information about the resistance programs, contact Louise (insecticides) or Sharon (Bt toxins) at the ACRI, Narrabri, Ph: 02 6799 1500.

Louise or Sharon can also be contacted regarding collection requirements, which are detailed in a previous article in *The Australian Cottongrower*, October–November 2004.

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1. NSW Dept of Primary Industries.

2. CSIRO Entomology.