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Pyrethroid and carbamate resistance in Australian *Helicoverpa armigera* (Lepidoptera: Noctuidae) from 2008 to 2015: what has changed since the introduction of Bt cotton?

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Abstract

Pyrethroid and carbamate resistance was evaluated in *Helicoverpa armigera* from 2008 to 2015. Insects were collected as eggs primarily from cultivated hosts in the major cropping areas of New South Wales and Queensland, Australia. Larvae reared from eggs were tested for resistance to fenvalerate, bifenthrin or methomyl in the F_0 generation using a topical application of a discriminating dose of insecticide. In 2008–2009, resistance to fenvalerate was 71% and no resistance to bifenthrin was recorded. In the following two seasons, resistance to pyrethroids was relatively stable with fenvalerate resistance ranging from 63% to 67% and bifenthrin resistance ranging from 5.6% and 6.4% in 2009–2010 and 2010–2011, respectively. However, in 2011–2012, pyrethroid resistance had increased to 91% and 36% for fenvalerate and bifenthrin, respectively. Resistance remained above 90% for fenvalerate and above 35% for bifenthrin in the following three seasons from 2012 to 2015. In 2008–2009, methomyl resistance was 33% and declined to 22% and 15% in 2009–2010 and 2010–2011, respectively. Methomyl resistance remained at moderate levels from 2011–12 to 2014–15, ranging from 21% to 40%. Factors that influenced selection pressure of pyrethroid and carbamate insecticides and impacted resistance frequency in *H. armigera* may have been associated with changes in the composition of the cropping landscape. The rapid expansion of the pulse industry and the commensurate increased use of insecticide may have played a role in reselection of high-level pyrethroid resistance, and highlights the need for an urgent and strategic response to insecticide resistance management in the Australian grains industry.

Keywords: cotton bollworm, transgenic crops, insecticide resistance monitoring, resistance management

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Introduction

The cotton bollworm, *Helicoverpa armigera* (Hübner) is one of the most serious global insect pest species of agriculture having by far the most reported cases of insecticide resistance worldwide, including field-evolved resistance to pyrethroids, carbamates, cyclodienes, and organophosphates (McCaffery, 1998), spinosad (Gunning, 2002), indoxacarb (Bird, 2016) and toxins derived from *Bacillus thuringiensis* (Mahon *et al.*, 2007). This capacity to develop resistance is associated with its highly polyphagous nature, wide geographical distribution (Zalucki *et al.*, 1986; Fitt, 1989) and an ability to migrate long distances (Feng *et al.*, 2005). Historically, *H. armigera* had a range of distribution throughout the Old World, with more recent work confirming a major incursion into South America (Czepak *et al.*, 2013; Mastrangelo *et al.*, 2014; Murúa *et al.*, 2014).

In Australia, there is considerable experience in managing *H. armigera* in agricultural systems. Following the widespread

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failure of insecticides to control field populations of *H. armigera* in the 1980s (Gunning *et al.*, 1984), a windows-based Insecticide Resistance Management Strategy (IRMS) was designed and implemented to manage resistance by restricting the use mode-of-action group by rotation, and the number of applications (Forrester *et al.*, 1993). In addition, a monitoring programme was established to detect changes in insecticide resistance frequencies based on a discriminating dose technique highly effective for detecting incipient resistance. This programme was important for assessing the effectiveness of IRMS and also provided a basis for the timely implementation of pre-emptive responses to control resistant populations (Forrester & Bird, 1996; Rossiter *et al.*, 2008). Although the IRMS did not overcome pyrethroid resistance in Australian *H. armigera*, it proved to be an effective tactic for delaying resistance and extending the useful life of these insecticides prior to the commercialization of genetically modified cotton producing the δ-endotoxin genes of *B. thuringiensis* subsp. *kurstaki* (Bt). The phased introduction of commercial scale Bt cotton to control *H. armigera* and the native *Helicoverpa punctigera* (Wallengren) began with the single gene variety, Ingard® (producing Cry1Ac toxin) in 1996, followed by two gene cotton, Bollgard II® (producing Cry1Ac and Cry2Ab) in 2003.

Concurrent with the introduction of Bt cotton was a reduction in the number of insecticide sprays for *Helicoverpa* spp. on a per hectare basis by 59% in the first 8 years of deployment when the cotton industry was reliant upon the single gene variety Ingard® (Fitt, 2008). During this time, Ingard® cotton was restricted to 30% of the total area of the cotton crop in Australia as a precautionary measure to reduce the risk of resistance to the Cry1Ac toxin. However, strong reliance on insecticides continued on the remaining 70% of non-Bt cotton, particularly during the latter part of the growing season due to the gradual decline of expression of Cry1Ac in Ingard® cotton, resulting in ongoing selection for resistance to insecticides in *H. armigera*. Following the introduction of Bollgard II®, which provided season-long protection of the crop from *Helicoverpa* spp., there was a further reduction (up to 90%) in the total number of spray applications required (Fitt, 2008). This resulted in tangible benefits to the environment (Knox *et al.*, 2006), provided a platform for integrated pest management (IPM) programmes and reduced selection pressure for conventional insecticide resistance in *H. armigera* (Wilson *et al.*, 2013).

Prior to the introduction of Bt cotton, heavy reliance on broad-spectrum insecticides selected for high resistance to pyrethroids (Forrester & Bird, 1996) and moderate resistance to carbamates (Gunning *et al.*, 1992) in *H. armigera*. Preferential use of relatively new classes of insecticides such as spinosad, emamectin benzoate and indoxacarb led to incipient resistance to all three insecticides by 2002. However, following the reduction in insecticide sprays targeting *Helicoverpa* spp. associated with high uptake of transgenic varieties, and particularly after commercialization of Bollgard II®, resistance to all chemical classes of insecticide declined and in some cases reverted to baseline levels. For example, resistance to bifenthrin increased steadily from 30% to over 60% over six seasons from 1996 to 2002 and then declined steadily during the following five seasons to 2007 when resistance to bifenthrin could not be detected (Rossiter *et al.*, 2008).

The present study reports pyrethroid and carbamate resistance collected from a wide range of cropping hosts intensively managed for *H. armigera* in eastern Australia from 2008 to 2015, and compares annual frequencies over this 7-year period to frequencies observed prior to the introduction of Bollgard II®. Methods of resistance monitoring have remained consistent with those used since the monitoring programme was first implemented, therefore ensuring continuity and compatibility of monitoring outcomes from this and previous studies. The work was also interested in exploring factors that may have influenced selection pressure imposed by pyrethroids and carbamates and associated changes in resistance frequency to these insecticides, particularly changes in the proportion of alternative host crops managed for *H. armigera* as a possible explanation for increased insecticide use.

Materials and methods

General rearing methods

Methods used to rear *H. armigera* were similar to those described by Teakle & Jensen (1985), except that formalin was omitted, and soybean flour was baked at 200°C for 10 min to remove enzyme inhibitors which can interfere with bioassays. Neonates were individually transferred to 45-well plastic trays (Tacca Plastics, Sydney, Australia) containing approximately 1.5 ml of diet and heat-sealed with perforated lids (Oliver Products, Grand Rapids, MI, USA). When larvae reached the late fourth or early fifth instar, they were transferred to fresh plastic trays containing diet. Larvae were allowed to pupate in these trays and when hardened they were removed, washed in 1% bleach solution and transferred to 5 litres round, plastic containers (21 cm diameter). A 14 cm diameter hole was cut into the lid of the container and used to secure a cloth liner which acted as an oviposition substrate for the emerging moths. Moths were provided with approximately 30 ml of 4% honey/sugar solution held in 50 ml plastic containers with a hole in the lid to accommodate a cotton wick. Eggs were collected daily by replacing the cloth liners which were washed in a 1% bleach solution and collected onto a Whatman No. 54 filter paper by vacuum filtration. Filter papers were allowed to air dry and were then placed in sealed plastic bags until neonates hatched.

In the larval stage, insect strains were maintained in a laboratory environment of 25 ± 2°C with 14:10 (L: D) hour photoperiod and ambient RH. Adults were maintained in a separate facility under the same conditions of light and temperature and RH.

Laboratory reference strains

Discriminating doses of pyrethroids and carbamates used in this study were based on data accumulated during the 1980s and 1990s (Gunning *et al.*, 1987, 1992; Forrester *et al.*, 1993) which was similar to a laboratory reference strain, known as the SUS strain, established from *H. armigera* collected from sorghum at Gatton, southern Queensland in 1979. The SUS strain was replaced by the New GR strain established from a series of collections made in cotton fields in the Namoi Valley, northern New South Wales during the mid-1980s and was supplemented with field-collected insects intermittently from 1990 to 2000. The New GR stain was bioassayed at intervals throughout this study to compare the dose response with that of the original SUS strain.

Sampling locations

Six geographically distinct areas across New South Wales and Queensland were sampled between September 2008 and March 2015 (fig. 1). The majority of samples were collected in

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Fig. 1. Map of eastern Australia depicting the major cropping areas, which correspond to those listed in table 2.

the Namoi and Gwydir valleys in northern New South Wales (30°19′57″S, 149°46′52″E; elevation 216 m), with smaller scale sampling conducted in the central New South Wales region of the Macquarie valley (31°42′9″S, 147°48′58″E; elevation 198 m) and in the Murrumbidgee Irrigation Area (MIA) (34°16′48″S, 146°2′44″; elevation 144 m) in the south of the state. Sampling locations close to the New South Wales and Queensland border are hereafter referred to as the Border Rivers region (28°29′12″S, 149°28′12″E; elevation 189 m). A further two regions were regularly sampled throughout the study; the Darling Downs region in southern Queensland (27°11′40″S, 151°15′57″E; elevation 341 m) and the Emerald region in central Queensland (23°31′38″S, 148°9′52″E; elevation 179 m).

Field sampling procedure

Visual searches for *Helicoverpa* spp. eggs were conducted in a range of cultivated hosts (primarily cotton, sorghum, maize and pulses) and the scrophulariaceous weed host, *Verbascum virgatum*. Collection sites had not been treated with insecticide at the time of sampling. Eggs (comprising a mixture of both *H. armigera* and *H. punctigera*, in unknown proportions) were collected at random across a wide geographical range in each sampling region and from a range of *H. armigera* host crops. The objective of each field collecting trip was to source between 100 and 200 eggs from any one individual farm location.

Field-collected eggs were removed from plant material using a fine hair paintbrush, transferred, one egg per well, to 45-well plastic trays and sealed, as above. Eggs were checked for the presence of parasites. The egg parasite, *Trichogramma* spp., occasionally caused high levels of egg mortality in samples. The solitary egg-larval braconid wasp parasite, *Chelonus* spp. was also occasionally present in samples but easily identified by characteristic developmental arrest in *H. armigera* larvae and the precocious onset of metamorphosis in parasitized larvae which were subsequently removed from the test cohort. Hatched larvae were identified to species at the second or third instar as either *H. armigera* or *H. punctigera*. The *H. armigera* larvae were then reared, as described above, to the appropriate size for resistance testing.

Insecticides

Insecticide solutions used in all bioassays were prepared from technical material dissolved in analytical grade acetone. Fenvalerate (95.3%) was provided by Sumitomo Chemical (Sydney, Australia); bifenthrin (93.3%) was provided by FMC (Brisbane, Australia); methomyl (98%) was provided by Bayer CropScience (Melbourne, Australia). Discriminating dose bioassays were performed using concentrations previously determined for fenvalerate as 0.125 µg/larva (Gunning *et al*., 1984), bifenthrin as 0.1 µg/larva (Forrester *et al*., 1993) and methomyl as 1 µg/larva (Gunning *et al*., 1992). The insecticide solutions used in control bioassays of the susceptible strain were prepared as twofold serial dilutions corresponding to six or seven insecticide concentrations which were expected to induce 1–99% mortality in 30–40 mg *H. armigera* larvae.

The insecticidal classes available for management of *H. armigera* throughout the duration of this study were widely registered for use across all crop types sampled, with the exception of pigeon pea when used a structured refuge for transgenic cotton. Registered insecticidal classes included pyrethroids, carbamates, organophosphates, spinosad and indoxacarb. During the span of this study, there were no additional registrations of pyrethroid or carbamate insecticidal classes. The diamide class of insecticides was introduced in Australia 2008 with chlorantraniliprole initially registered in cotton and then extended to pulses in mid-2014. A second diamide insecticide cyantraniliprole was also first registered in cotton in late-2013.

Bioassays

Larvae collected as eggs from the field were reared to the third or fourth instar. Larvae within a weight range of 30–40 mg were transferred to fresh diet and allocated randomly to insecticide treatment groups. Each larva was treated by topical administration of 1 µl of acetone/insecticide solution applied to the dorsal thorax using a 50 µl micro-syringe in a repeating dispenser (Hamilton Company, Reno, NV, USA). Trays containing tested larvae were covered with heat-sealed perforated lids.

Control bioassays were performed in triplicate with individual treatments (insecticide concentrations) in replicates consisting of a minimum of 20–30 individuals; acetone alone was used as the control. Control bioassays were performed on three non-synchronous cohorts of New GR and the results

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were pooled in the final analysis because there were no significant differences between cohorts for any of the insecticides tested. The LD_{50} generated from the pooled result was used as the estimate of baseline susceptibility for the New GR strain. Pyrethroid and methomyl bioassays were maintained for 3 and 4 days, respectively, under the same conditions described above for larval rearing and assessed for mortality based on the inability to demonstrate coordinated movement when prodded with a blunt probe.

Insecticide usage data and cropping statistics

Data for the volume of pyrethroid and carbamate insecticides used by the cotton industry during the study period were provided by the association of Crop Consultants Australia Incorporated (CCA). The CCA coordinated the collection of survey data across key cotton growing regions of Australia through its annual cotton market audit survey. Data for the hectares sown to the major broad-acre crops in New South Wales and Queensland from 2001 to 2014 were obtained from the Australian Bureau of Agriculture and Resource Economics (ABARES, 2016).

Data analysis

The dose responses from bioassays of *H. armigera* to fenvalerate, bifenthrin and methomyl were corrected for control mortality using the formula of Abbott (1925). Slope, LD_{50} and $LD_{99.9}$ estimates, and associated 95% fiducial limits (FLs) were calculated by probit analysis using the POLO-PC software (LeOra Software, Berkeley, CA, USA). Toxicity ratios were calculated by dividing the LD_{50} value of the New GR strain (pooled result of three non-synchronous cohorts) by the LD_{50} value of the SUS laboratory strain. The proportions of annual larval survival at the discriminating dose for each insecticide were adjusted using a generalized linear model with year as the explanatory factor (R Development Core Team, 2012) to account for unequal sample size between regions and within years. Binomial standard errors were calculated using the formula of Forrester *et al.* (1993).

Results

Discriminating dose calibration

The comparison of dose responses of the reference strains, New GR and SUS, is shown in table 1. The median lethal concentration of fenvalerate was similar in both strains with LD_{50} values of 0.043 and 0.034 in the New GR and SUS strains, respectively, and resulted in a toxicity ratio of 1.3. There was significantly higher sensitivity to bifenthrin in the New GR strain compared with the SUS strain, which resulted in a toxicity ratio of 0.5. Tolerance to methomyl was considerably higher in the New GR strain compared with the SUS strain, with a toxicity ratio of 4.8.

Insect sampling

The regions sampled were largely mixed cropping landscapes comprising a continuum of hosts including cotton, pulses and coarse grains which were high value commodities managed intensively for *H. armigera*. The crops sampled annually in each region are shown in table 2 with data combined for all crop types, as in other similar studies (Forrester *et al.*, 1993; Mahon *et al.*, 2007) based on relative spatial homogeneity of resistance frequency due to the absence of reproductive isolation between cohorts of adults and a high level of gene flow between habitats (Gunning & Easton, 1989; Glenn *et al.*, 1994).

The range of crops sampled within regions varied between years with cropping diversity dependant largely on factors such as rainfall and commodity prices. A high level of sampling diversity was achieved in the Namoi/Gwydir region with insects routinely collected from cotton, maize, sorghum, mung beans, soya beans, pigeon pea, sunflowers and *V. virgatum*. There was moderate sampling diversity in the Macquarie Valley, Border Rivers and Darling Downs regions with regular seasonal planting of cotton, maize and pigeon pea and opportunistic plantings of sorghum and summer pulses in some years when timing of rainfall was favourable. The regions with the lowest sampling diversity were Emerald and the MIA. The total number of sites sampled in each region annually is shown in table 2. The numbers of insects sourced from sampling regions was influenced by population abundance but generally ranged between 100 and 200 eggs from each individual farm location; the minimum number sampled from any individual farm location was 20 eggs and the maximum number sampled from any individual farm location was 1482 eggs. The most intensely sampled region was the Namoi/Gwydir valley with the highest number of sites sampled in 2011–12. Extensive sampling was also conducted in the Darling Downs and Border Rivers regions in most years. However, due to low population abundance across many regions during 2013–14 testing was restricted to insects collected in New South Wales only.

Fenvalerate resistance

Annual resistance frequencies for fenvalerate at each region are shown in table 2. The average annual survival of larvae from all regions and crop types at the discriminating dose of fenvalerate are shown in fig. 2. During the seasons from 2008–09 to 2010–11, fenvalerate resistance ranged from 61% to 70% in the Namoi/Gwydir region and was similar to that recorded from populations sampled from the Border Rivers area, the only other region where fenvalerate resistance was monitored during this time period (table 2). In 2011–12, resistance increased to 90% in the Namoi/Gwydir region, which was consistent with elevated resistance in the other locations sampled in both New South Wales and southern Queensland. Average resistance to fenvalerate persisted at levels above 90% for the remainder of the study (fig. 2).

Bifenthrin resistance

While no resistance was detected to bifenthrin in 2008–09, resistance levels in 2009–10 and 2010–11 increased to 5.6% and 6.4%, respectively (fig. 2). In contrast to low-level bifenthrin resistance experienced prior to 2011, resistance to this insecticide increased markedly in 2011–12 to 39% in the Namoi/Gwydir region, 38% in the Macquarie valley and MIA, 45% in the Darling Downs and 50% in the Border River region (table 2). Elevated levels of resistance persisted in all locations during 2012–13 ranging from 30% in the Darling Downs to 48% at Emerald. Average annual bifenthrin resistance increased further in 2014–15 to 51% (fig. 2) with marked between-site sample size variability due to low insect pressure in some regions such as the Darling Downs and MIA (table 2).

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Table 1. Bioassays of fenvalerate, bifenthrin and methomyl against a susceptible laboratory strain of *Heliothis armigera*, New GR compared with historical baseline data for the SUS laboratory susceptible strain of *H. armigera*.

Insecticide	Strain	LD_{50} [μg ai/larva] (95% FL)	$LD_{99.9}$ [μg ai/larva]	Slope ± se	χ^2 (df)	*P*	Toxicity ratio[1]	Reference
Fenvalerate	New GR	0.043 (0.038, 0.048)	0.2691	3.9 ± 0.35	8.64 (4)	0.071	–	–
Bifenthrin	New GR	0.012 (0.010, 0.013)	0.0561	4.5 ± 0.49	6.84 (3)	0.077	–	–
Methomyl	New GR	1.067 (0.853, 1.421)	63.925	1.7 ± 0.22	2.82 (3)	0.420	–	–
Fenvalerate	SUS	0.034 (0.028, 0.040)	–	3.0	–	–	1.3	Forrester *et al.* (1993)
Bifenthrin	SUS	0.026 (0.023, 0.030)	–	3.7	–	–	0.5	Forrester *et al.* (1993)
Methomyl	SUS	0.220 (0.170, 0.280)	–	3.1	–	–	4.8	Gunning *et al.* (1992)

[1]Toxicity ratio is LD_{50} value of New GR/LD_{50} value of SUS strain.

Table 2. Pyrethroid and carbamate resistance in *Heliothis armigera* collected from a range of hosts in six sampling areas (Namoi/Gwydir and Macquarie River valleys, and Murrumbidgee Irrigation Area (MIA) in New South Wales, Border Rivers regions of northern New South Wales and southern Queensland, Darling Downs region of southern Queensland, and the Emerald irrigation area of central Queensland). Results are expressed as the percentage of larvae (reared from field-collected eggs) surviving the discriminating dose (0.125, 0.1 and 1.0 μg/larva of fenvalerate, bifenthrin and methomyl, respectively) ± pooled binomial standard error. *n* = number of larvae tested.

Sampling area	Year	Collection data		Fenvalerate		Bifenthrin		Methomyl	
		Number of farms sampled	Crop type sampled[1]	% survival ± se	*n*	% survival ± se	*n*	% survival ± se	*n*
Namoi/Gwydir	2008/09	48	C, M, S, PP, SF	70.4 ± 2.9	240	0	423	34.0 ± 2.0	544
	2009/10	28	C, M, S, PP, SF, V	64.8 ± 4.0	145	6.3 ± 1.7	205	24.8 ± 3.8	133
	2010/11	63	C, M, S, MB, PP, SF, V	61.4 ± 3.7	176	12.0 ± 2.3	200	17.3 ± 1.7	504
	2011/12	82	C, M, S, MB, PP, SF	89.6 ± 1.1	714	39.4 ± 1.6	923	34.3 ± 1.7	792
	2012/13	49	C, M, S, MB, SB, PP, V	89.3 ± 0.8	1547	38.5 ± 1.3	1320	22.1 ± 1.2	1196
	2013/14	26	C, M, S, PP	90.8 ± 1.3	491	44.1 ± 2.1	540	27.2 ± 2.0	504
	2014/15	42	C, M, S, MB	95.4 ± 0.8	755	58.3 ± 1.8	750	51.6 ± 1.9	701
Macquarie	2011/12	12	C, M, CP	95.6 ± 2.2	90	37.8 ± 4.5	119	28.1 ± 4.0	128
	2012/13	30	C, M, S, MB, PP	91.8 ± 1.3	417	43.0 ± 2.5	291	22.5 ± 2.2	351
	2013/14	2	M, PP	84.0 ± 5.2	50	44.6 ± 6.7	56	31.5 ± 6.4	54
	2014/15	8	C, M, PP	82.4 ± 6.6	34	44.4 ± 9.7	27	39.1 ± 10.4	23
MIA	2011/12	6	C, PP	90.0 ± 6.9	20	37.9 ± 9.2	29	–	–
	2013/14	4	C, PP	77.4 ± 7.6	31	22.6 ± 7.6	31	22.6 ± 7.6	31
Border Rivers	2008/09	8	C, M, S, PP	–	–	0	20	28.9 ± 4.3	114
	2009/10	18	C, PP	–	–	12.2 ± 5.2	41	29.3 ± 4.8	92
	2010/11	29	C	66.0 ± 6.6	53	3.1 ± 1.5	129	15.2 ± 4.4	66
	2011/12	12	C, M, PP	85.7 ± 7.8	21	50.0 ± 8.2	38	46.2 ± 9.9	26
	2012/13	52	C, M, PP	92.9 ± 1.4	336	38.7 ± 2.6	354	30.5 ± 2.7	292
	2014/15	19	C, M, S, PP	89.2 ± 2.4	166	51.1 ± 3.9	176	51.4 ± 4.2	144
Darling Downs	2010/11	11	C, M	–	–	0	60	12.0 ± 6.6	25
	2011/12	13	C, M, PP	92.9 ± 4.0	42	44.9 ± 6.0	69	42.6 ± 6.4	61
	2012/13	17	C, M	95.0 ± 2.0	119	30.2 ± 4.1	129	18.0 ± 3.7	111
	2014/15	10	C, M, MB, PP	75.9 ± 8.1	29	32.0 ± 9.5	25	26.1 ± 9.4	23
Emerald	2009/10	9	C, CP	85.3 ± 6.2	34	0	29	–	–
	2011/12	7	C	–	–	–	–	24.0 ± 8.7	25
	2012/13	5	C, CP	92.6 ± 5.1	27	47.6 ± 11.2	21	–	–
	2014/15	7	C, MB	92.8 ± 3.1	69	46.4 ± 6.0	69	30.0 ± 6.0	60

[1]Initials indicate crop types sampled.
C, cotton; M, maize; S, sorghum; CP, chickpeas; MB, mung beans; SB, soya beans; PP, pigeon pea; SF, sunflowers; V, *Verbascum virgatum*.

Methomyl resistance

Resistance declined from 29% in 2008–09 to 15% in 2010–11 (fig. 2). In 2011–12, industry-wide resistance levels increased to 32%, ranging from 24% in Emerald to 46% in the Border Rivers region (table 2). Average annual resistance declined in the seasons between 2012 (21%) and 2014 (25%), increasing again in 2014–15 to 40% (fig. 2).

Pyrethroid and carbamate use in Australian cotton production 2000–2014

Annual usage of pyrethroids and carbamates over a 15-year period between 2000 and 2014 is shown in figs 3 and 4, respectively, and was summarized from a cotton market audit survey conducted annually to provide data on product usage across the Australian cotton industry. Hence spray

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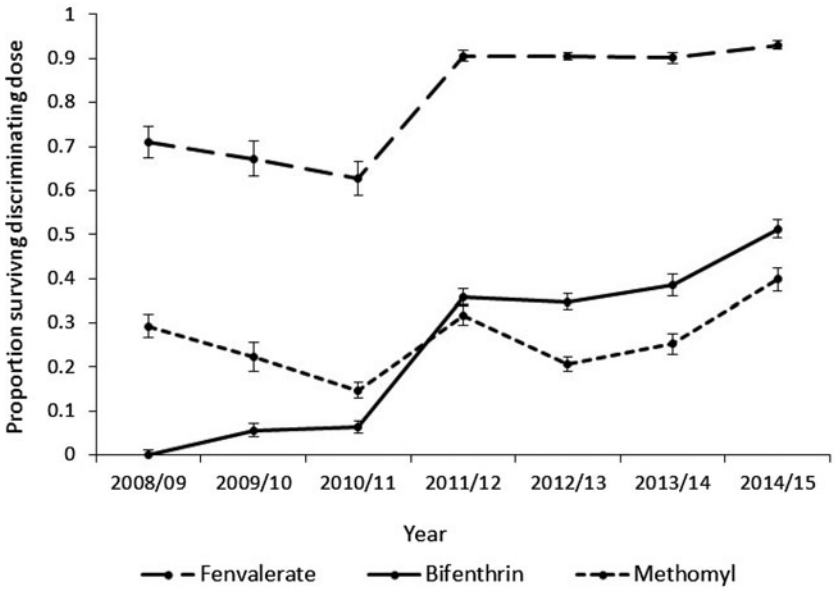

Fig. 2. Proportion of *Heliothis armigera* larvae surviving a discriminating dose of fenvalerate, bifenthrin or methomyl ± pooled binomial standard error. The data for each insecticide have been summed across sampling locations and combined for all crop types within each season from 2008 to 2015.

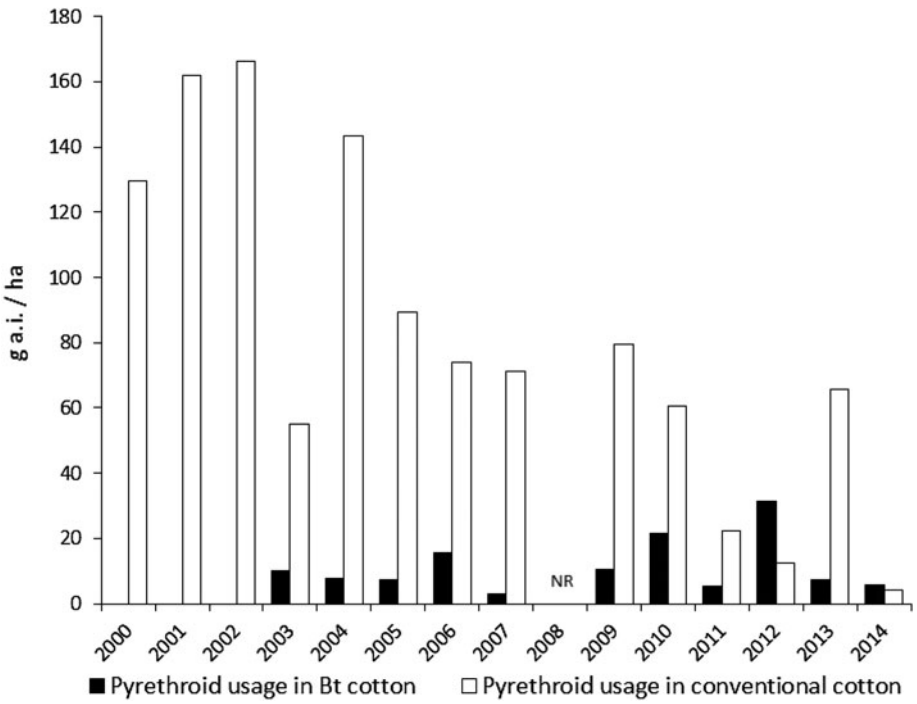

Fig. 3. Pyrethroid use on Bt (Bollgard II®) cotton and conventional cotton. NR = not recorded because cotton area was very small due to drought. Source: Cotton Research and Development Corporation (CRDC) Summary of Crop Consultants Australia (CCA) market audit, 2014.

volume data for pyrethroid and carbamate insecticides is restricted to usage in the cotton industry only as no data were available for insecticide use in the grains industry. Conventional cotton is grown by a small proportion of the industry and accounts for the majority of industry-wide pyrethroid and carbamate use. In the 3 years prior to the introduction of Bollgard II® in 2003, pyrethroid usage ranged from 129 to 166 g of active ingredient per hectare in conventional cotton

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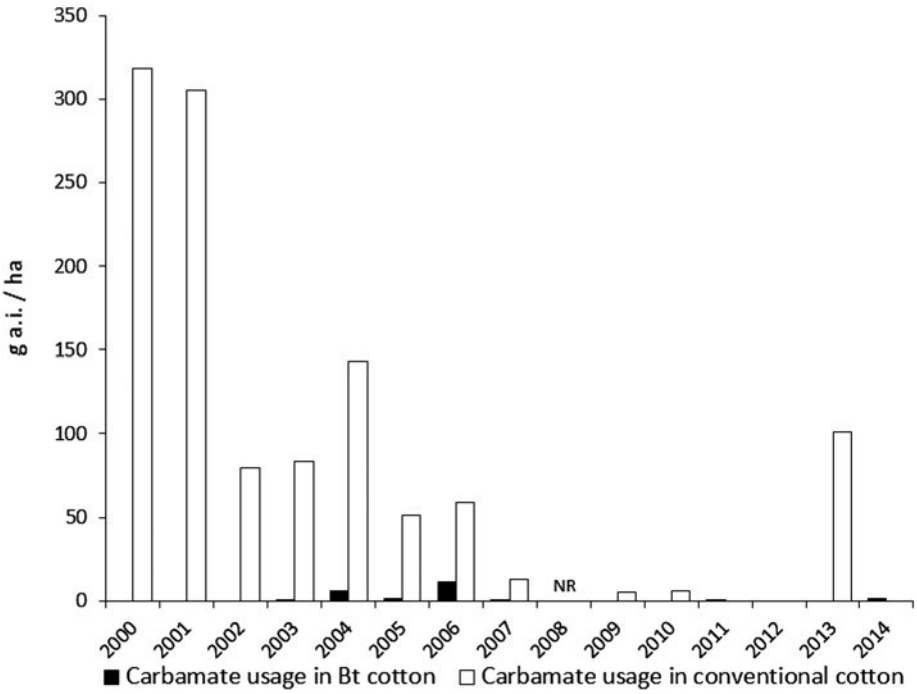

Fig. 4. Carbamate use on Bt (Bollgard II®) cotton and conventional cotton. NR = not recorded because cotton area was very small due to drought.
Source: Cotton Research and Development Corporation (CRDC) Summary of Crop Consultants Australia (CCA) market audit, 2014.

(fig. 3). Variation in annual pyrethroid use was presumably due to fluctuations in seasonal insect pressure, influenced largely by host availability. Nevertheless, there was an overall reduction in pyrethroid use in cotton of approximately 60% in the years following the commercialization of Bollgard II® (fig. 3). The decline in use of carbamate insecticides in conventional cotton was even more pronounced with an overall reduction of 80% in total amount of active carbamate applied to cotton following the introduction of Bollgard II® (fig. 4).

Cropping statistics and pyrethroid resistance in New South Wales and Queensland 2003–2015

The major broad-acre production systems managed for *H. armigera* by the use of insecticides in New South Wales and Queensland are shown in fig. 5. Cotton hectares were influenced by a period of prolonged drought and produced a corresponding trough in production from 2006 to 2010. Sorghum was the dominant summer crop prior to 2008–09, when water for irrigation was less assured for cotton growers. The total area planted to pulse crops (chickpeas, faba beans, field peas, lentils, lupins, mung beans and vetch) increased from the early 2000s and onward, peaking in 2010–11. Chickpea production accounted for 60–70% of pulse total pulse production in New South Wales and Queensland during the period of this study and is represented separately from other pulses in fig. 5.

Bifenthrin resistance in the 14 years from 2001–02 to 2014–15 is presented in fig. 5 to illustrate the long term trends in resistance spanning the period before the introduction Bollgard II® cotton, and comparing this with the results from the present study. In 2001–02, bifenthrin resistance was 60% (Gunning, 2002) and declined steadily over the following five seasons. Resistance could not be detected in the three seasons from 2006–2007 to 2008–2009 (Rossiter *et al.*, 2008) but re-emerged in the second year in the present study.

Discussion

There is little doubt that the introduction of transgenic cotton has reduced the overall need for insecticides that target *Helicoverpa* spp. The commensurate decline in insecticide resistance levels in Australian *H. armigera* were presumed to be associated with reduced selection for resistance in an industry dominated by transgenic cotton (Fitt, 2008; Wilson *et al.*, 2013); there was considerably lower survival at the discriminating dose of bifenthrin following the 2004–05 season, with resistance returning to baseline levels for two seasons from 2006 to 2008. Results from the present study indicate that, although *H. armigera* was still fully susceptible to bifenthrin in 2008–09, incipient resistance was detected in the following season at a level of 6%, increasing to 36% in 2011–12. This was accompanied by a similar increase in resistance to fenvalerate which exceeded 90% and which would have resulted in field failures of ester-bonded phenoxybenzyl alcohol pyrethroids (Forrester *et al.*, 1993). Moderate resistance to methomyl was also consistent with unreliable levels of control observed under field conditions (Gunning *et al.*, 1992).

Despite the dominance of transgenic varieties in Australian cotton production, conventional varieties are still grown by a small proportion (<10% since 2010) of the industry in regions with a preference for non-transgenic varieties (Constable *et al.*, 2011). However, the marked increase in bifenthrin and fenvalerate resistance occurred despite relatively low levels of

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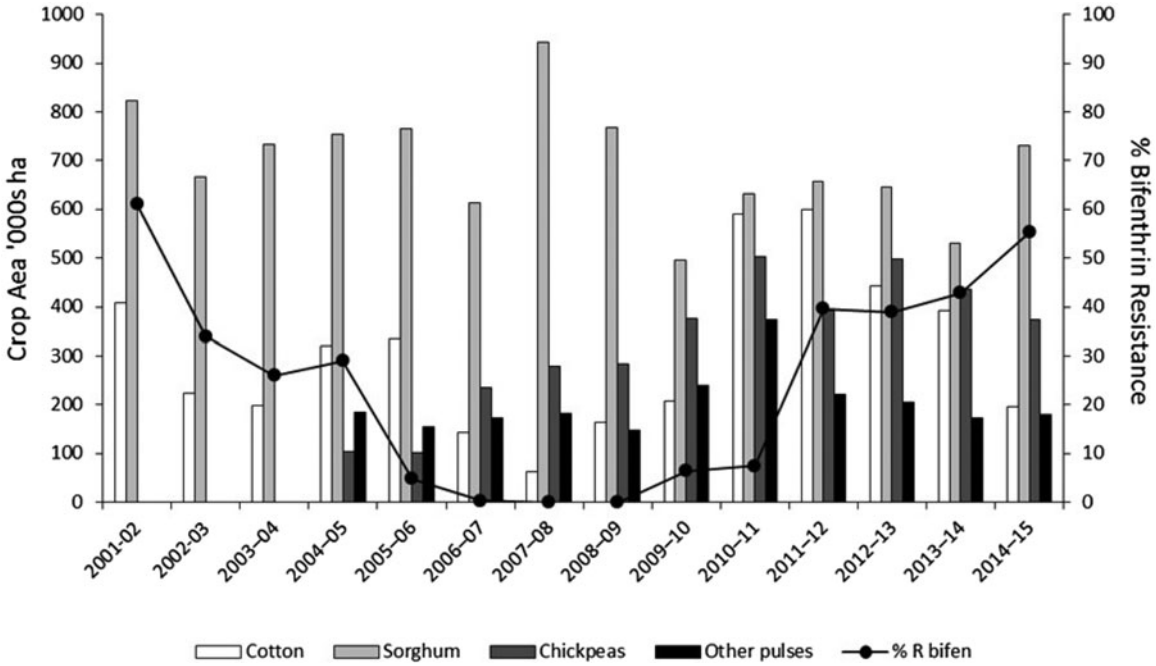

Fig. 5. Hectares of pulses, cotton and sorghum grown annually (2003–2015) in New South Wales and Queensland compared with annual bifenthrin resistance levels (resistance data have been summed across sampling locations and combined for all crop types). Sources: Commodities data from Australian Bureau of Agricultural and Resource Economics (ABARES, 2016); Bifenthrin resistance frequency data 2001–2002 to 2007–2008, from Rossiter *et al.* (2008).

pyrethroid use to target *Helicoverpa* spp. in cotton and was unlikely to have provided sufficient selection pressure to account for the magnitude of increase in resistance frequency. Likewise, but to lesser extent, resistance to methomyl, although variable over time, had increased significantly in 2014–15 despite very low levels of carbamate use in cotton, notwithstanding the exception of increased usage in 2013.

Although use of these insecticides declined in cotton, they remained important management options in the Australian grains industry which was reliant upon broad-spectrum insecticides largely because of the lack of selective options registered in summer and winter pulse crops at that time and also because broad-spectrum insecticides were a cost-effective measure for control of the susceptible species *H. punctigera* (Murray *et al.*, 2005; Brier *et al.*, 2008). The release of high yielding, disease-resistant chickpea varieties and expanding export markets for this and other pulses led to increased production in Australia during the mid-2000s, with pulse hectares peaking in 2010–11. However, pulse crops are hosts for both *H. armigera* and *H. punctigera*, with the crop most at risk from both species during the reproductive phase of plant development (Brier *et al.*, 2008). A mixed *Helicoverpa* spp. composition in flowering and podding winter pulses is a common occurrence (L.J.B. unpublished data) and the use of pyrethroids and methomyl for their control (based either on economic thresholds or used as a prophylactic application) may have provided a scenario for resistance selection in *H. armigera* and contributed to the observed increases in resistance frequency to pyrethroids and carbamates. Resistance management has been supported by the Australian cotton industry over the last 30 years by the development and implementation of a voluntary IRMS. On the other hand, the Australian grains industry has not had access to a formal strategy for mitigating resistance in *H. armigera*. Unregulated insecticide use during the rapid expansion of the pulse industry as a possible source of selection for resistance highlights the urgent need for a strategic approach to insecticide management to preserve the efficacy of other insecticides which are at similar risk of resistance from over-reliance in grains production systems.

The incremental increases in resistance observed in this study contrast markedly with the resistance trends observed in the preceding seven seasons from 2001–2002 to 2007–2008. The use of pyrethroids and carbamates was generally higher in the period before the introduction of Bollgard II® cotton in 2003, which likely reflect the continued reliance on these insecticides in Ingard® cotton, resulting in ongoing selection for resistance in *H. armigera*. It has been suggested that the replacement of Ingard® with Bollgard II® cotton may have contributed to population suppression by removal of a precautionary 30% cap on total area of Ingard® cotton grown (Baker & Tann, 2017). However, *H. armigera* population abundance may have also been impacted by prevailing climatic conditions at that time (Zalucki & Furlong, 2005). The size of *H. armigera* populations depends upon the availability of suitable hosts, which is closely linked with the quantity and timing of rainfall (Fitt, 1989). In temperate eastern Australia, *H. armigera* moths are generated from both immigrant origins and emergence from local overwintering pupae (Fitt & Daly, 1990) with establishment of spring cohorts on winter pulses (Murray *et al.*, 2005) and, to a lesser extent, uncultivated hosts such as roadside weeds (Wilson, 1983). During droughts, spring abundance of *H. armigera* is likely to be reduced due to resource bottlenecks such as the absence of suitable non-crop hosts and reduced plantings of cultivated hosts (Gregg *et al.*,

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1995), particularly dryland crops such as chickpeas and sorghum.

Plantings of major *H. armigera* hosts were relatively low from 2006 to 2009, with the exception of sorghum. Notwithstanding the challenging conditions for irrigated crops, plantings of sorghum, although variable, were relatively high through the period of this study. Although sorghum has a high carrying capacity for *H. armigera*, it can only support a single generation per season. Furthermore, without a sequence of suitable hosts for cohorts from sorghum to move into, the suppressive effect from Bollgard II® acting as a sink for the *H. armigera* population may have been compounded by bottlenecking events in years with low rainfall. The abundance of *H. armigera* may have also been impacted by an increased abundance of natural enemies such as pupal parasitoids due to reduced insecticide use in cotton (Baker & Tann, 2014) or selective mortality due to lower fitness of pyrethroid-resistant individuals following pupal diapause (Daly & Fisk, 1995).

The effect of population suppression, either by a landscape dominated by transgenic cotton, or by drought conditions which limit the abundance and quality of host crops, may have contributed to a reduction in resistance through founder effects and genetic drift. Reductions in the size of populations and subsequent genetic bottlenecks can result in changes in common allele frequency, leading to shifts in gene diversity (England *et al*., 2003). Genetic diversity is then predicted to influence responses to selection (Allendorf, 1986). A severe reduction in the size of the *H. armigera* population, such as was reported by in the Namoi valley during the seasons from 2006 to 2008 (Baker & Tann, 2017), may have impacted the frequency of common alleles, such as those that confer resistance to pyrethroids, through loss of genetic variation and allelic decay. Normally, the effect of this could be mitigated by immigration of populations from other areas which may have experienced greater suitability of rainfall and breeding conditions, ensuring sufficient levels of migration to maintain genetic similarity and widespread distribution of alleles (Daly & Gregg, 1985; Gregg *et al*., 1995). However, when host availability and diversity are severely limited by widespread and prolonged periods of drought, we might also expect immigration rates to be substantially affected.

Resistance monitoring is a key component of the IRMS in the Australian cotton industry. Ideally, the establishment of baseline susceptibility data and calibration of discriminating doses for resistance monitoring should be determined from a large number of geographically diverse field susceptible populations before product commercialization (ffrench-Constant & Roush, 1990). In the case of monitoring for resistance in Australian *H. armigera*, there were clear benefits associated with this approach as it provided a high level of confidence that survival at this dose indicated resistance evolution in the field (Forrester & Cahill, 1987). It is equally important to maintain a laboratory reference strain representative of baseline susceptibility to ensure validity of comparisons of long-term monitoring programmes over time. However, susceptible strains of Lepidoptera held for long periods in the laboratory may bear little resemblance to susceptible strains in the field because of the high likelihood of inbreeding depression which can lead to loss of hybrid vigour and impact on the outcome of bioassays (Santos *et al*., 2012). The laboratory reference strain used in this study (New GR) appeared to be distinct from the reference strain (SUS), previously described as having full susceptibility to broad-spectrum insecticides (Gunning *et al*., 1992; Forrester *et al*., 1993). The New GR strain demonstrated an increased sensitivity to bifenthrin and an increased tolerance to methomyl compared with the SUS strain. Presumably, this was due to the introduction of field insects into the New GR strain as a source of genetic variation, resulting in genetic divergence. Continued resistance monitoring will be important for assessing the effectiveness of the cotton IRMS. However, the results from this study provide evidence that the cotton IRMS alone is not sufficient to delay resistance development in insecticides that are utilized to target *H. armigera* in other commodities. Hence, there is an imperative for the Australian grains industry to support the development and implementation of management strategies to delay the development of resistance to key selective *Helicoverpa* spp. insecticides that are currently at high risk from over-reliance in grains production systems.

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