



# Abundance and mortality of overwintering pupae of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) on the Darling Downs, Queensland, Australia

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## Abstract

Surveys were conducted between 1997 and 2001 to investigate the incidence of overwintering *Helicoverpa* spp. pupae under summer crop residues on the Darling Downs, Queensland. Only *Helicoverpa armigera* was represented in collections of overwintering pupae. The results indicated that late-season crops of cotton, sorghum, maize, soybean, mungbean and sunflower were equally likely to have overwintering pupae under them. In the absence of tillage practices, these crops had the potential to produce similar numbers of moths/ha in the spring. There were expected differences between years in the densities of overwintering pupae and the number of emerged moths/ha. Irrigated crops produced 2.5 times more moths/ha than dryland crops. Overall survival from autumn-formed pupae to emerged moths averaged 44%, with a higher proportion of pupae under maize surviving to produce moths than each of the other crops. Parasitoids killed 44.1% of pupae, with *Heteropelma scaposum* representing 83.3% of all parasitoids reared from pupae. Percentage parasitism levels were lower in irrigated crops (27.6%) compared with dryland crops (40.5%). Recent changes to *Helicoverpa* spp. management in cotton/grain-farming systems in south-eastern Queensland, including widespread adoption of *Bt* cotton, and use of more effective and more selective insecticides, could lead to lower densities of overwintering pupae under late summer crops.

**Key words** cultivation, diapause, parasitoids, pupae busting.

## INTRODUCTION

The corn earworm or cotton bollworm, *Helicoverpa armigera* (Hübner) and the native budworm, *Helicoverpa punctigera* (Wallengren) (Lepidoptera: Noctuidae) are widely regarded as the main pests of cotton and grain crops in eastern Australia (Wardaugh *et al.* 1980; Zalucki *et al.* 1986; Fitt 1994). Both *Helicoverpa* spp. are capable of long-distance movement, but this characteristic is more typical of *H. punctigera* (Farrow & Daly 1987). Most *H. armigera* are thought to originate from within the farming systems of eastern Australia, although the contribution of locals vs. immigrants can vary greatly from season to season and within seasons (Scott *et al.* 2005a,b). This trait leads to *H. armigera* populations in Australia's major cropping regions being exposed to constant insecticide resistance selection pressure during the spring/summer cropping cycle and has resulted in widespread resistance to several key insecticide groups (Daly & Fitt 1990; Forrester *et al.* 1993; Gunning *et al.* 1996). In contrast, *H. punctigera* breeds during winter on flowering plants in arid areas of inland Australia, when there is sufficient rainfall to generate growth of broadleaf vegetation (Gregg *et al.* 1995). As this inland vegetation

senesces in late winter/spring, the moths migrate to the cropping areas of eastern Australia, carried on the warm winds preceding storm fronts in spring.

*Helicoverpa* spp. pupating during autumn enter an overwintering or diapausing phase (Wilson *et al.* 1979; Kay 1982; Murray 1991). The majority of overwintering pupae found under cultivated crops are *H. armigera* (Wilson 1983; Fitt & Daly 1990; Duffield 2004). Survival of overwintering *H. armigera* pupae is an important issue for both the grain and cotton industries as these pupae provide the main instrument to convey populations and insecticide resistance from one season to the next (Daly & Fitt 1990). Several studies in eastern Australian cropping regions have evaluated the contribution of overwintering *Helicoverpa* spp. pupae to this pest management threat (Wilson 1983; Fitt & Daly 1990; Sequeira & Playford 2001; Duffield 2004).

Intensive post-harvest cultivation, referred to as pupae busting, is a high-profile campaign promoted within the cotton industry aimed at reducing survival of overwintering *Helicoverpa* pupae (Fitt 1994; Murray *et al.* 1995; Forrester & Bird 1996; Slack-Smith *et al.* 1997). Management of resistance to both conventional insecticides and transgenic *Bt* toxins is fundamentally important for the profitability and sustainability of cropping on the Darling Downs. Recently, low-rainfall years have become more common, resulting in conservation tillage gaining greater acceptance in an attempt to conserve soil

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moisture. Grower sentiment has also begun to question the need for wide-scale cultivation of fields that may in fact carry no overwintering pupae.

No data were available on the abundance, species composition and biotic mortality factors of overwintering *Helicoverpa* pupae under autumn crop residues on the Darling Downs, a major mixed cropping region of south-eastern Queensland. This paper presents the results from a 5-year survey across the Darling Downs aimed at quantifying what crop types pose the greatest risk and their relative contribution to the spring generation of *Helicoverpa* moths. We also discuss the use of a predictive model for informed decision making regarding the need to cultivate by identifying the time frame when fields are more likely to produce overwintering pupae.

## MATERIALS AND METHODS

### Survey area

Pupae were sampled across much of the Darling Downs from Killarney (28°20'S, 152°18'E) in the south-east to Chinchilla (26°44'S, 150°38'E) in the north-west. The Darling Downs is predominately a dryland mixed cropping region interspersed with areas of intensive irrigated agriculture. Surveys were conducted between April and June in 1997–2001 with a minimum of 100 fields sampled per year (Table 1). In 1999–2001, the majority of sampling was conducted in two study areas that were part of a project developing a *Helicoverpa* Regional Management Strategy (HRMS). One was a largely irrigated area between Brookstead and Cecil Plains and the other encompassed the mostly dryland Jimbour Flood Plain north-west of Dalby. Preliminary scouting was normally carried out in early/mid-March each year and coordinates of crops of the appropriate growth stage capable of hosting *Helicoverpa* larvae were recorded for future sampling.

### Sampling procedure

Pupae were sampled from either 6 or 10 × 1 m (HRMS areas) and 15 × 1 m (non-HRMS areas) inter-row quadrats per field. In each field, two or three transects were assessed with random samples taken 50–75 m apart and >50 m from the field boundary to avoid edge effects. In single- and double-skip row cotton, samples were restricted to the narrow inter-row. Sampling each quadrat entailed carefully scraping off the top 1 cm of soil with a hand trowel to expose any emergence tunnels constructed by the larvae prior to pupation. Each tunnel was removed and the viable pupa or pupal remains recovered. The entire quadrat was then excavated to a depth of 12 cm, depending on soil structure, to reveal any further pupae. In the field, pupae were recorded as either (1) alive; (2) emerged; (3) damaged; or (4) parasitised and emerged. Pre-pupae in pupal chambers were included in the live pupal counts. Classification of the pupal state was similar to that described by Duffield (2004). Estimates of the density of viable pupae per hectare were calculated as:

$$\frac{(\text{no. alive} + \text{no. damaged})/\text{row metres sampled} \times (10\,000 \times (100/\text{cm. row spacing}))}{10\,000 \times (100/\text{cm. row spacing})}$$

Viable pupae (alive and damaged) were returned to the laboratory and undamaged pupae were held at the diapause threshold temperature of 18°C for 2 weeks and then identified to species and examined for eyespot movement and fat bodies to determine diapause incidence (Murray & Wilson 1991). Pupae were then stored at 12°C for at least 2 weeks to expose them to 'cold shock' before being returned to 25°C until moths or parasitoids emerged. Pupae damaged during collection were dissected to determine their developmental state and if parasitised, identification was made using either immature parasitoid morphology or colour of meconium (J Hopkinson unpubl. obs. 1999).

### Model predictions

For each year of our study, daily predictions of the proportion of pupae entering diapause were made for Dalby, using the diapause induction algorithm (D Murray unpubl. data 1997) contained in the HEAPS model (Dillon & Fitt 1997). This model uses the environmental parameters of daily average temperature derived from maximum and minimum temperatures, and daylength (sunrise to sunset) as the principal triggers for diapause induction. Predictions were also made for Dalby using long-term average temperature data. Yearly predictions were calculated from data stored on the Bureau of Meteorology (BOM) Data Drill database, whereas long-term model predictions were based on 20-year average meteorological data (1982–2002) derived from a BOM Patched Point Data set.

### Data analyses

All mortality variables and the proportion of sites with viable pupae were analysed using logistic regression incorporating a generalised linear model (GLM) with binomial distribution and logit link function (GenStat Release 7.2). We assumed a binomial distribution as our data consisted of many cases of 0/1 data (e.g. each pupa is observed to be dead or alive, parasitised or not). The model terms included in all analyses were: year + crop + water regime. Two-way interaction terms (e.g. crop and water regime) were tested and included where significant. Where a factor (e.g. crop) had a significant effect, *t*-test pair-wise comparisons were made between factor levels. Pupal density data were analysed using residual maximum likelihood (REML). Pigeon pea, lucerne and lablab sites were excluded from the analyses due to the small number of samples (four in total).

## RESULTS

### Pupal abundance

The crop type data, number of sites sampled with viable pupae and pupal densities are presented in Table 1. Of the 575 sites sampled during the 5 years of the study, cotton crops repre-

**Table 1** Abundance of overwintering *Helicoverpa armigera* pupae under autumn crop residues on the Darling Downs, 1997–2001

Year	Crop	Water regime	No. sites sampled	% Sites with viable pupae	No. viable pupae/ha			
					Mean†	SE	Range‡	
1997	Cotton	Irrigated	22	0.0	0			
1998			18	55.6	1 778	684	667–10 000	
1999			24	87.5	9 708	2123	1 000–42 000	
2000			54	75.9	4 370	927	667–42 000	
2001			15	26.7	7 689	6150	667–92 000	
1997	Cotton	Dryland	8	25.0	389	284	889–2 222	
1998			27	55.6	1 016	311	333–7 333	
1999			32	68.7	6 047	2112	500–54 000	
2000			28	46.4	633	156	667–2 667	
2001			19	36.8	1 234	628	333–8 889	
1997	Maize	Irrigated	2	50.0	1 333	1333	2 667	
1998			2	50.0	444	444	899	
1999			6	33.3	5 167	4778	2 000–29 000	
2000			12	66.7	7 361	2857	1 333–28 000	
2001			9	88.9	3 926	1762	667–14 667	
1997		Maize	Dryland	6	50.0	444	222	667–1 333
1998				10	50.0	756	351	889–3 556
1999				14	64.3	2 944	881	667–9 000
2000				14	71.4	1 786	819	667–10 667
2001				13	92.3	2 135	436	667–5 833
1997	Sorghum	Irrigated	5	100.0	1 200	389	667–2 667	
1998			1	0.0	0			
1999			4	50.0	2 500	2179	1 000–9 000	
2000			1	100.0	6 667			
1997			Sorghum	Dryland	31	16.1	122	51
1998		34			17.6	484	291	667–8 889
1999		30			46.7	8 263	4499	667–119 000
2000		5			20.0	133	133	667
2001		20			30.0	400	234	667–4 667
1997		Sunflower	Irrigated	1	0.0	0		
2000	1			100.0	3 333			
1997	Sunflower		Dryland	5	80.0	978	317	889–1 667
1998				2	50.0	444	444	889
1999				5	40.0	4 267	3942	1 333–20 000
2000	8	25.0	292	213	667–1 667			
2001	7	57.1	722	293	833–2 000			
1999	Mungbean	Irrigated	2	50.0	1 500	1500	3 000	
2000			1	100.0	26 667			
1997		Mungbean	Dryland	4	25.0	3 333	3333	13 333
1998				6	0.0	0		
1999				2	50.0	3 500	3500	7 000
2000	5	40.0	2 200	1812	1 667–9 333			
2001	12	25.0	463	274	667–2 667			
1997	Soybean	Irrigated	15	6.7	267	267	4 000	
1998			1	0.0	0			
1999			8	37.5	1 889	1094	1 778–8 000	
2000		14	64.3	7 976	4957	995–70 000		
2001		2	0.0	0				
1998	Pigeon pea	Irrigated	4	0.0	0			
1997			1	100.0	2 667			
2001	1	100.0	1 333					
1997	Lucerne	Dryland	1	0.0	0			
2000	Lablab	Dryland	1	0.0	0			

†Mean of all sites sampled, not only sites with viable pupae.

‡Density range in sites sampled with viable pupae.

sented 43% of sites, followed by sorghum (22.8%), maize (15.3%) and soybean (7.7%). Over all years, cotton, sorghum and maize were the major crops and represented 81% of the fields sampled. The contribution of irrigated and dryland crops

to the annual total of sampled sites varied considerably from year to year. The majority of all sampled crops (61.6%) were dryland. While each of the major summer crops were represented by both irrigated and dryland sites, some crops

**Table 2** The proportion of sampled sites with viable overwintering *Helicoverpa armigera* pupae, the number of viable overwintering *H. armigera* pupae/ha and the number of emerged moths/ha in each year of the 1997–2001 survey period

Year	Proportion	No. pupae/ha	No. moths/ha
1997	0.2336 <sup>a</sup>	361.3 <sup>a</sup>	185.4 <sup>a</sup>
1998	0.3867 <sup>ab</sup>	859.0 <sup>ab</sup>	267.1 <sup>a</sup>
1999	0.6028 <sup>b</sup>	5960.2 <sup>d</sup>	2049.0 <sup>c</sup>
2000	0.5687 <sup>b</sup>	3014.3 <sup>c</sup>	1035.4 <sup>b</sup>
2001	0.4382 <sup>ab</sup>	2337.3 <sup>bc</sup>	1183.7 <sup>bc</sup>

Values in a column followed by different letters are significantly different ( $P < 0.05$ ).

were predominately dryland as represented by sunflower (93.1%), sorghum (91.6%), mungbean (90.6%) and maize (64.8%). The predominately irrigated crops were soybean (86.4%) and cotton (53.8%).

All of the 1761 pupae recovered in our surveys were *H. armigera*. Across the 5 years of the survey, viable pupae were recovered from 52.7% of sites. The proportion of sites with viable pupae varied significantly ( $P < 0.05$ ) with year (Table 2), but neither crop nor irrigation was a significant ( $P > 0.05$ ) main effect.

Across all sampled fields, the density of viable pupae/ha was  $2940 \pm 386$  (mean  $\pm$  SE). The highest recorded density of viable pupae was 119 000/ha from a field of dryland sorghum in 1999. The density of viable pupae/ha varied significantly ( $P < 0.05$ ) with year (Table 2). There were significantly more ( $P < 0.05$ ) viable pupae/ha under irrigated crops (3934 pupae/ha) compared with dryland crops (1404 pupae/ha). Crop type had no significant effect ( $P > 0.05$ ) on the number of viable pupae/ha.

### Emerged moths

Moths emerged from 44.0% of pupae (Table 3). A significantly higher proportion ( $P < 0.05$ ) of pupae produced moths from maize than from each of the other crops (cotton, soybean, sorghum, sunflower and mungbean) (Table 4). Year and irrigation did not significantly ( $P > 0.05$ ) influence the proportion of pupae that produced moths.

The number of emerged moths/ha was significantly different ( $P < 0.05$ ) between years, with the lowest number of moths/ha in 1997 and 1998 (Table 2). The year of highest moth production/ha was 1999 (2049.0 moths/ha), but this was not significantly different ( $P < 0.05$ ) from that of 2001 (1183.7 moths/ha).

Although there were large differences between crop types in the number of emerged moths/ha, crop type was not a significant ( $P > 0.05$ ) main effect (Table 4). Irrigation significantly ( $P < 0.05$ ) affected moth production, with 2.5 times more moths from irrigated fields (1247.3 moths/ha) compared with dryland fields (509.2 moths/ha).

### Mortality factors

Partitioning of pupal mortality between parasitism and other causes is presented in Table 3.

#### Unknown dead

Many pupae failed to produce moths or parasitoids, and the cause of their death was not determined (Table 3). These deaths were assigned an 'unknown' category. While year and crop were not significant ( $P > 0.05$ ) main effects for the incidence of 'unknown' pupal mortality, there were significantly more ( $P < 0.05$ ) pupae dead from 'unknown' causes in irrigated crops (26.1%) than in dryland crops (14.4%).

#### Parasitism

Of all the collected pupae, 44.1% were parasitised (Table 3). *Heteropelma scaposum* (Morley) was the most common parasitoid, representing 83.3% of all parasitoids reared from pupae. Crop type had a significant ( $P < 0.05$ ) effect on parasitism levels, with significantly lower levels of parasitism from maize than each of the other crops (Table 4). The highest levels of parasitism were recorded from mungbean (71.2%), but these were not significantly different ( $P > 0.05$ ) from those recorded in sunflower, soybean or cotton. While parasitism levels were not significantly different ( $P > 0.05$ ) between years, parasitism levels were significantly higher ( $P < 0.05$ ) in dryland crops (40.5%) compared with those in irrigated crops (27.6%).

### Model predictions

The typical trend was for increased diapause induction levels from low levels (<2%) in early March to high levels (>80%) by late April (Fig. 1). The transition from low to high levels of diapause was relatively rapid over a period of approximately 6 weeks, commencing about 9 March. Within each season there was substantial variation about the long-term average incidence of diapause. These variations were due to daily temperature fluctuations and their profound effect on diapause induction levels. During the period 1997–2001, predicted diapause levels were mostly below the levels predicted using long-term averages. These data suggest these seasons were mostly warmer than average, and could result in lower densities of overwintering pupae compared with the longer-term average.

### DISCUSSION

The number of sites sampled for each crop reflected that crop's potential contribution to the total number of fields that could harbour overwintering pupae in a particular year. While areas and sowing dates of irrigated crops tend to remain relatively stable from year to year, areas sown and sowing dates of dryland crops tend to be much more variable. Late planting rains can push dryland crops into the high-risk window when

Table 3 Fate of overwintering *Helicoverpa armigera* pupae collected on the Darling Downs, 1997–2001

Year	Crop	Total pupae collected	% Emerged as moths	% Dead (unknown causes)	Total % parasitism†	% Contribution to total parasitism			
						Hs‡	Ip§	Other Hymenoptera	Tachinidae††
1997	Cotton	6	16.7	16.7	80.0	100.0	0.0	0.0	0.0
	Maize	8	87.5	0.0	12.5	100.0	0.0	0.0	0.0
	Sorghum	12	41.7	0.0	58.3	85.7	0.0	0.0	14.3
	Sunflower	6	50.0	0.0	50.0	100.0	0.0	0.0	0.0
	Mungbean	2	50.0	0.0	50.0	0.0	100.0	0.0	0.0
	Soybean	6	66.7	16.7	20.0	100.0	0.0	0.0	0.0
	Total	40	52.5	5.0	44.7	88.2	5.9	0.0	5.9
	Cotton	84	35.7	34.5	45.5	96.0	4.0	0.0	0.0
	Maize	10	80.0	20.0	0.0	0.0	0.0	0.0	0.0
	Sorghum	14	7.1	21.4	90.9	100.0	0.0	0.0	0.0
1998	Sunflower	1	0.0	0.0	100.0	100.0	0.0	0.0	0.0
	Pigeon pea	2	50.0	0.0	50.0	100.0	0.0	0.0	0.0
	Total	111	36.0	30.6	48.1	97.3	2.7	0.0	0.0
	Cotton	452	40.9	16.8	50.8	79.6	12.6	3.7	4.2
	Maize	82	61.0	22.0	21.9	100.0	0.0	0.0	0.0
	Sorghum	271	29.2	31.7	57.3	85.8	9.4	0.9	3.8
	Sunflower	24	20.8	33.3	68.8	90.9	9.1	0.0	0.0
	Mungbean	6	16.7	50.0	66.7	100.0	0.0	0.0	0.0
	Soybean	22	4.5	59.1	88.9	100.0	0.0	0.0	0.0
	Total	857	37.5	23.8	50.8	83.4	10.5	2.4	3.6
2000	Cotton	217	42.4	23.5	44.6	90.5	6.8	1.4	1.4
	Maize	128	65.6	15.6	22.2	70.8	20.8	4.2	4.2
	Sorghum	11	0.0	27.3	100.0	100.0	0.0	0.0	0.0
	Sunflower	2	0.0	0.0	100.0	50.0	50.0	0.0	0.0
	Mungbean	16	0.0	6.3	100.0	100.0	0.0	0.0	0.0
	Soybean	52	36.5	15.4	56.8	100.0	0.0	0.0	0.0
	Total	426	45.8	19.5	43.1	89.9	7.4	1.4	1.4
	Cotton	207	53.1	21.7	32.1	57.7	23.1	3.8	15.4
	Maize	95	83.2	3.2	14.1	69.2	23.1	0.0	7.7
	Sorghum	12	50.0	25.0	33.3	100.0	0.0	0.0	0.0
2001	Sunflower	7	28.6	0.0	71.4	80.0	0.0	0.0	20.0
	Mungbean	4	25.0	0.0	75.0	33.3	33.3	0.0	33.3
	Pigeon pea	2	0.0	50.0	100.0	100.0	0.0	0.0	0.0
	Total	327	60.6	15.9	28.0	62.3	20.8	2.6	14.3
	5-year total	1761	44.0	21.3	44.1	83.3	10.5	2.0	4.3

†Total % parasitism = no. parasites × 100/no. collected – no. dead (unknown causes).

‡*Heteropelma scaposum*.§*Stenoneumon promissorius*.||Includes *Lissopimpla excelsa* and probable hyperparasites of key parasitoids.††Mainly *Carcelia* spp.

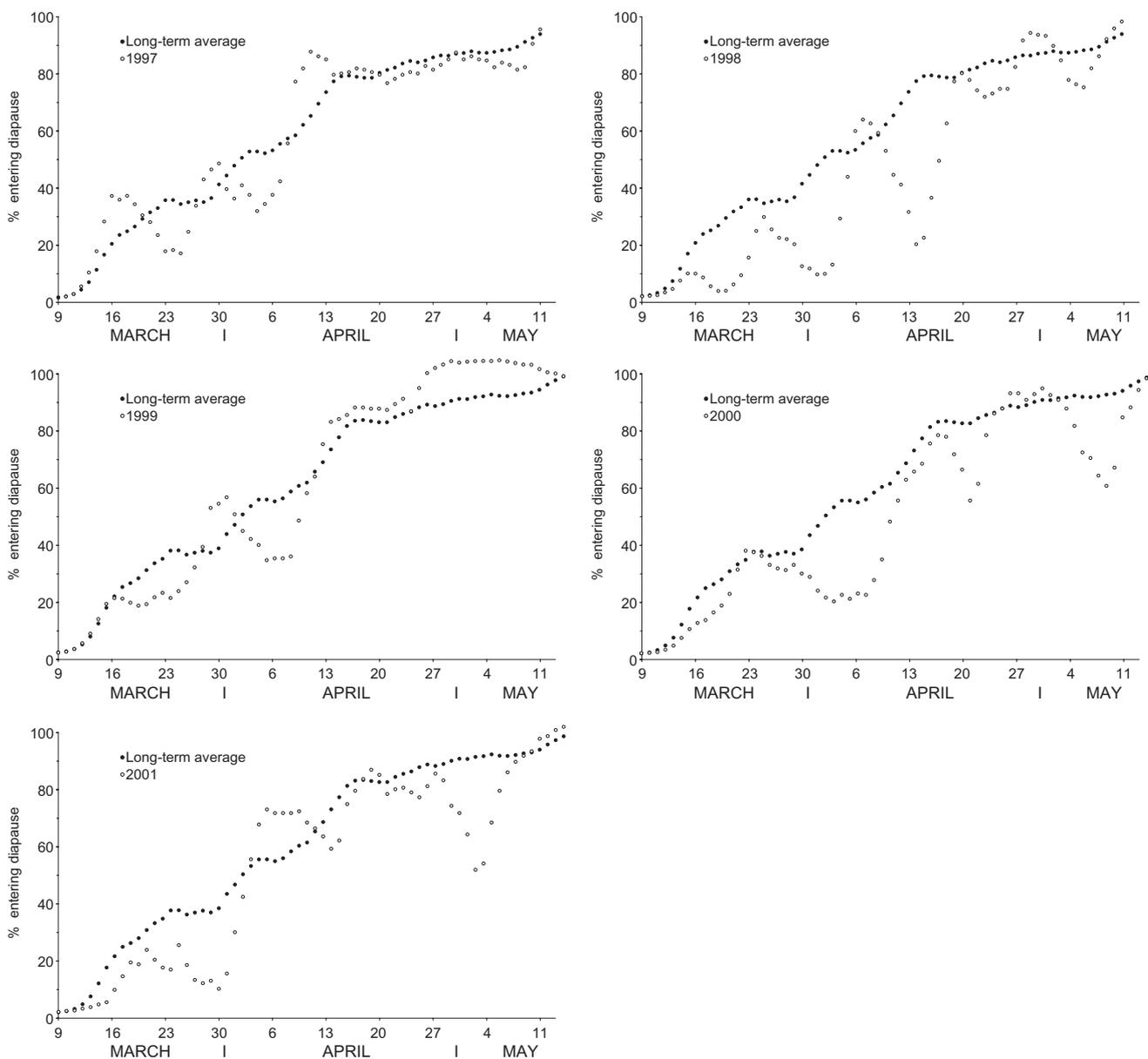
**Table 4** Effect of crop type on the proportion of overwintering pupae emerging as moths, the number of emerged moths/ha and the proportion of pupae parasitised during the 1997–2001 survey period

Crop	Proportion emerging as moths	No. emerged moths/ha	Proportion pupae parasitised
Cotton	0.4309 <sup>a</sup>	847.4	0.3742 <sup>bc</sup>
Maize	0.6923 <sup>b</sup>	1223.5	0.1619 <sup>a</sup>
Mungbean	0.1020 <sup>a</sup>	252.3	0.7118 <sup>c</sup>
Sorghum	0.3039 <sup>a</sup>	654.8	0.3534 <sup>b</sup>
Soybean	0.3049 <sup>a</sup>	496.7	0.4408 <sup>bc</sup>
Sunflower	0.2310 <sup>a</sup>	269.6	0.4807 <sup>bc</sup>

Values in a column followed by different letters are significantly different ( $P < 0.05$ ). No letters indicate no significant difference.

overwintering pupae are formed, and increase their likelihood of harbouring overwintering pupae as described by Duffield (2004) for farming systems in southern New South Wales.

Data are available on the annual areas designated to each crop, but care should be exercised in extrapolating from annual crop area statistics to estimate the overall threat of overwintering pupae. The reason for this, particularly for dryland crops, is that the majority of crops are no longer attractive and supporting *H. armigera* during the period when diapause could be induced. Only in occasional dry years is a substantial proportion of the summer crop planting delayed beyond December. In such cases the late planted crops are pushed into the high-risk window of producing overwintering pupae. For cotton, agronomic factors such as excess nitrogen, poor fruit



**Fig. 1.** Daily model predictions for diapause induction for each of the survey years 1997–2001 compared with the long-term average at Dalby.

retention, cool season or hail damage can delay crop maturity and also serve to push the crop into the high-risk window. In addition, cotton regrowth can support larval populations that subsequently pupate with a high incidence of diapause (Fitt & Daly 1990).

The failure to find any overwintering *H. punctigera* pupae in our pupal surveys concurs with findings from other regions (Wilson 1983; Fitt & Daly 1990; Duffield 2004). It is apparent that in most years *H. punctigera* do not overwinter under crop residues on the Darling Downs. In contrast, local crops can serve as a major source of overwintering *H. armigera*.

The survey data indicated that in the absence of tillage practices, late-season cotton, sorghum, maize, soybean, mung-bean and sunflower were equivalent with respect to the potential number of *H. armigera* moths/ha produced in spring. These contributions varied enormously both within and between seasons. The critical issue for any particular field is the likelihood that it will fall into the high-risk category. In most years crop development means that the majority of grain crops are mature and no longer capable of supporting *Helicoverpa* spp. larvae during late summer and autumn, so they pose negligible risk of producing overwintering pupae and do not need to be pupae busted. Such fields can be safely zero tilled or opportunity cropped using minimum tillage sowing equipment. High-risk grain fields can be identified based on scouting history and timing, and pupae busting action taken where considered appropriate.

In contrast, Australian cotton industry guidelines recommend pupae busting of all conventional cotton fields as soon as possible after harvest, and no later than the end of August (Farrell 2007). This is a voluntary guideline that historically has had a relatively high level of compliance as part of the Insecticide Resistance Management Strategy. Pupa busting has disadvantages associated with it. To be effective, it requires full soil surface disturbance to a depth of 10 cm. This is a costly operation made more difficult by soil conditions that are either too dry or too wet. Opportunities to double crop into a winter cereal may be lost due to this pupae busting requirement. Minimum tillage planters do not always provide sufficient soil disturbance to effectively reduce survival of overwintering pupae (Marshall *et al.* 1996; Duffield & Dillon 2005).

Single-gene transgenic *Bt* cotton containing the gene expressing the Cry1Ac toxin (Ingard<sup>®</sup>) was commercially released in Australia in 1996 (Fitt & Cotter 2005). During the years of our study, Ingard<sup>®</sup> represented no more than 30% of the cotton crop on the Darling Downs. No attempt was made to separate Ingard<sup>®</sup> and conventional fields in our surveys because the levels of expression of the Cry1Ac toxin in Ingard<sup>®</sup> typically decline through the boll maturation period, allowing late-season survival of *Helicoverpa* spp. larvae (Fitt *et al.* 1998; Olsen *et al.* 2005). For the purposes of our survey, the late-season performance of Ingard<sup>®</sup> against *Helicoverpa* spp. was considered similar to conventional cotton and was assumed not to have altered densities of overwintering *H. armigera* pupae under these crops.

There were major differences between years in the proportion of sites with viable pupae, the number of viable pupae/ha and the number of emerged moths/ha. A high proportion (47.3%) of sampled fields had no overwintering pupae under them. In contrast, some fields had extremely high densities of overwintering pupae. Seasonal conditions, timing of crop development and levels of *H. armigera* infestation are major determinants of densities of overwintering pupae under crops. The two key factors that influence diapause induction in *H. armigera* are daylength and temperature (Wilson *et al.* 1979; Kay 1982). While daylength on a particular day does not change from one year to the next, day-to-day fluctuations in temperature can dramatically alter the proportion of pupae entering diapause. On any day, lower-than-average temperatures can greatly increase diapause levels, while higher-than-average temperatures result in lower levels of diapause. Weather systems usually result in periods of higher or lower temperature that may persist for 5–10 days. These effects are clearly shown on the predictions of diapause for each year of this study (Fig. 1).

Crops that could support larvae after early March are identified as high risk and most likely to have overwintering pupae under them. Duffield (2004) used a similar approach in southern New South Wales, to suggest that crops that flowered after the beginning of February were high risk. This risk is manifested only if larval infestations subsequently develop on crops. High levels of larval infestation coupled with low levels of diapause induction, as can occur in March, represent a serious overwintering risk situation. Conversely, low levels of larval infestation at a time when high levels of diapause induction occur, as in late April–early May, represent an equally serious situation. The development of a predictive model for diapause induction provides this additional detail, which when used with information about levels of larval infestation after February, provides a more robust guide to identify high-risk fields.

While irrigated and dryland crops were equally likely to have overwintering pupae under them, irrigated crops had more viable pupae/ha, and produced 2.5 times more emerged moths/ha. From the perspective of population dynamics and resistance management, it is the number of moths/ha that emerge from overwintering pupae in spring that is of greatest importance. Based on our findings, irrigated crops are more likely to contribute to the spring moth population, and the potential carry-over of inherited resistance traits.

Because yield expectations and profit margins are higher for irrigated crops, particularly cotton, they have a greater reliance on insecticides for pest management compared with dryland crops. Irrigated crops are often in better agronomic condition than dryland crops, and may be more attractive to ovipositing moths and favour larval survival (Slosser 1980). Provided the applied insecticides are effective in reducing larval infestations, fewer pupae could be expected under irrigated crops. However, with high *H. armigera* pressure and declining performance of some older insecticide groups due to insecticide resistance, more *H. armigera* larvae may survive in irrigated crops.

## Mortality of pupae

The proportion of moths emerging from pupae was not influenced by year or water regime, suggesting a level of relative stability in pupal survival. The proportion surviving did not take into account the cultural practice of pupae busting, which, if carried out successfully, could reduce pupal survival to less than 20%, with the best treatments providing almost total control (Fitt *et al.* 1993; Murray *et al.* 1995; Marshall *et al.* 1996; Duffield & Dillon 2005). Survival of pupae to produce emerged moths was influenced by 'unknown' mortality and parasitism. Whereas there were more 'unknown' deaths in irrigated crops, parasitism was higher in dryland crops. Combined, 'unknown' mortality and parasitism resulted in similar survival levels for pupae in irrigated and dryland crops.

The higher incidence of 'unknown' mortality in irrigated compared with dryland crops may be due to consistently higher soil moisture conditions under irrigated crops favouring soil-borne micro-organisms such as nematodes, bacteria and protozoa (Barbercheck 1992). No attempt was made to identify these organisms using the post-sampling pupal handling procedures used in this study. While white mycelial growth of organisms such as *Beauveria bassiana* Balsamo is obvious during sampling, organisms such as nematodes, bacteria and protozoa require more detailed examination. Failure of pupae to successfully emerge as moths has been reported in other studies. In a study of overwintering *Helicoverpa* spp. in the Namoi Valley, Wilson (1983) found 44% of dead pupae contained dead parasitoids, mainly *Ichneumon promissorius* Erichson. Another study of overwintering pupal abundance and mortality in the Namoi-Gwydir region of New South Wales found the proportion of dead pupae was generally low, but ranged from 0 to 66.7% (Fitt & Daly 1990). Murray and Zalucki (1994) reported average mortality from unknown causes of 8.8% in combined within-season and overwintering pupal populations on the Darling Downs.

Parasitoids made a relatively consistent contribution to pupal mortality across years. However, higher parasitism levels were recorded in dryland crops compared with irrigated crops. Higher reliance on insecticides in irrigated crops, particularly cotton, may have adversely affected parasitoid activity. During the period of this survey, pyrethroids and organophosphates were often used on late-season cotton, and these groups are known to be highly disruptive to natural enemies (Farrell 2007).

A higher proportion of viable pupae emerged as moths from maize than from any other crop. The possible disruptive effect of insecticides on parasitoids is not implicated in this outcome as maize is very rarely treated to control *H. armigera*. Larvae feeding within cobs are protected from parasitoids and, as large larvae, are exposed to parasitism for only a brief wandering period before burrowing into the soil to pupate. From this regard, maize is quite different from most other crops. For some larval parasitoids of *Helicoverpa* spp. there is also evidence of interference by other factors. For example, long-distance foraging by *Cotesia kazak* (Telenga) in maize was reduced (Cameron *et al.* 2006) and may be related to lack of

food resources, as has been found for *Microplitis croceipes* (Cresson) in sweet corn (Takusu & Lewis 1995). In laboratory experiments, maize volatiles were shown to interfere with host location by *M. croceipes* (Keller & Lewis 1989).

Parasitism explained a high proportion (44.1%) of pupal mortality (non-emergence of moths from pupae). The levels of parasitism recorded in this study were similar to those reported for both diapausing (Wilson 1983; Fitt & Daly 1990) and non-diapausing pupae (Murray & Zalucki 1994; Sequeira & Playford 2001), but much higher compared with studies by Duffield (2004) for overwintering *H. armigera* in southern New South Wales and Schneider (2003) for overwintering *Helicoverpa zea* (Boddie) in Mississippi.

There was no evidence from our field sampling to suggest predation of pupae was an important mortality factor. This is in agreement with other published findings for overwintering populations (Wilson 1983) and combined populations (Murray & Zalucki 1994).

## Recent changes to *Helicoverpa* spp. management in farming systems

A number of recent developments could lead to lower densities of overwintering pupae under late summer crops than those reported in our surveys during 1997–2001.

Two-gene cotton containing genes expressing Cry1Ac and Cry2Ab toxins (Bollgard II<sup>®</sup>) replaced Ingard<sup>®</sup> in 2003 and has captured about 85% of the Australian cotton acreage (Farrell 2007). Compared with Ingard<sup>®</sup>, Bollgard II<sup>®</sup> is much more efficacious against *Helicoverpa* spp. and provides full season protection, so very few overwintering pupae should be encountered under Bollgard II<sup>®</sup> crops. Despite the low risk of overwintering pupae being present under Bollgard II<sup>®</sup>, the Resistance Management Plan (Farrell 2007) has a zero risk attitude towards pupae under Bollgard II<sup>®</sup> and it is mandatory that all Bollgard II<sup>®</sup> crops be pupae busted as soon as practical after harvest.

There is also speculation that the strong performance of Bollgard II<sup>®</sup> against *H. armigera*, coupled with its high level of adoption, could alter the population dynamics of *H. armigera* in cotton/grain-farming systems with cotton now acting as a large sink for ovipositing moths.

Conservation of natural enemies is also favoured in Bollgard II<sup>®</sup> crops as they receive 70% fewer sprays per season compared with conventional crops (Anon. 2006). It is not known how effective parasitoids of *H. armigera* pupae will be in response to the very low densities of surviving larvae that may occur in Bollgard II<sup>®</sup> crops, but lower levels of *H. armigera* egg and larval parasitism have been shown in *Bt* transgenic cotton in China (Yang *et al.* 2005). In some grain crops, new and more efficacious insecticides have been registered against *Helicoverpa* spp. (Murray *et al.* 2005; Franzmann 2006), leading to further reductions in the numbers of survivors in insecticide-treated crops. Some of these products, e.g. indoxacarb, spinosad and *Helicoverpa* nucleopolyhedrovirus, are also less disruptive to natural enemies (Farrell 2007), and could lead to higher levels of parasitism in treated fields.

Notwithstanding some possible changes in the densities of overwintering *H. armigera* pupae under crops, irrigated crops are more likely to have overwintering pupae under them compared with dryland crops. In most years the majority of summer grain crops, which are predominately dryland, represent a low risk of harbouring overwintering *H. armigera* pupae, and have no requirement for post-harvest cultivation. In such cases, conservation tillage can be practised without risk of jeopardising insecticide resistance management strategies. Tools such as the diapause induction model (Farrell 2007) are available online to aid the decision-making process on the need for pupae busting. Resistance management strategies for both conventional and transgenic toxins should take these factors into consideration. The recent stabilisation or decline in resistance levels to most conventional insecticides (Rossiter *et al.* 2007) has been a catalyst to relax the requirement to pupae bust sprayed conventional cotton. Any moths emerging from these crops could contribute to the pool of *Bt* susceptible moths and effectively help delay the development of resistance to *Bt* toxins. Pupa busting to reduce survival of overwintering *H. armigera* pupae remains the most valuable non-chemical tool available to combat insecticide resistance. These changes to the pupae busting guidelines demand the careful and ongoing monitoring of resistance levels to conventional insecticides. Any increase in resistance levels should be a trigger for immediate resumption of pupae busting recommendations.

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