CHAPTER 7
LARVAL DIET AND FLIGHT PERFORMANCE

7.1 Introduction

The nutritional ecology of immature insects may influence such important adult life-history attributes as the timing and magnitude of reproductive effort (Englemann 1970, McCaffery 1976), adult body size, energy reserves, and longevity (Slansky & Rodriguez 1987). These factors may in turn significantly influence an insect's propensity for flight and migratory activity (Dingle 1985, 1989).

High polyphagy has been identified as a major contributor to the pest status of *H. punctigera* and *H. armigera* in Australia (Fitt 1989). Zalucki *et al.* (1986) recorded 159 species of plant as hosts, 78.6 % (125 / 159) of these being exotic, including many non-cultivated and weed hosts. Weeds and non-cultivated hosts are of significant importance to *Helicoverpa* spp, providing refuge for adults and alternate hosts for immatures (Wardhaugh *et al.* 1980, Coombs & Ramsey 1991). For *H. punctigera* and *H. armigera*, such hosts may, in addition, play an important role in early season establishment and build-up of populations prior to crops becoming attractive (Fitt 1989).

On the Northern Tablelands of New South Wales, *Helicoverpa* hosts are represented by weeds (eg. chicory, *Oenothera* spp), plants of improved pasture (eg. lucerne, *Medicago sativa* , and red clover, *Trifolium spp*) and some cultivated hosts (eg. sunflower, *Helianthus annuus*). Each of these plants are known to act as hosts for *Helicoverpa* immatures (Zalucki *et al.* 1986, P.Gregg pers. comm.). The aim of this chapter is to investigate the influence of larval diet on adult body size, the timing of reproductive effort and tethered-flight ability of *H. punctigera and H. armigera.*

7.2 Methods

The study was conducted in two parts. Firstly, larvae were bagged individually on plants in the field (experiment 1), where they remained until pupation. Secondly,

larvae, on individual diets, were maintained under constant conditions of photoperiod and temperature in the laboratory. These individuals were fed excised plant material brought in from the field on a daily basis (experiment 2). Full details are given below.

7.2.1 Larval treatments

All larvae used in these experiments were derived from 4th generation adults maintained under constant conditions of photoperiod (14:10 L:D), temperature (25 ± 1°C) and relative humidity (70 %). Newly laid eggs were transferred to and maintained on artificial diet (Teakle & Jensen 1985).

7.2.1.1 Experiment 1 - Larvae caged on plants in the field

Following development on artificial diet to late 2nd instar, cohorts of larvae were transferred to and contained in groups of 3 - 4 in nylon mesh bags (mesh diam. 1.0 mm) on chicory, sunflower, red clover or lucerne plants. The artificial diet was used in the early larval stages to reduce mortality. Sunflower, red clover and lucerne were grown on adjacent plots at the Laureldale Rural Research Station of The University of New England while chicory occurred on road-sides or as a weed of uncultivated land. For sunflowers, larvae were bagged on inflorescences only at or shortly after the commencement of anthesis, and each bag enclosed an entire inflorescence. For lucerne, red clover and chicory, bags enclosed an entire plant. Larvae were only bagged on plants that bore growing tips (floral and vegetative). *Helicoverpa* immatures preferentially feed on these structures (Zalucki *et al.* 1986). Larvae remained bagged until pupation. Pupation occurred within the frass accumulated at the bottom of each bag. Following pupation, pupae were returned to the laboratory and stored in groups of 10 -12 in 125 ml plastic containers at 25 ± 1 °C and 14:10 L:D until emergence. Two measurements of body size were recorded; pharate pupal weight (PPW) (mg), measured on the afternoon prior to emergence, and adult fore-wing length (FWL) (mm)(see section 4.3.1 for rationale). The time to first oviposition (days) was recorded for all flight tested moths.

7.2.1.2 Experiment 2- Larvae fed excised plant material

Following development of larvae to late 2nd instar on artificial diet, cohorts of larvae were either maintained on artificial diet (control) or transferred to one of four plant diets (treatments); sunflower, chicory, red clover or lucerne, which were presented as freshly excised plant material (flowers and buds). Larvae were held individually in 125 ml plastic cups. Excess plant material and frass were removed daily and replenished with fresh plant material. This continued until pupation occurred. Desiccation of the plant material was reduced by the close fitting lids on each of the plastic cups and the humidity (70 %) of the culture room. Following pupation, pupae were stored in groups of 10 - 12 in clear plastic cups. Larvae and pupae were held at a temperature of 25 ± 1 °C and a 14:10 L:D regime. Pharate pupal weight (PPW), fore-wing length (FWL) and pre-oviposition period were measured as above.

7.2.2 Flight testing

All moths were supplied with 10 % honey solution from the time of emergence (see section 3.2.1 for methodology). The honey solution was renewed daily. Flight testing involved tethering moths in a manner identical to that described in chapter 3. Only virgin females were flight tested and were flown on nights 1, 2, 4, 6 and 8 following emergence. Moths were flight tested over a 3 hr period, flights being initiated within 45 mins of the commencement of the dark phase (see section 3.2.2 for rationale). The duration of the longest flight exhibited was used in statistical analyses.

7.2.3 Statistics

Flight distributions (duration longest flight) were analysed using product limit survival analyses (Dixon 1981). Comparison of median flight durations (min) between treatment and control groups were made using the non-parametric Breslow (Generalised Wilcoxon) test (Dixon 1981). Potential differences in morphometric parameters and the time to first oviposition between treatment groups and controls

were examined using analysis of variance. Multiple range comparisons (SNK test) were subsequently used to identify where significant differences lay (Zar 1974).

7.3 Results

7.3.1 Experiment 1

Recoveries of *H. armigera* and *H. punctigera* pupae from bagged plants were very low. Percent recovery of pupae from different plants (number of pupae recovered as a function of numbers of larvae bagged) is presented in Table 7.1. Insufficient adults were generated to conduct meaningful experiments.

7.3.2 Experiment 2

7.3.2.1 Survival of immatures

Percent survival of *H. punctigera* and *H. armigera* immatures (measured as the proportion of larvae surviving to adult eclosion) fed excised plant material from selected wild hosts and artificial diet are presented in Table 7.2. Percent survival to adult eclosion was greatest on sunflower for both *H. punctigera* (88.2 %) and *H.armigera* (85.7 %). Percent survival of *H. punctigera* while lowest on lucerne (55.7 %), was substantially greater than that of *H. armigera* (4.9 %) on the same host. Survival of *H. punctigera* and *H. armigera* immatures fed on red clover and on chicory were intermediate between the values recorded for each species on lucerne and sunflower.

7.3.2.2 Body size and pre-oviposition period

Mean pharate pupal weights (PPW), fore-wing lengths (FWL) and pre-oviposition periods of female *H. punctigera* and *H. armigera* moths reared on selected wild hosts (treatments) or on artificial diet (control) are presented in Table 7.3. Analysis of variance indicated significant differences between treatment groups for pharate pupal weights of *H. punctigera* ($F_{4,70} = 57.2$, $P < 0.001$) and *H. armigera* ($F_{3,56} = 56.6$, $P < 0.001$), and fore-wing lengths for *H. punctigera* ($F_{4,70} = 31.3$, P

< 0.001) and *H. armigera* ($F_{3,56}$ = 94.5, P < 0.001). Pre-oviposition period was found to differ significantly between treatment groups for *H. punctigera* ($F_{4,70}$ = 3.67, P < 0.01), but not *H. armigera* ($F_{3,56}$ = 1.38, P > 0.05).

For *H. punctigera*, both PPW and FWL of adults derived from larvae that had fed on excised plant material were significantly lower (P < 0.05) than those of adults derived from larvae that had fed on artificial diet. Among plant diets, adults reared from larvae fed on red clover and lucerne exhibited the smallest PPW and FWL (minimums of 91 mg and 11.5 mm). Adults reared from larvae fed on chicory and sunflower were intermediate in size between adults reared on red clover or lucerne and adults reared on artificial diet. The shortest mean time to first oviposition (2.1 days) was recorded in females reared on lucerne. The longest mean time to first oviposition (3.9 days) was recorded in females reared on chicory (see Table 7.3).

For *H. armigera*, PPW and FWL of adults reared on excised plant material were significantly (P < 0.05) less than those of adults derived from larvae that had fed on artificial diet. The smallest adults (FWL and PPW) were from larvae that had fed on red clover (minimums of 153 mg and 13.6 mm). Adults derived from larvae that had fed on chicory and sunflower were intermediate in size between those reared on red clover and on artificial diet (Table 7.3). Pre-oviposition period did not differ significantly between treatment and control groups (P > 0.05).

7.3.2.3 Flight performance

Median flight durations (min) and estimated standard errors (Dixon 1981) for moths reared on excised plant material and artificial diet are shown in Table 7.4, and Figs. 7.1 (*H. punctigera*) and 7.7 (*H. armigera*). Flight distribution curves are shown in Figs. 7.2 - 7.6 for *H. punctigera* and Figs. 7.8 - 7.12 for *H. armigera*. Data are presented for moths flown on nights 1, 2, 4, 6 and 8 after emergence. Rank tests identified significant differences between flight distributions only on nights 1 and 2 for *H. punctigera*. Examination of flight distributions on these nights (Fig 7.2 and 7.3) show that substantially more of the moths that were reared on artificial diet

undertook long flights (> 3hr) in comparison with moths derived from larvae fed on excised plant material. No significant differences were detected between flight distribution profiles on any night for *H. armigera* (see Table 7.4). For *H. punctigera* females that had fed on red clover, lucerne, sunflower or artificial diet as larvae, median flight durations (min) peaked on nights 4 to 6. For those derived from larvae that had fed on chicory, median flight duration was greatest on night 8 (Table 7.4, Fig 7.1). For *H. armigera* females that had fed on chicory, red clover, or artificial diet, median flight durations peaked on night 4 (Fig 7.7). For females that had fed on sunflower as larvae, median flight duration was greatest on night 2.

7.4 DISCUSSION

Larvae of both species readily established on all excised plant material offered, with the exception of *H. armigera* larvae supplied with excised lucerne. Significant differences in adult body size parameters (PPW and FWL) were detected among treatment groups (Table 7.3), suggesting differing host plant qualities. In all cases larvae reared on excised plant material displayed lower body sizes than larvae reared on artificial diet, which can be regarded as more nutritionally complete. This is consistent with the findings of other studies estimating the nutritional qualities of wild hosts versus artificial diets (for example, Gross & Young 1977, Nadguada & Pitre 1983).

Though all of the plant species used in the study are recorded hosts (see Zalucki *et al.* 1986) and the food plants were generally accepted when presented as excised buds and tips, only a small number of pupae were recovered when larvae were bagged in the field. Escape from the mesh bags, and the lack of a suitable pupation site are possible causes of the low yields. Though, *H. armigera* larvae readily chew through the sides of plastic rearing containers, *H. punctigera* larvae rarely did so. In addition, though both species normally pupate within cells constructed within the soil (Zalucki *et al.* 1986) they readily pupated in frass and plant remains in the laboratory. The accumulated frass and plant debris at the bottom of the mesh bags should have

provided an acceptable pupation site. An additional factor resulting in low pupal yields may have arisen as a consequence of enclosing the larvae on individual plants or plant parts. Feeding by *Helicoverpa* spp is characterised by extensive movement of the larvae within and between plants (Zalucki *et al.* 1986). This may be a response by the larvae to avoid secondary metabolites mobilised by the plant; such chemicals may serve to decrease or suppress the feeding response or may be toxic (Berenbaum 1986). Restricting larvae to an individual plant may result in sufficient quantities of these substances being ingested to disrupt growth and function, eventually resulting in high mortality. Providing freshly excised plant material on a daily basis to larvae maintained in the laboratory apparently prevented this occurring to those insects.

Insect larvae in general, require a minimum critical weight for pupation, and to produce a pupae capable of metamorphosing to a 'functional' adult that will survive and reproduce (Slansky & Scriber 1985). *H. punctigera* and *H. armigera* larvae have the ability to pupate even when they are very small (PPW of 91 - 372 mg for *H. punctigera,* and 153 - 413 mg for *H. armigera,* see Table 7.3). Clearly, both species possess plasticity in the ability of larvae to produce viable pupae, and of pupae to produce viable adults. This plasticity leads to wide variation in adult body size (as measured by pharate pupal weight and adult fore-wing length). The lower pupal weights that permitted adult emergence were approximately 24 % (91 / 372) and 37 % (153 / 413) of the maximum pupal weight recorded for *H. punctigera* and *H. armigera*, respectively. Mukerji & Guppy (1970) recorded a lower pupal weight threshold (as a percentage of the maximum observed) of 36 % for the noctuid, *Pseudaletia unipuncta.* The ability of *H. punctigera* and *H. armigera* larvae to pupate at a very small size may be of adaptive value in that it confers the ability to withstand food shortage. Slansky & Rodriguez (1987) suggest that such behaviour may have evolved within the context of a high probability that larvae will be subject to prolonged periods of starvation during their development. *H. punctigera* , and to a lesser extent *H. armigera,* inhabit semi-arid regions of inland Australia breeding on native ephemerals (Gregg *et al.* 1989). The patchy and intermittent distribution of rainfall on

which these plants are dependent for growth and reproduction may lead to temporal variation in the availability and nutritional quality of these plants and the host plant complex itself. Low thresholds and high plasticity of pupal weights may have evolved in *H.punctigera* and *H. armigera* in such habitats. Other noctuids (see Farrow & McDonald 1987) inhabiting the semi-arid zone would be expected to have similar attributes, though this remains to be determined.

As found for other heliothine spp (eg. Gross & Young 1977, Nadguada & Pitre 1983), pre-oviposition periods were largely not influenced by differing larval diets. For *H.armigera* females, mean pre-oviposition period ranged between 2.0 to 2.7 days. However, for *H. punctigera* females that were reared on chicory, oviposition did not commence on average until 3.9 days after emergence, compared with the 2.1 - 2.9 days for females reared on the other plant diets and artificial diet. The delay in the onset of reproduction for females reared on chicory also coincided with a delay in the peak median flight duration for these moths (see Fig 7.1). As shown in Chapter 3, peak tethered-flight ability appears to coincide closely with the onset of reproductive activity for these moths.

Summary

Despite significant differences in body size and reproductive parameters of females reared on differing plant diets, there was no evidence of a significant effect on the tethered-flight abilities for either *H. punctigera* or *H. armigera* moths on any of the nights on which moths were flight tested (Table 7.4). Median flight durations increased during early adult life in both species in a manner consistent with that predicted from the tethered flight studies presented in Chapter 3. In addition, comparisons of flight distribution profiles show a consistently greater proportion of *H. armigera* females undertaking long flights (> 3 hr) in comparison with *H. punctigera* females. These results also support the findings on the differing tethered-flight abilities of the two species as determined in Chapter 3.

7.5 References

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Table 7.1 Numbers of second instar *H. punctigera* and *H. armigera* larvae caged on, and the numbers surviving to adult eclosion recovered from various plant hosts growing in the field. Red clover - RC, Lucerne - LUC, Chicory - CHIC, Sun Flower - SF.

	RC	LUC	CHIC	SF
H. punctigera				
No. of larvae	115	109	95	106
No. eclosing	3	7	4	2
% survival	2.6	6.4	4.2	1.9
H. armigera				
No. of larvae	86	75	85	112
No. eclosing	0	0	4	6
% survival	0	0	4.7	5.3

Table 7.2 Numbers of second instar *H. punctigera* and *H. armigera* larvae supplied with, and the numbers surviving to adult eclosion that had been fed with various plant hosts (excised tips and buds) and artificial diet. Red clover - RC, Lucerne - LUC, Chicory - CHIC, Sun Flower - SF, and artificial diet - ART.

	RC	LUC	CHIC	SF	ART
H. punctigera					
No. of larvae	108	104	65	110	106
No. eclosing	68	58	48	97	93
% survival	62.9	55.7	73.8	88.2	87.7
H. armigera					
No. of larvae	88	102	60	84	94
No. eclosing	53	5	34	72	81
% survival	60.2	4.9	56.7	85.7	86.2

Table 7.3 Pharate pupal weight (PPW) (mg), forewing length (FWL) (mm) and preoviposition period (days) for *H. punctigera* and *H. armigera* adults reared on various larval diets. Red clover - RC, Lucerne - LUC, Chicory - CHIC, Sun Flower - SF, and artificial diet - ART. Values represent mean ± s.d., (range). Values followed by differing superscripts differ significantly at $P < 0.05$.(Within row comparisons only).

		Treatment				Control
		RC	LUC	CHIC	SF	ART
H. punctigera	n	15	15	15	15	15
	PPW	$133.5^{a} \pm 19.7$	$152.6^{a} \pm 23.7$	$193.3^{b} \pm 25.3$	$199.4^{b} \pm 33.7$	$275.1^{c} \pm 34.3$
		(91 - 177)	(118 - 187)	(154 - 231)	(159 - 269)	(235 - 372)
	FWL	$12.9^{a} \pm 0.8$	$13.2^{a} \pm 0.9$	$13.9^{b} \pm 0.8$	$14.9^{c} \pm 0.8$	$15.9^{d} \pm 0.9$
		(11.5 - 14.1)	(11.0 - 15.0)	(12.4 - 15.4)	(13.1 - 16.4)	(15.0 - 17.6)
	Preovip.	$2.8^{ab} \pm 1.3$	$2.1^{a} \pm 0.8$	$3.9^{b} \pm 1.8$	$2.9^{ab} \pm 1.4$	$2.5^{a} \pm 1.1$
		(0 - 5)	(1 - 4)	(1 - 7)	(1 - 5)	(1 - 4)
H. armigera	n	15	-	15	15	15
	PPW	$212.8^{a} \pm 36.0$	-	$234.7^{a} \pm 42.9$	$289.0^{b} \pm 37.6$	$366.0^{c} \pm 20.2$
		(153 - 272)		(175 - 295)	(209 - 368)	(327 - 413)
	FWL	$14.4^{a} \pm 0.5$	-	$14.9^{b} \pm 0.45$	$15.6^{c} \pm 0.8$	$17.9^{d} \pm 0.7$
		(13.6 - 15.0)		(14.1 - 15.5)	(13.6 - 17.1)	(16.9 - 18.9)
	Preovip.	$2.6^{a} \pm 1.0$	-	$2.7^{a} \pm 1.2$	$2.4^{a} \pm 1.1$	$2.0^{a} \pm 0.9$
		(1 - 5)		(1 - 4)	(1 - 5)	(1 - 4)

Table 7.4 Median flight durations (min) for *H. punctigera* and *H. armigera* adults flown on nights 1, 2, 4, 6 and 8, reared on various larval diets. Red clover - RC, Lucerne - LUC, Chicory - CHIC, Sun Flower - SF, and artificial diet - ART. Values represent median ± s.e.

		Treatment				Control	Breslow	P
	Night	RC	LUC	CHIC	SF	ART	Statistic	
H. punctigera	1	1.3 ± 1.1	2.2 ± 0.7	2.3 ± 0.2	2.2 ± 0.6	3.7 ± 2.4	9.7	P < 0.05
	2	3.7 ± 1.1	4.3 ± 4.0	5.0 ± 2.0	3.0 ± 3.0	12.5 ± 3.8	18.7	P < 0.001
	4	17.0 ± 5.5	11.5 ± 3.1	7.2 ± 4.7	12.0 ±7.4	18.3 ± 2.9	8.4	P > 0.05
	6	19.0 ± 10.2	10.7 ± 4.9	10.2 ± 15.1	8.9 ± 2.0	16.7 ± 6.3	2.6	P > 0.5
	8	16.0 ± 9.7	11.0 ± 1.1	19.7 ± 2.4	6.5 ± 2.6	12.0 ± 4.1	2.3	P > 0.5
H. armigera	1	21.3 ± 31.7	-	16.3 ± 19.0	26.2 ± 27.9	11.3 ± 3.5	6.3	P > 0.05
	2	53.2 ± 19.3	-	92.5 ± 21.0	75.0 ± 5.9	30.0 ± 8.7	6.0	P > 0.1
	4	90.2 ± 67.1	-	114.0 ± 46.2	36.0 ± 8.0	52.0 ± 49.3	0.4	P > 0.75
	6	51.3 ± 24.0	-	88.0 ± 20.6	35.6 ± 7.6	33.0 ± 6.5	6.9	P > 0.05
	8	27.5 ± 10.4	-	31.5 ± 23.2	31.3 ± 9.2	41.3 ± 8.6	0.7	P > 0.75

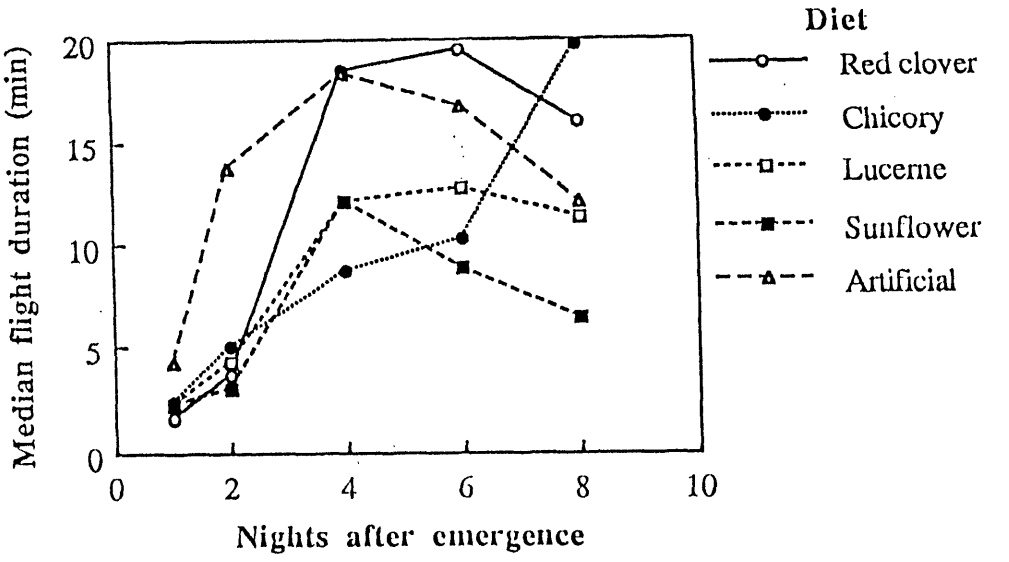

Fig 7.1 Median flight durations (mins) for *H. punctigera* females on nights 1, 2, 4, 6, and 8 following emergence. Moths reared on selected host plants or artificial diet (see legend).

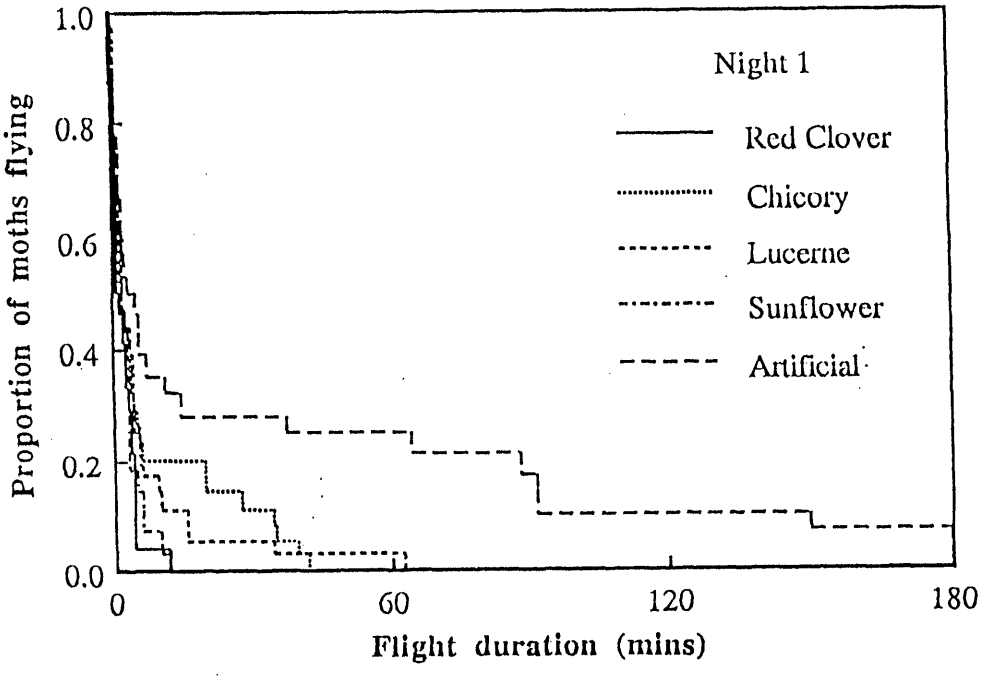

Fig 7.2 Flight distribution profiles for *H. punctigera* females on night 1 following emergence. Moths reared on selected host plants or artificial diet (see legend).

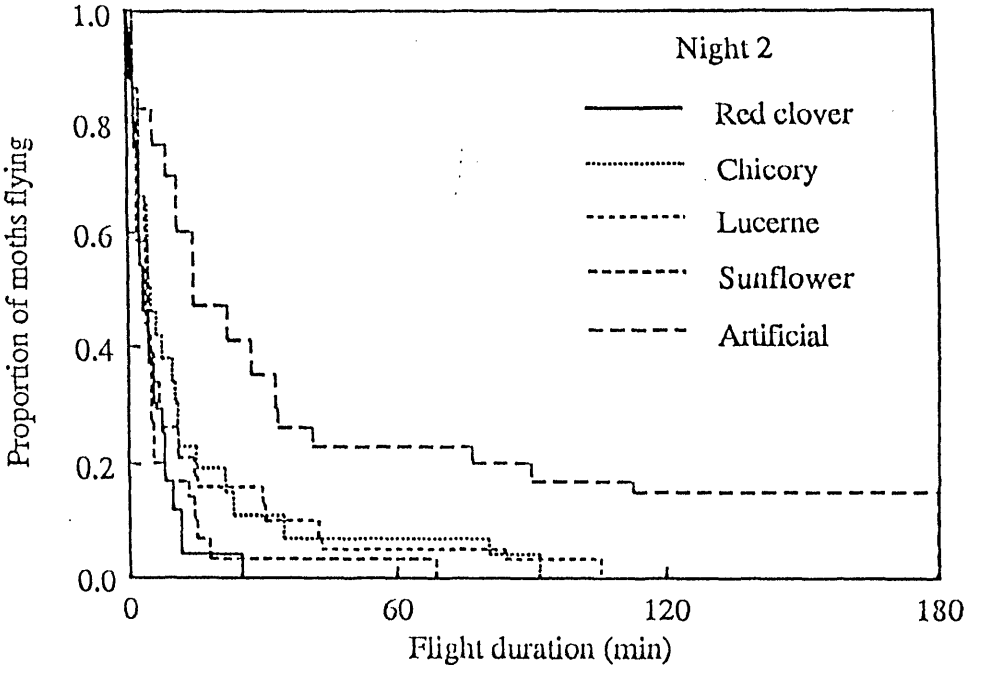

Fig 7.3 Flight distribution profiles for *H. punctigera* females on night 2 following emergence. Moths reared on selected host plants or artificial diet (see legend).

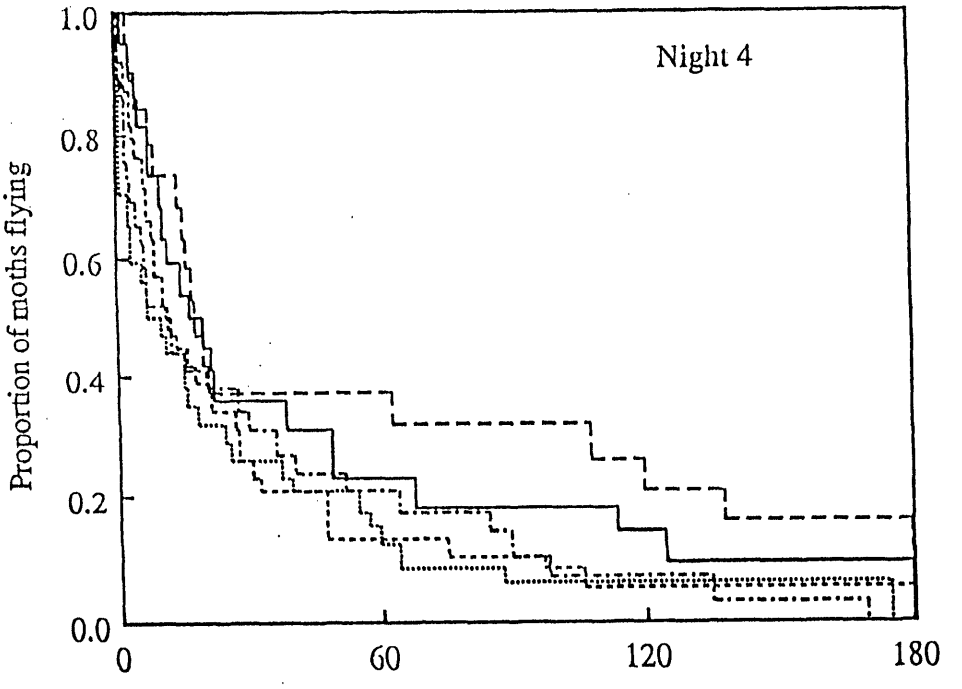

Fig 7.4 Flight distribution profiles for *H. punctigera* females on night 4 following emergence. Moths reared on selected host plants or artificial diet (see legend).

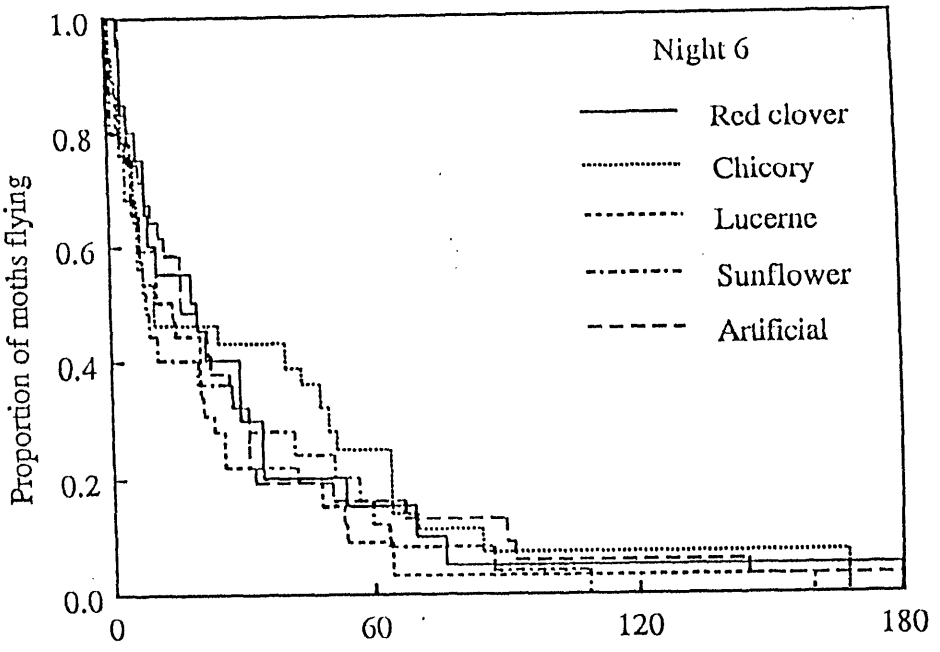

Fig 7.5 Flight distribution profiles for *H. punctigera* females on night 6 following emergence. Moths reared on selected host plants or artificial diet (see legend).

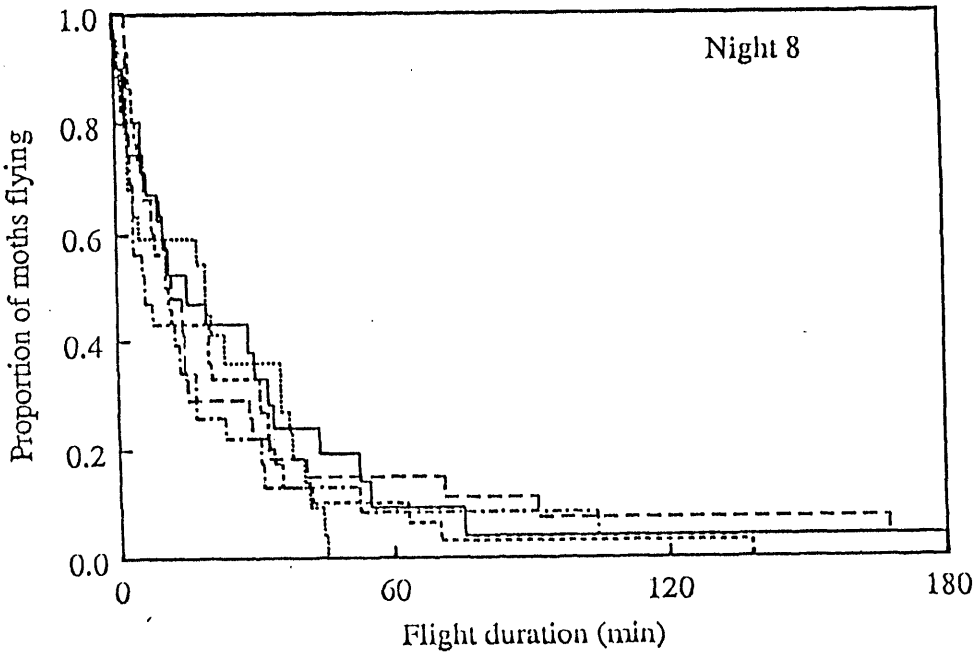

Fig 7.6 Flight distribution profiles for *H. punctigera* females on night 8 following emergence. Moths reared on selected host plants or artificial diet (see legend).

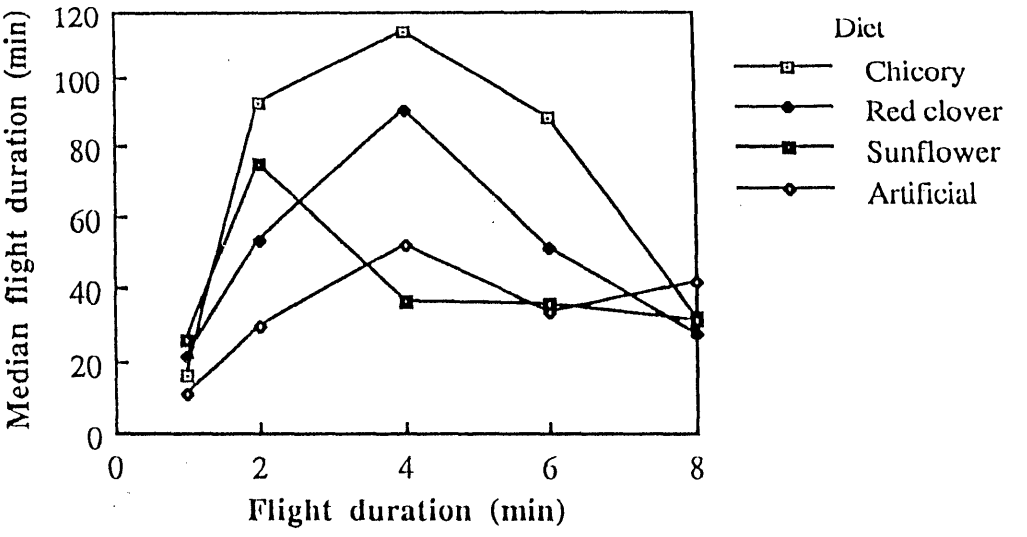

Fig 7.7 Median flight durations (mins) for *H. armigera* females on nights 1, 2, 4, 6, and 8 following emergence. Moths reared on selected host plants or artificial diet (see legend).

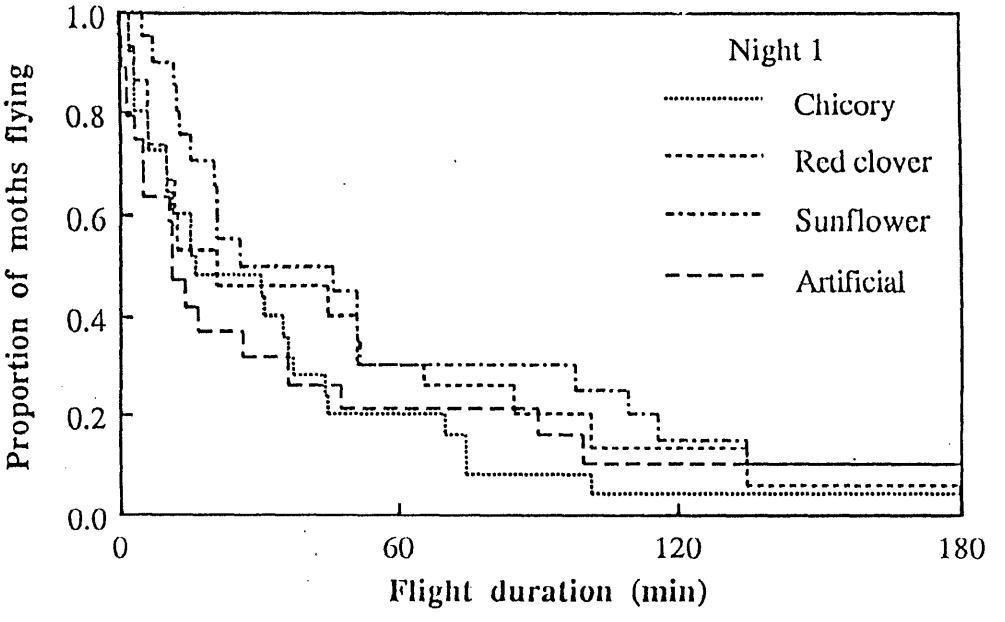

Fig 7.8 Flight distribution profiles for *H. armigera* females on night 1 following emergence. Moths reared on selected host plants or artificial diet (see legend).

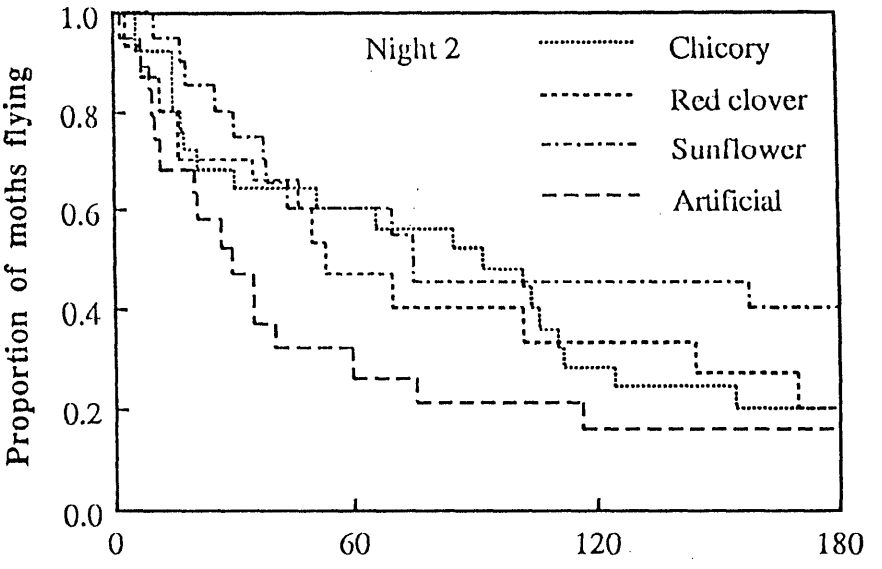

Fig 7.9 Flight distribution profiles for *H. armigera* females on night 2 following emergence. Moths reared on selected host plants or artificial diet (see legend).

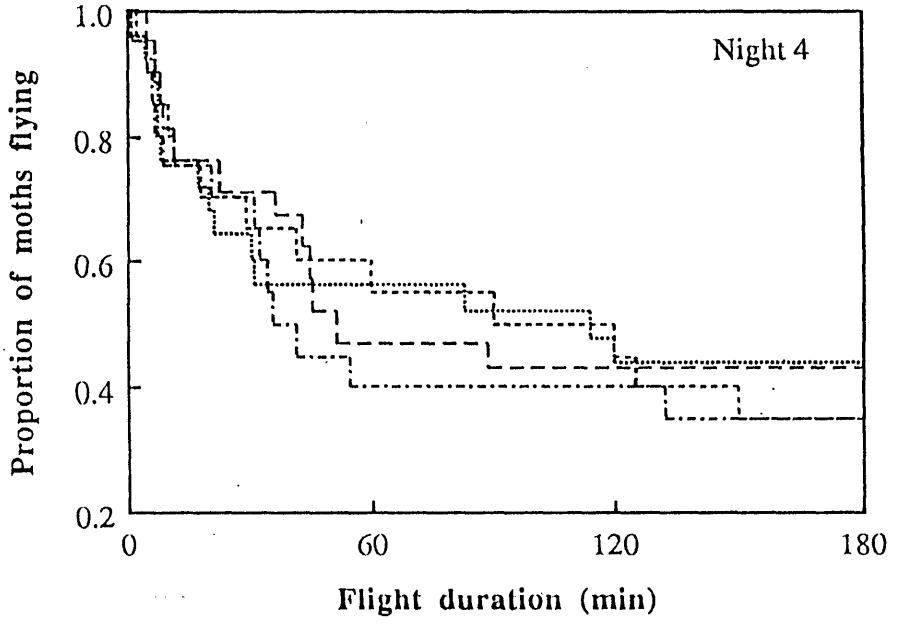

Fig 7.10 Flight distribution profiles for *H. armigera* females on night 4 following emergence. Moths reared on selected host plants or artificial diet (see legend).

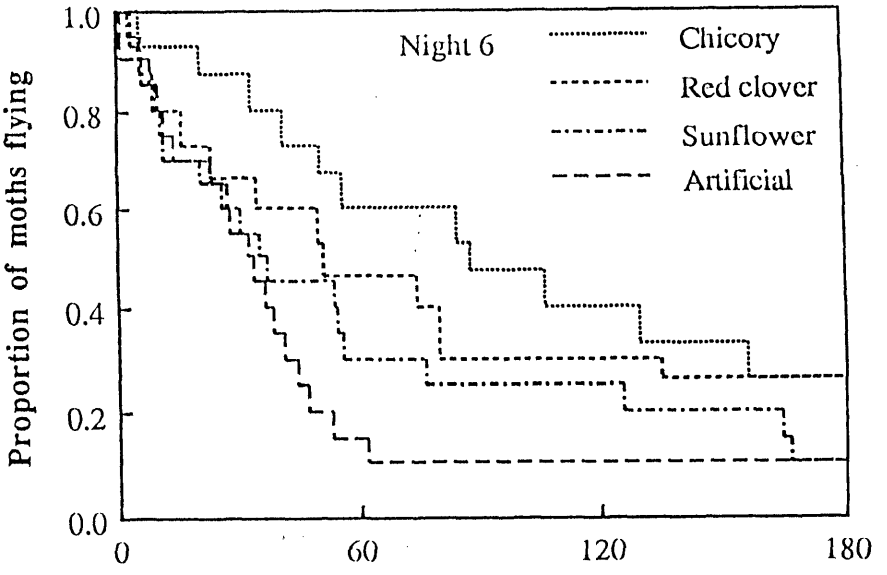

Fig 7.11 Flight distribution profiles for *H. armigera* females on night 6 following emergence. Moths reared on selected host plants or artificial diet (see legend).

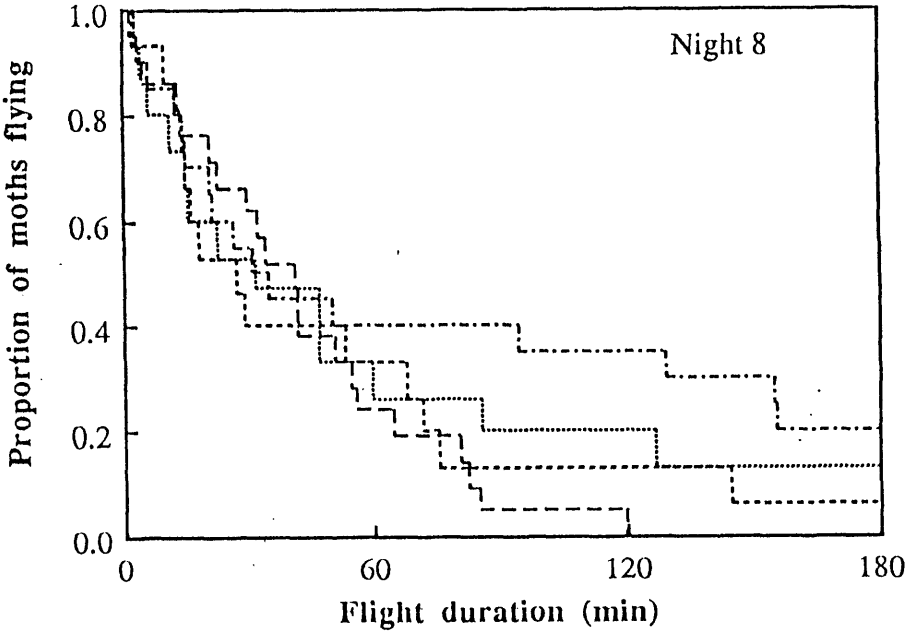

Fig 7.12 Flight distribution profiles for *H. armigera* females on night 8 following emergence. Moths reared on selected host plants or artificial diet (see legend).

CHAPTER 8
SEASONAL POLYPHENISM OF H. PUNCTIGERA

8.1 INTRODUCTION

H. punctigera is primarily an early to mid-season pest in major cropping regions of eastern and southeastern Australia (Zalucki *et al.* 1986, Fitt & Daly 1990), with adults usually appearing in mid to late September (early spring). *H. punctigera* populations typically persist for one or two generations in these regions before either disappearing or declining to low levels. The decline in numbers of *H. punctigera* during late summer and early autumn is unlikely to result from the deterioration of habitat quality. *H. armigera* maintains local populations throughout summer and into late autumn (Wilson 1983, Fitt & Daly 1990), and of the 159 host plants recorded for *H. punctigera* and *H. armigera* in Australia, 43 are shared by both species (Zalucki *et al.* 1986). Also, whereas *H. armigera* overwinters in these regions as diapausing pupae, *H. punctigera* either does not appear to do so or in very low numbers (Wilson 1983, Fitt & Daly 1990). The influx of *H. punctigera* into eastern cropping regions during spring, therefore, appears to be the result of immigration from other source areas. These spring influxes typically precede the expected period for local emergences of *H. punctigera* (Fitt & Daly 1990).

Recent surveys (Gregg *et al.* 1989, P. Gregg pers. comm.) have identified substantial winter breeding populations of *H. punctigera* in arid and semi-arid regions of inland Australia. The location of these populations are dependent on the growth of native emphemerals as hosts, which are in turn dependent on the geographic occurrence of autumn and winter rains. There is increasing evidence to suggest that these inland populations represent a major source of the spring immigrants arriving in to eastern cropping zones (Fitt & Daly 1990, Gregg unpubl. MS). However, the nature of these inland habitats suggest that they are themselves ephemeral. In early spring temperatures increase rapidly and daily maxima in excess of 30 ^{o}C are frequent. Also, rainfall is often very low (Division of National Mapping, 1986). Annual plants senesce quickly, leading

to deterioration in habitat over wide areas. It is unlikely that breeding populations of *H. punctigera* can persist in inland regions under these conditions during the summer period, or at levels sufficient to re-establish winter breeding populations. Emigration from eastern cropping zones may, therefore, be essential to re-establish inland populations.

Such return migrations have been demonstrated for some noctuid species. In the armyworm, *Mythimna separata*, round-trip migrations spanning several generations occur in eastern China (Chen & Bao 1987). In the latter species, seasonal changes in the direction of prevailing winds aid firstly movement to higher latitudes during spring and secondly return migrations to low latitudes during autumn. Many insects, including some Lepidoptera, have been shown to demonstrate seasonal changes in life-history traits (seasonal polyphenism) that facilitate such phases of migratory activity (Danthanarayana 1976, Tauber *et al.* 1986).

The aim of this chapter is to determine if there were any changes in life-history traits of *H.punctigera* that may facilitate emigration away from eastern regions and enable, in part, a seasonal redistribution of the species. Changes in photoperiod and temperature were considered as the most likely cues. The study did not attempt to address the problem of identifying a behavioural mechanism for the spring immigration of *H. punctigera* in to eastern cropping zones.

8.2 METHODS

8.2.1 Insect material

A culture of *H. punctigera* was established from field caught adults during early September 1989 (spring). Adults were contained as single male and female pairs in 325 ml plastic containers at 25 ± 1 °C and 14:10 L:D. Adults were supplied with 10 % honey solution, via soaked cotton wool plugs fitted to glass vials, and paper towelling as an oviposition surface, both of which were renewed daily. Offspring of these moths were reared individually in 35 ml plastic containers under the same conditions of photoperiod and temperature. Larvae were provided with artificial diet based on the formula provided

by Teakle & Jensen (1985). Pupation occurred within excess diet and frass. Following pupation pupae were transferred to and maintained in groups of 10 -12 in clear 125 ml plastic containers. Pupae were maintained under the same conditions as those for adults and larvae. The emerging adults were supplied with 10 % honey solution from the time of emergence, and maintained as above. These adults were designated as the F_0 generation. F_0 adults were maintained as single male and female pairs. Cohorts of 1st instar larvae (F_1 generation) from each F_0 adult pair (minimum of n = 30) were split equally to be either retained at 25 ± 1 °C and 14:10 L:D (control) or transferred to a field cage subject to natural diel fluctuations in temperature and photoperiod (treatment). F_1 generation larvae from the control group contributed to the subsequent F_2 generation for both control and treatment groups (see Fig 8.1). The rate at which generations were placed in the field cage was dependent on generation time in the control group.

8.2.2 Life-history parameters

The following parameters were measured for both control and treatment groups: larval and pupal duration (days), pharate pupal weight (mg) and length (mm), adult forewing length (mm), pre-oviposition period (days) and tethered-flight duration (min). These measurements were carried out for successive generations, and were documented for both sexes, other than for tethered flight duration where only females were tested.

8.2.3 Flight testing

Moths were tethered and flown by the same method as described in Chapter 3. Only females were flown. Moths were flown on night 4 following emergence as flight ability of *H. punctigera* adults is well developed at this age (see Section 3.3.1). Moths were held at 25 ± 1 °C, for a period of 1 hr prior to flight testing and during flight tests. All moths were flight tested for a period of 2 hr. The duration of the longest flight exhibited was used in statistical analyses. At the end of flight testing moths were returned to either the field cage or constant temperature room as appropriate.

8.2.4 Environmental parameters

Daily records of maximum and minimum temperature (°C) and daylength (hours of light) were recorded. These parameters were recorded throughout the duration of the study.

8.2.5 Statistical analyses

Analysis of covariance (Dixon 1981), with temperature (daily max °C) and photoperiod as covariates, was performed on each measured life-history parameter. Multiple range tests were used to identify where significant differences occurred (Zar 1974). Raw data were $\log_e$ transformed prior to analysis. Product-limit survival analyses (Dixon 1981) were employed to compare flight distribution profiles.

8.3 RESULTS

The influence of temperature and photoperiod on the measured life-history traits were investigated for 3 successive generations of *H. punctigera* under field cage conditions (treatment) and under constant laboratory conditions (control). The correspondence of these three generations (F_1 - F_3) with changes in photoperiod and temperature (as 10 day averages ± s.d) is shown in Fig 8.2. F_1 individuals experienced a phase of increasing photoperiod and temperature, including peak photoperiod (approx. 14 hr day^{-1}). F_2 individuals experienced the least change in photoperiod and temperature. F_3 individuals experienced decreasing temperature and photoperiod. Results of covariance analyses are presented in Table 8.1. Photoperiod and temperature were identified as significantly influencing larval and pupal duration and pupal weight. Forewing length was significantly influenced by temperature but not photoperiod, whereas pre-oviposition period was influenced by photoperiod, but not temperature.

The direction of differences within main effects (life-history parameters) are presented in Table 8.2. Within main effects, there were significant differences between sexes for pupal duration, pupal weight and pupal length. Significant differences existed between control and treatment groups for each parameter other than forewing length. A

significant generation effect was detected for pupal weight, pupal length and forewing length.

Untransformed means (± s.d.) of measured life-history traits are shown in Table 8.3. Values represent pooled data for males and females (directions of change were similar for both males and females). For cohorts of larvae maintained under constant photoperiod and temperature, larval and pupal durations and pre-oviposition period of females did not differ significantly with successive generations. Significant differences, however, were detected for pupal length and weight, and forewing length (the significant generation effect from Table 8.1). The differences, however, were overlapping, suggesting close similarities of means (Zar 1974).

For cohorts of larvae maintained in field cages significant differences were recorded for each of the measured life-history parameters other than pupal length and pupal weight. Mean larval and pupal durations were greatest in F_1 individuals and smallest in F_3 (for larval duration) and F_2 (for pupal duration) individuals. Mean forewing lengths increased progressively from F_1 through F_3 individuals. Pre-oviposition period similarly increased significantly from F_1 through F_3. Frequency histograms showing variability in pre-oviposition period in F_1, F_2 and F_3 are shown in Fig 8.4.

Most F_1 individuals (76 %) commenced oviposition within 1-2 nights of emergence, with all individuals ovipositing by night 4 (Fig 8.3a). For F_2 and F_3 individuals this value decreased to 50 % and 15 % respectively by 2 nights post-eclosion. A small percentage of F_2 and F_3 individuals did not commence oviposition until nights 7 and 8 respectively (Figs. 8.3 b and c).

Product-limit survival analyses indicated significant differences (Breslow = 6.26, d.f. = 2, P = 0.04) between flight distribution profiles of successive generations held in the field cage, but not between control generations (Breslow = 4.49, d.f. = 2, P = 0.11). Flight distribution profiles (females only) for treatment and control groups are shown in Figs. 8.4a and b. The proportions of females undertaking flights in excess of 2 hours increased with successive generations of moths maintained under field conditions.

Overall, the percentage of moths undertaking 'long flights' (> 2 hr) under field conditions (approx 4 % - 15 %) were considerably lower than those of females from control groups (20 % - 45 %) (Fig 8.4).

8.4 DISCUSSION

In temperate regions, seasonal changes in daylength are wide ranging (Beck 1980) and highly correlated with seasonal changes in temperature, moisture, and food supply. As a consequence, photoperiod plays an important role as a 'token stimulus' (Fraenkel & Gunn 1940) in regulating seasonal polyphenism of insects (Tauber *et al.* 1986, Gatehouse 1989, Dingle 1989). This study has shown that *H. punctigera* displays such seasonal polyphenism, whereby photoperiod and temperature were identified as significantly influencing such important life-history traits as development time of immatures, adult body size parameters, and time to first oviposition. In addition, significant changes in tethered-flight duration, used here as a presumptive index of migratory potential, were detected. Corresponding sibling generations held under fixed photoperiod and temperature conditions did not display any consistent phenotypic changes. This suggests that the results are due largely to polyphenic (environmental) influences rather than polymorphic (genetic) influences. Further, the experimental design negated possible maternal effects (Mousseau & Dingle 1991) as a likely contributor to the observed seasonal polyphenism. These influences are, however, as yet of undetermined importance in influencing the expression of seasonal polyphenism in *H. punctigera.*

The observed polyphenic changes in *H. punctigera* are consistent with a suite of traits associated with an oogenesis-migration syndrome (Johnson 1969, Tauber *et al.* 1986, Dingle 1986, 1989); characterised primarily by shortened generation time, delayed reproductive development, and increased flight propensity. In this study, successive generations of *H. punctigera*, subject to seasonal fluctuations of photoperiod and temperature, displayed a progressively increasing incidence of these traits.

Mean pre-oviposition period of *H. punctigera* females increased from 1.9 days (F_1 individuals) to 4.2 days (F_3 individuals) during the course of the study. If, as

indicated in Chapter 2, migratory flights are pre-reproductive in *H. punctigera* , then this represents a substantial increase in the time span available in which to migrate. The ratio of forewing to body length and body weight was also found to alter significantly. Whereas, body length and body weight remained constant, mean forewing lengths increased from 14.8 mm (F_1 individuals) to 16.1 mm (F_3 individuals), representing an 8 % gain. In *H. punctigera* , forewing length is highly correlated with gross wing area (see Ch 4). This clearly implies progressively lower wing loadings for F_2 and F_3 individuals; lower wing loadings make for more efficient flight (Johnson 1969, Pennycuik 1972). The progressively longer winged F_2 and F_3 individuals produced in late summer and early autumn (Fig 8.2) are, therefore, likely to be more efficient fliers than those produced during early summer (F_1).

Concomitant with changes in body size parameters, and delayed ovarian maturation, was a presumptive change in migratory propensity, as indicated by tethered-flight duration. Statistical analyses indicated significant changes between flight distribution profiles of generations (only females were tested) subject to fluctuating photoperiod and temperature, that were not detected between control generations. Although the statistical analysis employed (product-limit survival procedure) does not indicate the direction of differences between test groups, there was a clear trend towards progressively increasing flight duration between F_1 and F_3 individuals. This was evidenced by an increase in the proportion of F_2 and F_3 individuals undertaking flights that were in excess of 2 hours duration (Fig 8.5).

Although the results of this study demonstrate clear inter-generational changes in a suite of factors that can potentially extend the duration and efficiency of migratory flight, the interpretation of the significance of such changes in relation to the seasonal changes in the distribution of favourable breeding areas for *H. punctigera* is not clear.

Though return migrations among insects are well documented (see Dingle 1989), there is little direct information to support the occurrence of a return migration of *H. punctigera* from southeastern to inland regions of Australia. In general, the decline in populations of *H. punctigera* in eastern Australia generally precedes the time at which

breeding populations are recorded from the inland (Gregg *et al.* 1989). Recent studies by Gregg *et al.* (1993) suggest migration of *H. punctigera*, among other noctuids, from eastern cropping zones toward the semi-arid and arid pastoral zones during summer and early autumn.

Successful movement of *H. punctigera* moths inland will be subject to two factors; the existence of a suitable life-history phase for migration and secondly, the existence of a suitable transport mechanism. This study has demonstrated the existence of suitable life-history traits that may facilitate more efficient migratory flight of *H. punctigera* moths. A possible transport mechanism may be provided by winds from the south and southeast which are common during late summer and autumn in eastern Australia (Symmons 1986, Division of National Mapping 1986). These winds are typically much lighter than those associated with migrations of noctuids during spring and summer. If return migrations occur during autumn they are likely to be a slower and more indirect process possibly spanning several generations, although westward movements of up to a few hundred kilometres on sea breezes are also possible (Farrow & McDonald 1987).

Summary

Current evidence indicates movement of *H. punctigera* from winter breeding populations located in inland regions to eastern cropping zones during spring (September - October) (Fitt & Daly 1990, Gregg *et al.* 1989, Gregg Unpubl. MS). The ephemeral nature of host plants (Gregg *et al.* 1989), and general aridity of inland regions during late summer (January and February) (Bureau of National Mapping 1986), make it unlikely that breeding populations of *H. punctigera* can persist in these regions during this period. These inland breeding populations must consequently be re-established each year, suggesting a further seasonal redistribution of *H. punctigera.* Eastern cropping zones and improved pastures represent potential source areas for such a return migration. Such a re-establishment of inland winter breeding populations from coastal populations has been proposed for other noctuids (Farrow & McDonald 1987).

The observed seasonal polyphenism of life history traits displayed by *H. punctigera* are consistent with a suite of traits associated with an oogenesis/migration syndrome. These traits, coupled with seasonally favourable wind systems may enhance the seasonal re-establishment of *H. punctigera* populations in inland Australia.

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Table 8.1 Results of three way analyses of covariance of *H. punctigera* life-history parameters, with temperature (°C) and photoperiod (hours of light) as covariates. Pooled data for all generations.

	L.d (days)	P.d (days)	PPL (mm)	PPW (mg)	FWL (mm)	Preov. (days)
Covariates						
Temp.	***	***	n.s.	*	***	n.s.
Photo.	***	***	n.s.	*	n.s.	***
Main Effects						
Sex	n.s.	***	***	***	n.s.	----
Cntrl / Treat.	***	***	*	**	n.s.	**
Generation	n.s.	n.s.	**	*	**	n.s.

n.s. Not significant, * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

L.d - Larval duration, P.d - Pupal duration, PPL - Pharate Pupal Length, PPW - Pharate Pupal Weight, FWL - Forewing Length, Preov. - Preoviposition period.

Table 8.2 Direction of differences within main effects for each of the developmental and body size parameters measured.

	Larval duration	Pupal duration	Pupal length	Pupal weight	Forewing length	Preovip. period
Sex M/F	M=F	M>F	M>F	M>F	M=F	---
Cntr / Treat	C<T	C<T	C>T	C>T	C=T	C<T
Generation	$F_1=F_2=F_3$	$F_1=F_2=F_3$	$F_3<F_1<F_2$	$F_3<F_1<F_2$	$F_2<F_3<F_1$	$F_1=F_2=F_3$

Table 8.3 Immature and adult developmental and body size parameters for successive generations of *H. punctigera* reared under constant (control) and fluctuating environments. Values represent mean ± s.d. Values followed by differing superscripts differ signiifcantly at $P < 0.01$ (Within row comparisons only).

		F_1	F_2	F_3
Larval duration	Control	22.3 ± 1.5^{a}	21.8 ± 1.5^{a}	22.1 ± 1.5^{a}
(days)	Treatment	37.5 ± 3.1^{a}	28.7 ± 2.3^{b}	24.6 ± 1.4^{c}
Pupal duration	Control	17.4 ± 1.6^{a}	17.7 ± 1.8^{a}	18.1 ± 2.1^{a}
(days)	Treatment	36.2 ± 3.1^{a}	22.2 ± 2.3^{b}	26.4 ± 2.3^{c}
Pharate pupal	Control	283 ± 25^{ab}	288 ± 21^{b}	277 ± 23^{a}
Weight (mg)	Treatment	263 ± 23^{a}	268 ± 28^{a}	271 ± 26^{a}
Pharate pupal	Control	18.4 ± 0.7^{ab}	18.6 ± 0.6^{b}	18.1 ± 0.7^{a}
Length (mm)	Treatment	18.4 ± 0.8^{a}	18.3 ± 0.9^{a}	18.2 ± 0.8^{a}
Fore-wing	Control	16.1 ± 0.7^{a}	15.6 ± 0.7^{b}	16.0 ± 0.6^{a}
Length (mm)	Treatment	14.8 ± 0.7^{a}	15.4 ± 0.8^{b}	16.1 ± 0.7^{c}
Preoviposition	Control	1.2 ± 0.5^{a}	1.3 ± 0.4^{a}	1.2 ± 0.5^{a}
Period (days)	Treatment	1.9 ± 0.6^{a}	2.6 ± 0.4^{b}	4.2 ± 0.9^{c}

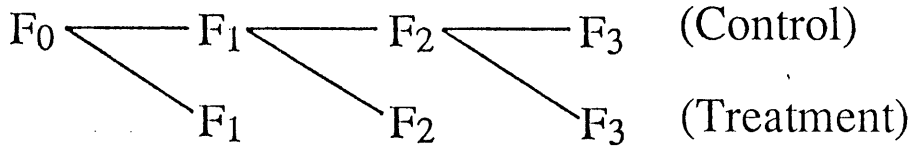

Fig 8.1 Experimental design employed to investigate seasonal polyphenism of *H. punctigera*. Moths reared at 25 ± 1 °C and 14:10 L:D (Control) or seasonal fluctuations of temperature and photperiod (Treatment).

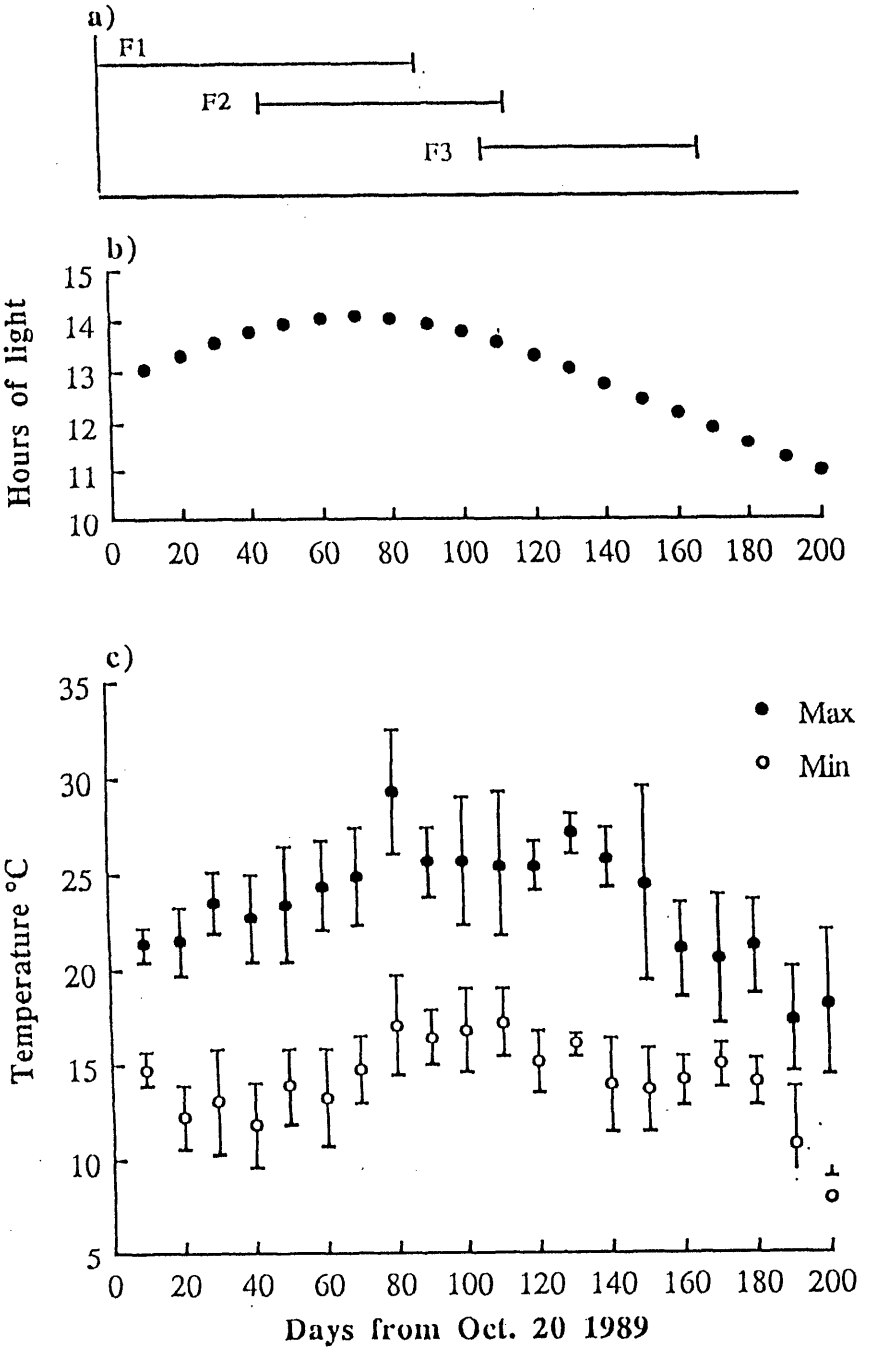

Fig 8.2 Concordance of a) *H. punctigera* generations reared in field cage (treatment) with, b) photoperiod (hours of light), and c) maximum and minimum temperature (°C). Values represent 10 day averages ± s.d. (s.d. bars for photoperiod smaller than data points).

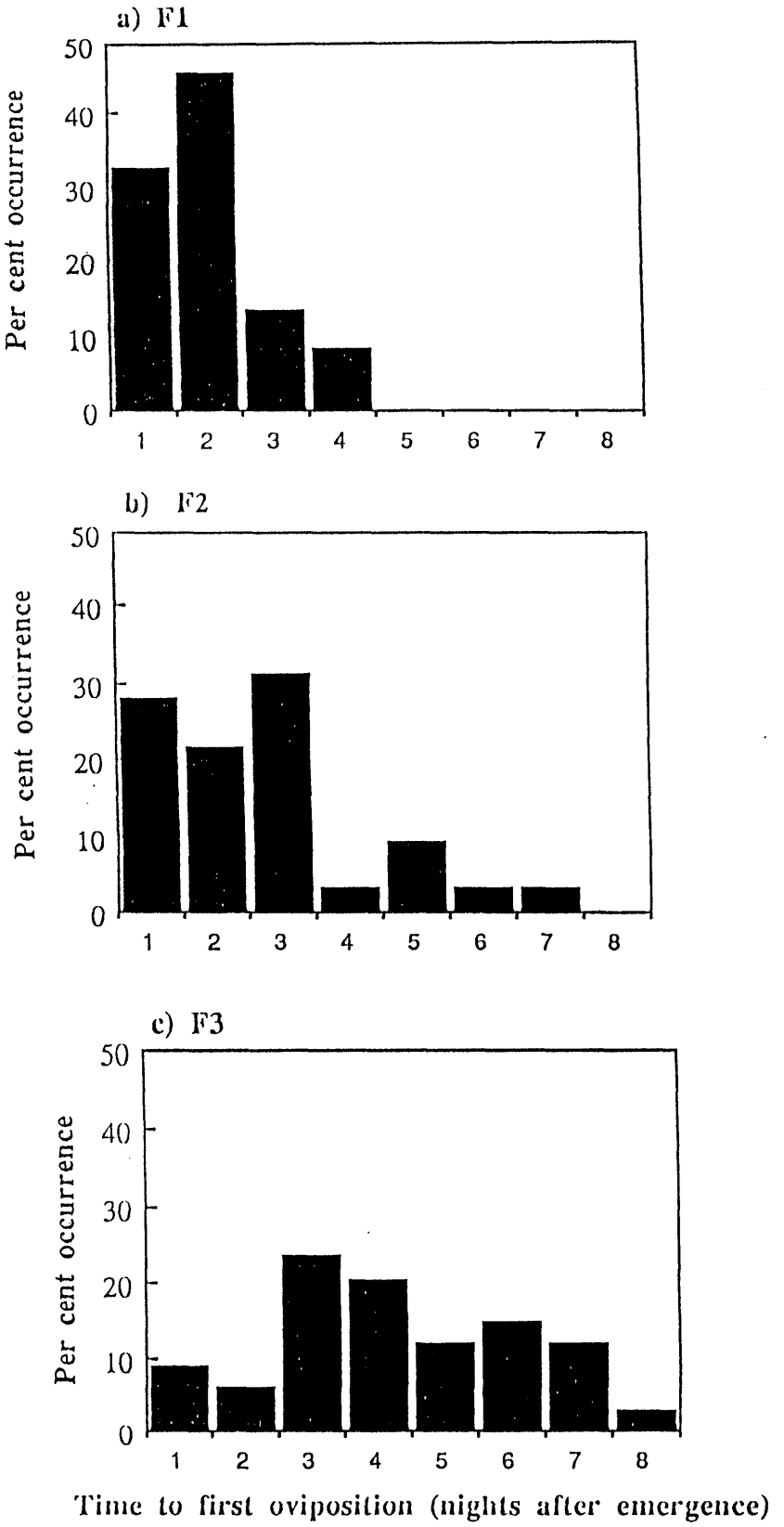

Fig 8.3 Frequency histograms of time to first oviposition for F_1, F_2, and F_3 females subject to seasonal fluctuations of temperature and photoperiod (treatment).

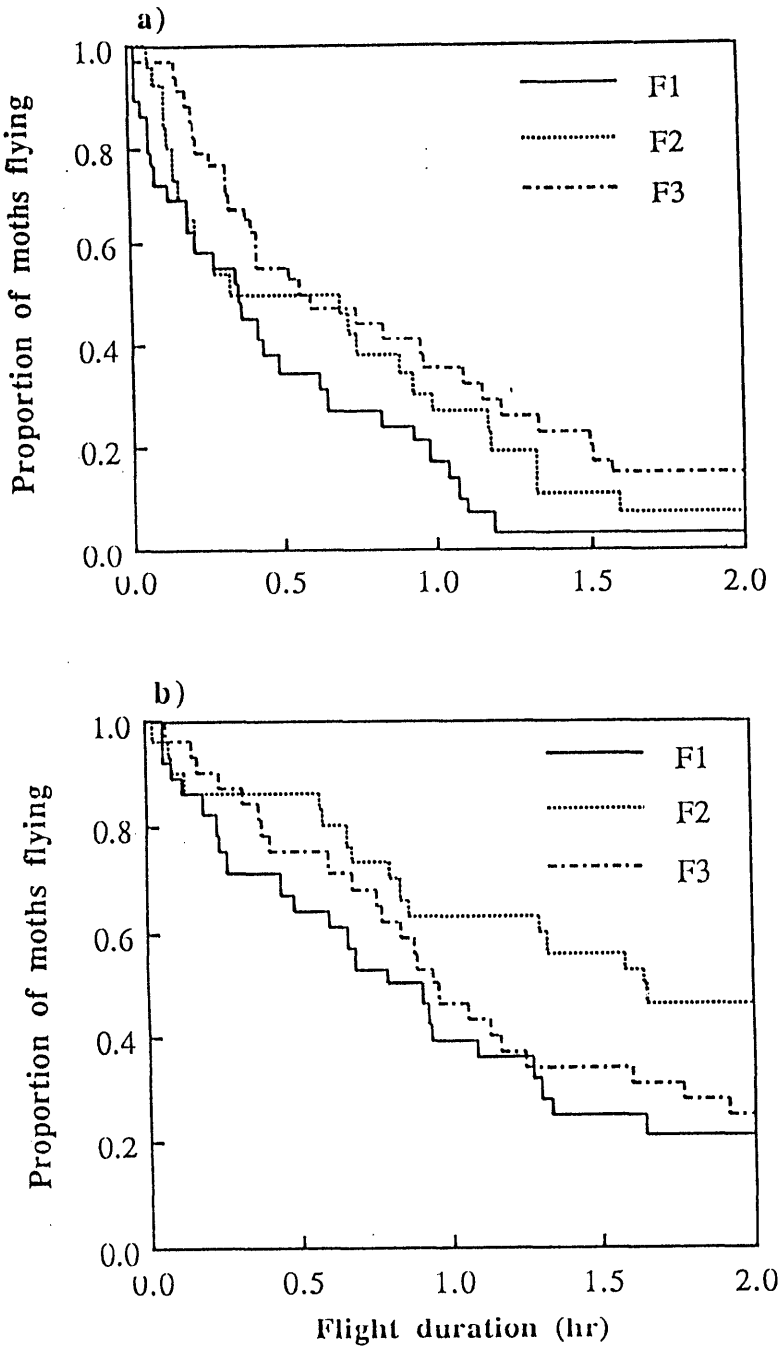

Fig 8.4 Flight distribution profiles for F_1, F_2, and F_3 females from a) treatment and b) control groups.

CHAPTER 9

GENERAL CONCLUSIONS

9.1 General conclusions

The purpose of this study was to consider the role of environmental factors in modifying the flight capacity of the noctuids, *Helicoverpa punctigera* (Wallengren) and *H. armigera* (Hübner).

The experiments described in chapter 3 were conducted to examine the association of flight capacity (tethered flight duration) with reproductive development. For *H. punctigera* and *H. armigera,* increases in flight capacity were coincident with the onset, and peak, in reproductive activity (as marked by the deposition of eggs). Among heliothine moths, males are sexually mature on the night following emergence (Henneberry & Clayton 1984), and females commence calling and mating once initial maturation of the ovaries has occurred (Kou & Chow 1987). In the tethered flight studies of chapter 3, initial maturation and deposition of eggs occurred within 2 - 3 nights of emergence for most *H. punctigera* and *H. armigera* females.

Determination of the reproductive status of migrant *H. punctigera* and *H. armigera* collected from a tower-mounted light trap (chapter 2), showed that nearly all moths were reproductively immature (they contained no full sized eggs). The rapid onset of reproductive activity by *Helicoverpa* females recorded in this study and elsewhere (Kou & Chow 1987), suggests that these migrant moths were likely to be only 1 - 2 days old (nights 2 & 3 after emergence). The tethered flight studies of chapter 3 showed that a small proportion of *H. punctigera* and *H. armigera* individuals were capable of prolonged flights (> 5 hr duration) from the night following emergence. Clearly, these moths represent those individuals most likely to undertake, and with the greatest capacity for pre-reproductive migration. Alternatively, the pre-reproductive moths trapped on the tower may represent the small proportion of individuals that delay oviposition until nights 4 and 5 after emergence (see Table 3.1). Migratory flight at this age would coincide with the period of maximum flight capacity observed during tethered flight experiments (see

chapters 3 & 4). Until an estimate of age can be made which is independent of ovarian development, it will be impossible to accurately assign a chronological age to field caught moths.

Long flying moths were present throughout the twelve nights on which moths were flight tested. If migratory flights serve, among other things, to take insects beyond their current habitat to allow colonisation of new habitats (Johnson 1969, Dingle 1985, Tauber *et al.* 1986), clearly, *H. punctigera* and *H. armigera* adults possess the capacity for such flights throughout their life-time. This is well supported by field observations that mature females undertake extensive intercrop movements (Topper 1987). For such highly mobile insects as *Helicoverpa* spp, there may be no clear distinction between the scale of movements associated with low level migratory versus vegetative flights. Farrow & Daly (1987) consider that such movements represent opposite ends of a continuum, the scale of flights being dependent on the spatial distribution of suitable habitats, the behaviour of the moth, and prevailing atmospheric conditions.

A feature common to most studies of insect tethered flight (including this study) has been the documentation of great variation in flight duration between individual moths in the laboratory. Typically, few moths are capable of long duration flights with the majority exhibiting only short duration flights. The range of this variation is generally perceived to reflect adaptation to the pattern of distribution of habitat patches in the environment (Davis 1980, Gatehouse 1989). Outside of cropping regions in Australia, *Helicoverpa* spp breed largely in spatially and temporally heterogeneous environments (see Gregg *et al.* 1989, Coombs & Ramsey 1991). High phenotypic variance for flight capacity is characteristic of species which inhabit such environments (Dingle 1989). Hence, variation in the flight capacity of *Helicoverpa* spp may be a result of the selective pressures imposed by such environments.

The reproductive success of females of both species was dependent on carbohydrate availability. Fecundities were reduced when adults were fed with water only. Denying access to carbohydrates did not influence flight capacity during early adult life in either species; however, continued starvation eventually depressed flight.

Successful mating increased both the fecundities and oviposition rates while reducing the longevities of females. Mating did not influence flight capacity during early adult life (night 4), but eventually depressed flight in later adult life. This is presumably a consequence of the diversion of energy to the increased reproductive effort that follows mating.

There was no correlation between the flight capacity and body size parameters of *H. punctigera* or *H. armigera* adults evident from the experiments conducted in chapter 4. Further, though *H. punctigera* and *H. armigera* exhibited phenotypic flexibility of adult body size in response to differing larval hosts (Chapter 7), no clear differences in flight capacity were evident. It must be concluded from these results that flight capacity cannot be predicted from body size and morphometric parameters.

In *H. punctigera* and *H. armigera* average flight speeds and overnight (10 hour) flight distances achieved on flight mills increased between night 1 and night 4 following emergence. *H. punctigera* moths maintained average flight speeds of 3.2 and 3.6 km hr $^{-1}$ and *H. armigera* moths 4.8 and 5.2 km hr $^{-1}$ on night 4. Individuals of both species produced maximum overnight flight distances of more than 60 km. These moths would have flown throughout the 10 hour test period to achieve these flight distances. These results show that both species certainly possess the capacity for intercrop movements and possibly inter-regional movements during the reproductive phase of their lives.

Populations of *H. punctigera* and *H. armigera* originating from widely separated geographical regions showed significant variations in some life history traits and in flight capacity during part of their adult lives (chapter 5). Maintenance of these populations under uniform laboratory conditions indicates that the observed differences were genetically based. Prior studies of genetic (electrophoretic) variation among *Helicoverpa* populations (Daly & Gregg 1985) suggests that, although populations are genetically differentiated, considerable gene flow occurs between widely separated regions. This is consistent with the migratory nature of *Helicoverpa* spp, and may account for the low

number of life history traits that were found to differ significantly between populations in this study.

In chapter 6, I examined the role of environmental temperature on flight of *H. punctigera* and *H. armigera* adults. Environmental (ambient) temperature exerts its influence primarily through an insect's body temperature (May 1985). During free flight both species maintain body temperature relatively independent of environmental temperature. Restraining moths by a tether acted to impair thermoregulatory ability as evidenced by increasing dependence of body temperature on environmental temperature. Impairment of normal thermoregulatory ability may have inadvertent effects on other physiological processes (for example, energy transfer) which may in turn influence flight performance parameters (duration and speed). Elevation of body temperature, and its maintenance above ambient, is achieved through endothermic mechanisms (wing shivering) of heat production. Endothermy by *H. punctigera* and *H. armigera* adults becomes energetically more expensive as the difference between body temperature and ambient increases. Neither species were able to initiate flight or maintain flight at ambient temperatures below 5 °C. Such physiological constraints may act to limit the periodicity of flight and the vertical distribution of insects during long distance migrations at high altitude. Field observations show that the diel periodicity of *Helicoverpa* flight is limited by low ambient temperatures (Coombs 1992).

The geographic range of *Helicoverpa* spp covers approximately 27 ° of latitude 11 ° to 38 °) in continental Australia and a further 2° 30' of latitude in Tasmania (41° to 43° 30') (Zalucki *et al.* 1986). Seasonal changes in photoperiod and temperature are large at higher latitudes (Beck 1980). As Armidale (NSW) is situated at approximately 35 ° S., changes in photoperiod and temperature at this latitude should be large enough to act as reliable cues of impending habitat change. The experiments described in Chapter 8 were undertaken to determine if seasonal changes in photoperiod and temperature influenced the expression of life-history traits for *H. punctigera*. The study documented clear inter-generational changes in the following traits: increased time to first oviposition, an increase in the ratio of wing length to body length, and increased flight capacity. How

or whether these phenotypic changes relate to the seasonal movement of *H. punctigera* populations is unclear. It is possible that winter breeding populations of *Helicoverpa* spp in inland regions are, in part, re-established by movement of adults from eastern cropping and pasture regions during early autumn. The suite of life-history traits exhibited by late season (late summer/ early autumn) *H. punctigera* adults on the Northern tablelands may facilitate more efficient migratory flight and enable such a re-distribution.

Throughout the study I have sought to minimise the likelihood of inbreeding depression and inadvertent selection of genotypes by maximising the number of mated pairs contributing to successive laboratory generations. In all cases at least 25 to 30 pairs of each species were maintained. This number is consistent with other researchers breeding Noctuids for behavioural research (A. G. Gatehouse pers. comm.). In addition I sought to minimise the number of laboratory generations that were required to complete experiments. During this study *Helicoverpa* cultures were maintained for not more than four generations prior to experimentation. Cultures of both species were regularly invigorated by addition of wild stock. Limiting experiments to insect material that had been in culture for a maximum of four generations is conservative when compared with the methodology of other Noctuid researchers. For example, Leppla *et al.* (1979), Gunn & Gatehouse (1987) and Sappington & Showers (1991) all used insect material that was in culture from between 8 to 10 generations prior to experimentation. It is common for some researchers to use insects that have been in culture for much longer periods. Sharp *et al.* (1975) and Lopez (1986) experimented with laboratory stock of three years of age, and Willers et al. (1987) experimented with *Heliothis virescens* that had been in culture for over 40 generations. In some instances, generation number is not reported (eg. Hill & Hirai 1986, Kawasaki 1986).

9.2 Directions for Future Research

Though significant differences in flight capacity are evident between *H. punctigera* and *H. armigera* under laboratory conditions, similar evidence from field situations is lacking. *H. punctigera* is generally assumed to exhibit greater migratory

propensity than *H. armigera* (Farrow & Daly, 1987). Potential differences in flight ability at the intra- and inter-crop level have not been investigated. Current attempts at modelling adult *Helicoverpa* movement (Dillon & Fitt, 1990) assume identical flight speeds and maximum flight duration for the two species. The results of this study have clearly shown *H. armigera* to be the stronger flier. Whether similar differences can be quantified in the field remains to be determined. The use of optical and video imaging techniques, such as those used by Riley *et al.* (1992), should enable potential differences to be detected.

The use of mark and recapture techniques, as outlined by Raulston (1979), may provide insight into differences in migratory propensity between *H. punctigera* and *H. armigera.* A mark and recapture study initiated by Fitt & Pinkerton (1990) incorporated radioisotope into larval food plants with the resulting adults being radioactively labelled. Dispersal of adults from an emergence site was then monitored by recovery of labelled adults from traps set at varying distances from the source crop. Their results indicated that *H. punctigera* was more mobile than *H. armigera*; the majority of *H. punctigera* which emerged appeared to leave the source area.

Analyses of the influence of environmental factors on flight capacity have been considered largely one at a time during the course of this study. It is reasonable to suppose that these factors do not operate independently of one another under field conditions. Tethered flight studies of adults derived from larvae reared under various agronomic situations (varying stages of host plant development, differing crop regimes) may provide insight into the interaction of environmental factors.

H. punctigera has been shown to display inter-generational changes in life-history traits under seasonal variation of photoperiod and temperature. Evidence to demonstrate similar changes in field populations remains to be documented. Sampling field populations for behavioural and morphological traits at regular intervals through the spring to autumn period should enable such changes to be detected. Such an approach would require a stable breeding population with little or no migratory input.

It is apparent from the results of this study that although environmental factors may influence flight capacity and other life-history traits, intrinsic variation in flight capacity is high, indicating an underlying genetic basis. Dingle (1991) stressed the need to understand not only the influence of environmental factors on flight and migration, but also the genetic variance underlying migration. Such an analysis of genetic variation can be determined from estimates of the heritability of flight performance, artificial selection, and cross breeding (Falconer 1981). This approach has yet to be undertaken for *Helicoverpa* spp.

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