Chapter 6

Experimental Section

6. The Distribution and Abundance of Predators Relative to *Helicoverpa* spp. and Alternative Prey

6.1 Introduction

This is the first of three chapters presenting the results of the approaches used in this study to measure the impact of endemic predators on *Helicoverpa* spp. in Australian cotton fields. This chapter discusses the methods of analysing field abundance data to identify the impact of suspected predators on their prey. The suitability of these analyses to *Helicoverpa* spp. and their predators on cotton is discussed. The results of spatial correlations calculated between the numbers of various predator groups and the numbers of *Helicoverpa* spp. or the numbers of various alternative prey are presented.

The distribution of predators across the fields was also investigated to search for colonisation patterns or gradients in abundance which might suggest important predator source areas surrounding the fields. The variation and distribution of predators on a realistic field scale is important for devising sampling strategies to estimate predator abundance for inclusion in IPM monitoring programmes.

6.2 The Relative Abundance of Predators and *Helicoverpa* spp.

After only limited sampling it became apparent that *Helicoverpa* spp. represented only a very small proportion of the available prey within the cotton system. *Helicoverpa* spp. larvae only contributed between 1 and 3% to the total arthropods when tallied over the season from the most comprehensive set of data collected, Midkin 1992/3 (Figures 6.1 to 6.2). Alternative prey, including aphids, thrips, cicadellids, mites, psyllids and other soft-bodied insects such as beetle larvae comprised 60 to 90% of the total arthropods. These might be expected to be the most acceptable prey for many generalist predators.

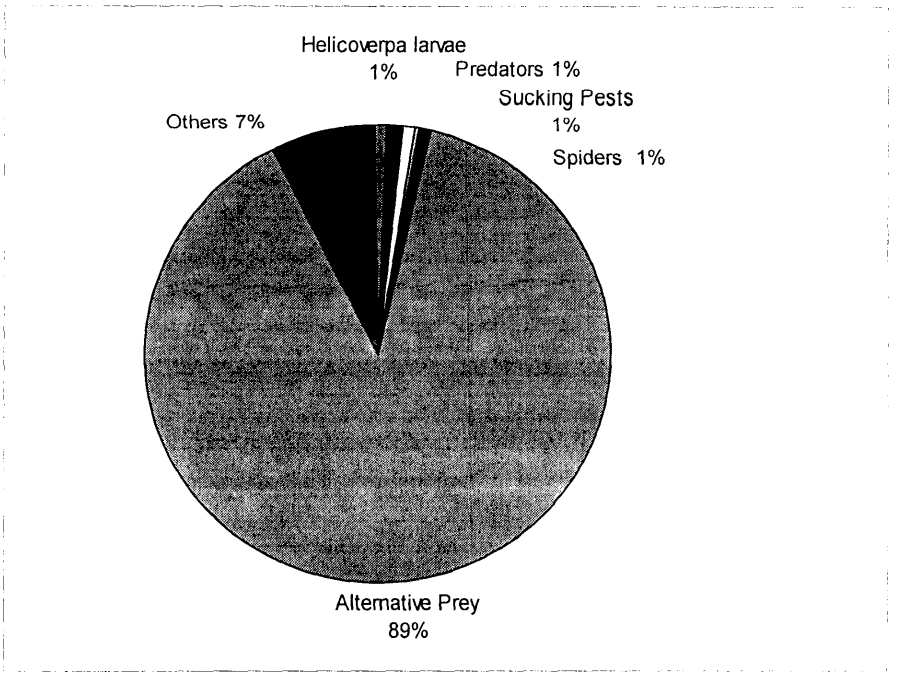

Figure 6.1 The relative abundance of **predators** and ***Helicoverpa* spp.** calculated from samples collected over the cotton growing season at Midkin 1992/3 from the soft-option field. The 'sucking pests' are *Campylomma* spp. and *Creontiades dilutus* which can be considered as predatory. 'Alternative prey includes abundant arthropods such as cicadellids, thysanoptera, aphids and diptera. 'Others' are mostly Hymenoptera.

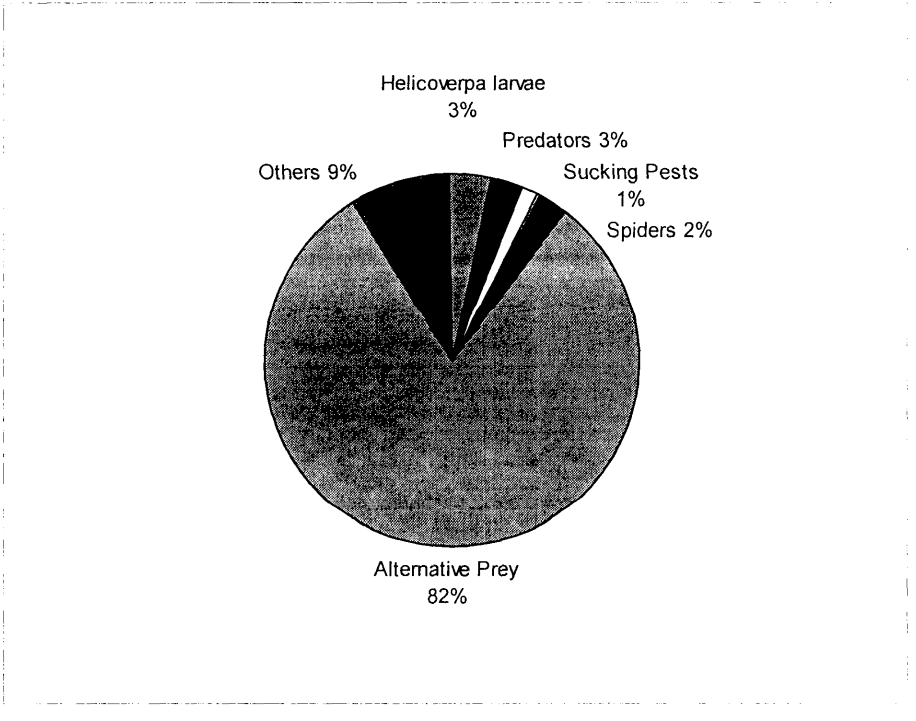

Figure 6.2 The relative abundance of **predators** and ***Helicoverpa* spp.** calculated from samples collected over the cotton growing season at Midkin 1992/3 from the conventional field. The 'Sucking pests' are *Campylomma* spp. and *Creontiades dilutus* which can be considered as predatory. 'Alternative prey' includes abundant arthropods such as cicadellids, thysanoptera, aphids and diptera. 'Others' are mostly Hymenoptera.

Such an abundance of alternative prey is likely to be an important influence on the impact of predators. Firstly it suggests that it is unlikely that the numerical responses of the generalist predators inhabiting cotton fields would be demonstrably linked to the abundance of *Helicoverpa* spp. alone. Secondly, the alternative prey may increase the impact on *Helicoverpa* spp. by arresting large numbers of predators in cotton fields. Thirdly, alternative prey may reduce that impact by distracting predators.

6.3 Population Studies to Identify the Impact of Predators

The analysis of predator and prey populations begins with the direct comparison of the seasonal abundance of the prey and the prevailing abundance of suspected predators. This comparison can be made as:

a) Correlations between populations recorded at the same time. This indicates whether the abundance of the predators and the prey are related but is confined to comparisons of estimates of the population taken at the same point in time.

b) Delayed density dependence. This approach endeavours to detect characteristic patterns in records of prey density and mortality which are unique to the action of natural enemies. Typically, delayed density dependence is a feature which is considered to display this.

c) Correlations between populations over a series of time delays. This is referred to as time series analysis and is capable of detecting statistical correlations which are not synchronised.

d) Spatial correlations which relate the abundance of predators and their prey within samples collected from the same position compared to those collected from other places.

The seasonal abundance of generalist predators in the cotton growing areas of Australia generally reveals that predators build up to a maximum in mid season and then decline (Chapter 5). In contrast, *Helicoverpa* spp. are in low abundance early but increase later in the season, that is, when predators are far less abundant (Figure 6.3 and 6.4). This pattern is necessary to suggest that predators are effective at controlling *Helicoverpa* spp. but, alone, is not convincing evidence for this. There may be several explanations which are not related to predators. For example, there may simply be greater *Helicoverpa* spp. immigration or more suitable environmental factors and crop characteristics for the survival of

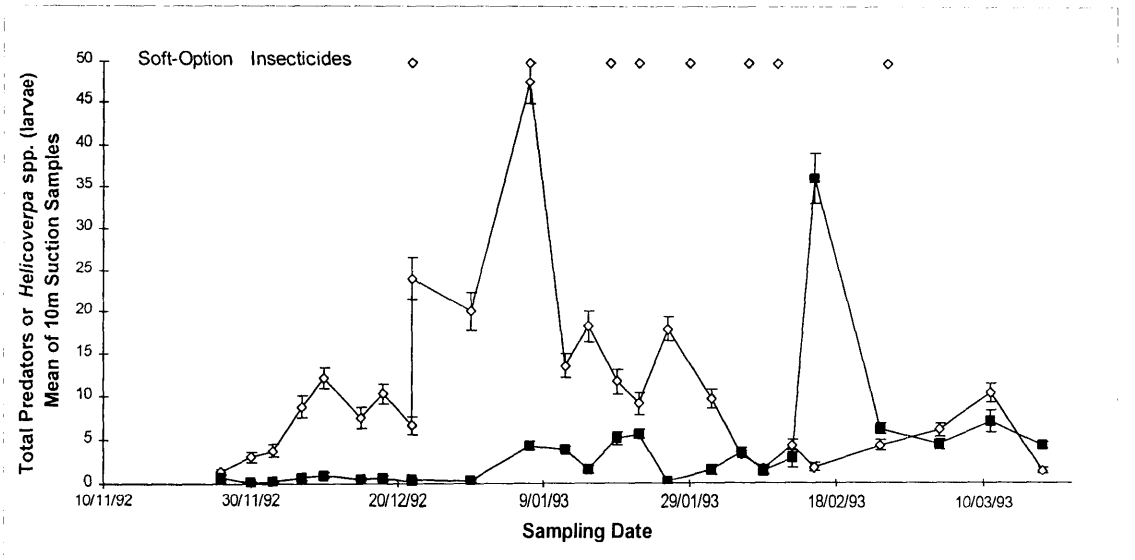

Figure 6.3 Total Predators and *Helicoverpa* spp. (larvae), Midkin 1992/3: The abundance of Total Predators (—◇—) compared to *Helicoverpa* spp. larvae (■)recorded by 10m suction samples from cotton field 1 treated with soft-option at Midkin over the 1992/3 cotton growing season. The timing of soft-option insecticide applications (◊) are shown above the chart. The details of the insecticide treatments are in appendix 5.1. The error bars represent the standard error of the means (n=16). The Elecvac was used up to and including the 22-12-92; the Bigvac was used from then on, including 22-12-92.

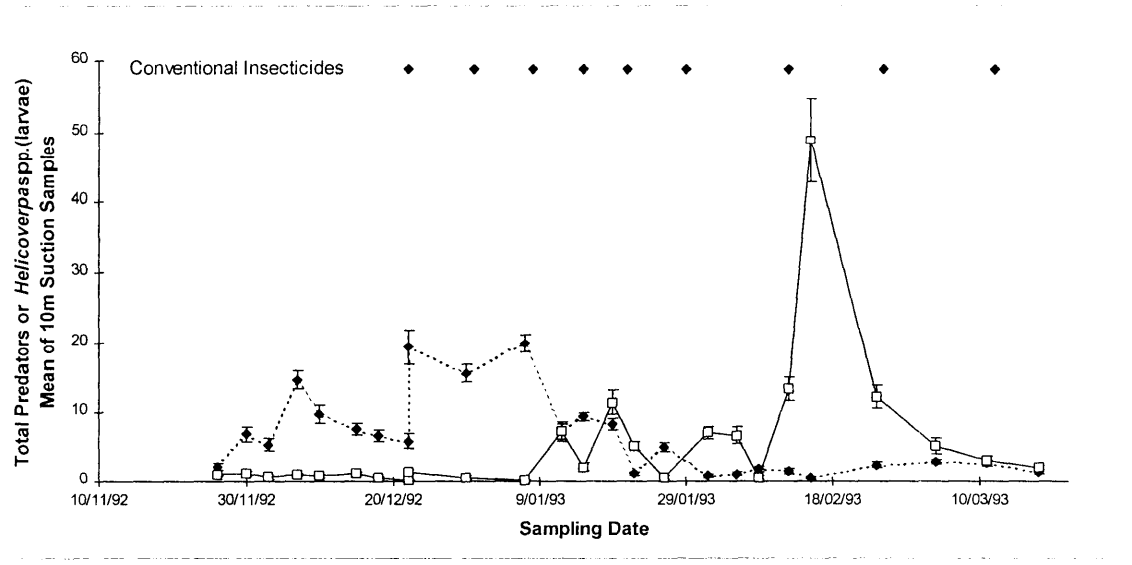

Figure 6.4 Total Predators and *Helicoverpa* spp. (larvae), Midkin 1992/3: The abundance of Total Predators (--◆--) and *Helicoverpa* spp. larvae (□) recorded by 10m suction samples from cotton field 2 treated with conventional insecticides at Midkin over the 1992/3 cotton growing season. The timing of conventional insecticide applications (♦) are shown above the chart. The details of the insecticide treatments are in appendix 5.1.. The error bars represent the standard error of the means (n=16). The Elecvac was used up to and including the 22-12-92; the Bigvac was used from then on, including 22-12-92.

Helicoverpa spp. towards the end of the season. This approach can only convincingly show the negative result, that is, if large numbers of *Helicoverpa* spp. survive in the presence of high predator densities the effect of predators can be deemed, at least as a sole mortality factor, to be insufficient.

At 'Alcheringa' the unsprayed plots harboured greater densities of predators than the organically treated areas and also had significantly lower populations of *Helicoverpa* spp. larvae (Figures 6.5 and 6.6). Seasonal and plant factors could reasonably be expected to be similar because these plots were close together and although the effects of the many organic treatments are not known, they could be expected, if anything, to reduce *Helicoverpa* spp. survival. Therefore the reduction in this case was likely to be have been due to predators.

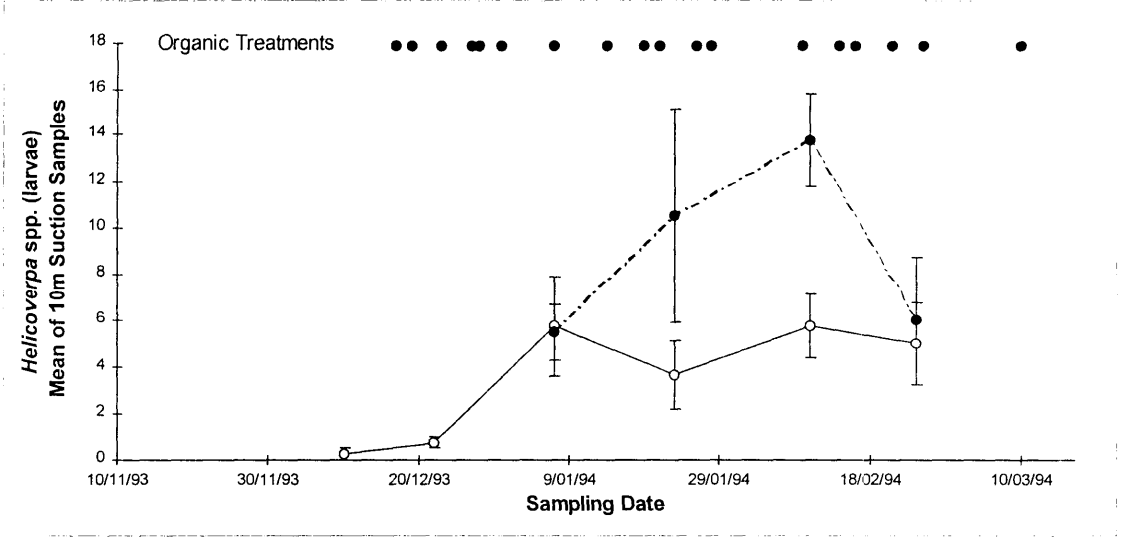

Figure 6.5 Helicoverpa larvae, Alcheringa 1993/4: The abundance of *Helicoverpa* larvae recorded by **Bigvac** 10m suction samples from cotton field 5 treated with no insecticides (-o-) or organic treatments (-•--) at Alcheringa over the 1993/4 cotton growing season. The timing of organic treatments are shown above the chart (•). The details of the treatments are in appendix 5.2. The error bars represent the standard error of the means (n=4).

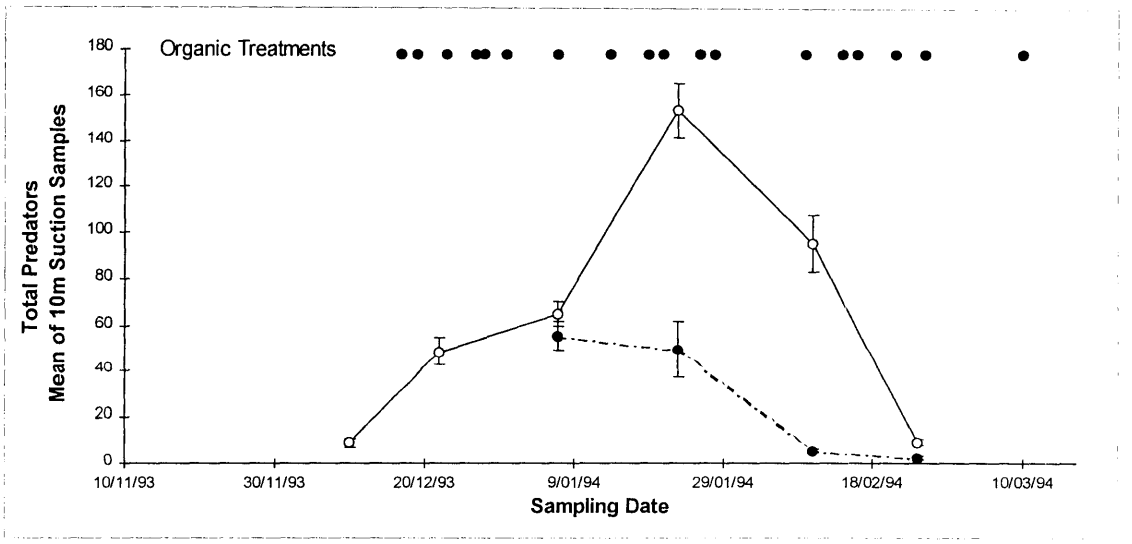

Figure 6.6 Total Predators, Alcheringa 1993/4: (not including small spiders). The abundance of Arthropod Predators recorded by **Bigvac** 10m suction samples from cotton field 5 treated with no insecticides (—o—) or organic treatments (--•--) at Alcheringa over the 1993/4 cotton growing season. The timing of organic treatments are shown above the chart (•). The details of the treatments are in appendix 5.2. The error bars represent the standard error of the means (n=4).

Despite this, even at predator densities of 16 *per meter*, which according to the suction efficiency experiments (Chapter 4.6) represents greater than 30 per meter in absolute terms, predators failed to maintain *Helicoverpa* spp. larvae at below commonly accepted economic thresholds. This predator density was greater than recorded at any time under either the soft-option or the conventional insecticide treatments at 'Midkin'. SIRATAC styled crop monitoring by the crop scouts of the organic area recorded 1.5 to 8 very small to small larvae per row-m at the time these suction samples recorded 0.6 to 1.4 larvae per meter. The presence of large *Helicoverpa* spp. larvae in these suction samples also implies that these estimates were highly conservative, because they are poorly collected by this method.

6.3.1 Approaches of Limited Value for Studying Predator Effectiveness in Cotton

Delayed Density Dependence

The detection of density dependent mortality does not necessarily indicate the action of a natural enemy because environmental factors combined with habitat limitations can also exhibit this. However, delayed density dependent mortality is a feature of prey populations in

which the majority of the mortality is caused by natural enemies. Graphically, by plotting the prey density (x axis) against the % mortality or *k*-value (y axis) and joining the points in chronological order (also called time series), an anti clockwise (negative) spiral will emerge if the prey is being affected in a delayed density dependant way (Varley *et al.* 1973).

If predation is the predominant mortality factor the interpretation is as follows:

In early prey generations, low densities of the prey experience low overall rates of predation due to density dependent mechanisms, such as an inability of the predators to find relatively scarce prey. Samples collected at this stage reveals a low population of prey with low mortality rates. Under this level of predation the prey population increases and sampling from these generations reveals higher prey densities but with low mortality rates. Low mortality persists because the predators have not had time to build up sufficient numbers to increase their impact on the prey population.

The larger prey densities of these subsequent generations may produce larger densities of predators which begin to impose a greater rate of mortality. The size of this impact will depend on how much the increasing density of the prey has relaxed the density dependent constraints on the predator. Therefore these generations register a larger population of prey but with high mortality rates. The predators eventually reach densities at which they decrease the prey abundance and therefore exhibit high mortality on low prey populations until declining prey density again reduces the predation rate. The density dependent response of the predators to the reducing prey density will be delayed because the predators surviving from the 'boom' period remain to inflict higher mortality rates than occurred as the populations were increasing. It is the time required by the predators to build a large enough population to impose the higher mortality on the prey and the persistence of these rates even after the prey has been considerably reduced which signifies the action of natural enemies.

This type of analysis is usually applied to sequential generations of the prey, often requiring many years to complete even one negative spiral (Varley *et al.* 1973). Specific predator-prey relationships are most suitable for analysis using this approach, but it can also be appropriate where a generalist predator can be treated as specific because a single identifiable predator to prey relationship predominates. The references to 'specificity' and 'predominant

prey' in the preceding sentence already suggest that this approach is not appropriate to the analysis of relationships between generalist predators and *Helicoverpa* spp. at the focus of this study.

Furthermore, before this method can be applied to studies of *Helicoverpa* spp. in cotton, the possibility of recording prey mortality rates and population densities in a sequence which could produce such patterns for other reasons, such as regional insecticide use, would need to be considered. It is even plausible that crop scouting in conjunction with residual insecticide effects may artificially produce this pattern. The density-dependent "predators" in this case would be humans applying chemicals when populations have increased to some level. The residual properties of the chemical or loosely synchronised regional applications could prolong the impact on the prey (pest) and therefore act in a delayed density dependent way. This may artificially produce the difference in the prey density at which the prey suffers an increased mortality and the density when it is released from such a high impact to create the spiral.

A further problem with this method is that it relies on a reproductive numerical response of the predatory species which is clearly associated with the density of the prey. Therefore for predators to exhibit such patterns they must be highly specific or generalists which lack alternative prey. As discussed before, *Helicoverpa* spp. contribute a very small proportion of the available prey within the cotton system. Therefore, if detected, negative spirals are more likely to indicate the presence of a specific agent such as a parasitoid than the activity of generalist predators.

Traditional Approaches using Numerical and Functional Responses.

The migratory nature of *Helicoverpa* spp. means that large numbers of eggs, exceeding economic thresholds, can appear in cotton crops over a single night. Predators could not possibly reproduce at a rate sufficient to contain this unless the numbers of predators were adequate beforehand. The *reproductive* numerical response of predators fed *Helicoverpa* spp. is therefore not considered a useful indication of the potential for control, and studies to determine this response were not conducted. Furthermore, the results of the field surveys clearly established that *Helicoverpa* spp. are a relatively unimportant proportion of the available food for predators within the cotton environment (Section 6.2). This again suggests that the reproductive numerical responses are likely to be driven by the abundant alternative

prey. The potential for predator populations to increase within cotton fields on these species might be worth studying, as a guide to the potential abundance of predators at times which may coincide with the arrival of *Helicoverpa* spp. However, such studies were not attempted in this thesis.

As discussed in the literature review (Chapter 1, section 1.4) the interpretation of laboratory-derived functional responses to infer the potential of particular predatory species also appears to provide little that is applicable to the measurement of generalist predation on *Helicoverpa* spp. in cotton. The effects of abundant alternative prey and the large complex arena of a cotton field would have seriously compromised the value of this approach to reach general conclusions. Such studies were therefore also omitted.

Threshold Analysis

A broad approach which might indicate the effectiveness of predators is to observe the rate at which the pests return to threshold levels where predators vary in abundance. If predators are having an impact, fields registering relatively high predator abundance would be expected to return to pest thresholds slowly compared to similar fields registering low predator numbers. The difficulty with applying this to *Helicoverpa* spp. in cotton is that the decision that a threshold has been reached is usually made within a day of the appearance of *Helicoverpa* spp. eggs. The threshold is based on the predicted potential of an egg abundance to lead to a population of larvae which would cause unacceptable damage. This decision is considered justified because the effect of insecticides is greatest when targeting newly hatched larvae and the risk of substantial losses if only one *Helicoverpa* spp. cohort survives is perceived to be high (Fitt 1994). This conservative approach is also expressed in the practical view that insecticides form a relatively small proportion of the variable costs of crop production and therefore provide 'cheap' insurance (I. Titmarsh pers. comm. 1994).

This approach reduces the period in which predators have time to operate to such an extent that the timing of thresholds bears virtually no relationship to the rates of *Helicoverpa* spp. mortality, whether due to predation or otherwise. It therefore appears reasonable to assume that the timing of insecticide applications on all the treatments described in Chapter 5 primarily reflects the arrival of high numbers of *Helicoverpa* spp. eggs, rather than the effectiveness of predators. Thus, the relative synchronicity of insecticide applications to the

soft-option and conventional plots in both years of the 'Midkin' survey does not necessarily indicate the relative effectiveness of predators.

Unfortunately, differences in the effective life of the insecticides used between treatments also cause difficulties with any such analyses. These differences are not well understood, though it is known that, for example, Chlorfluazuron (Helix®) used in the soft-option treatments may remain active for up to two months, and the pyrethroids have longer residual effects than most other groups. In addition, residual effects may influence aggregative and host-searching behaviour of predators, as well as their survival, further complicating the interpretation of the results.

The conclusion from the preceding sections is that traditional laboratory studies to understand functional and numerical responses, and methods of detecting density dependence do not appear to be efficient avenues for studying the predation affecting *Helicoverpa* spp. in cotton. Threshold analysis also suffers from serious difficulties. These approaches were therefore not applied. The question of whether predators might be able to control *Helicoverpa* spp. was investigated using population comparisons, particularly temporal and spatial correlations (discussed in the remainder of this chapter) and more mechanistic approaches using field cages which enclosed realistically complex searching arenas (Chapter 7).

6.4 Spatial Population Comparisons

If an ecologically significant predator-prey relationship exists between two species within a relatively uniform habitat such as a cotton field, one would expect spatial correlations between the abundance of the two species. For example, if the predators are attracted to areas of abundant prey, there would be a positive correlation. On the other hand, if predators were extremely effective in reducing the prey numbers in a given locality, one might expect a negative correlation. Indeed, both of these effects might be apparent within the same predatory relationship but in sequence. The polarity of the correlation will depend on the time in the sequence that the populations are estimated. For instance, initially, relatively abundant patches of prey may be located by searching predators. If the populations were measured at this stage,

relatively high number of predators will be present where there are relatively high numbers of prey and a positive correlation will be exhibited. Some time after this the predators may have removed so many prey that prey abundance in these patches is reduced to below background levels. If populations are measured at this stage the predators will be relatively abundant where the prey are relatively depleted, and so there will be a negative correlation. At some intermediate time in this sequence there will be a stage when no correlation exists or is clearly detectable. Discovery of such a sequence would provide strong evidence that the predators are reducing prey populations. However the reverse, that is, the absence of significant correlations within fields, does not necessarily indicate the absence of important predatory impacts on the prey, because a combination of unsynchronised positive and negative correlations in patches across a field may give the appearance of no correlation at all.

The interpretation of spatial correlations can be improved with knowledge of the behaviour of the suspected predators and their prey. Factors such as the movements of predators during searching and feeding and threshold densities of prey which elicit the emigration of predators may suggest whether spatial correlations are likely to be an indicator of predation by a particular insect. Sampling methods can also be improved by knowledge of the biology of the predators and prey, which can enable better spatial analysis. If feeding occurs at different locations to other activities of a predator throughout a diurnal period, the timing of sampling may alter the results of spatial correlations. For example, spiders may 'rest' during the day in low portions of the canopy and only reflect the spatial distribution of their prey during the night whilst hunting. The scale of sampling may also affect the detection of spatial correlations within a field. For example, if the samples are large compared to the size of the aggregations of predators and prey then relationships can be lost by combining so many aggregations in each sample that little variation in prey or predator numbers occurs between samples.

Within the cotton field there are other factors which must also be considered in determining which correlations are the signature of an effective generalist predator of *Helicoverpa* spp. The abundance of alternative prey may overwhelm or distract predators from aggregating in areas of relatively high *Helicoverpa* spp. abundance. This suggests that a positive correlation is unlikely unless there is a strong preference for *Helicoverpa* spp. Even with such a preference, the relatively low densities of *Helicoverpa* spp. and their even

distribution, suggest that low numbers of predatory events would cause a local depletion and therefore, also lead to a negative correlation.

Thus, both positive and negative correlations in abundance can indicate an ecologically significant relationship, but those between *Helicoverpa* spp. and predators in cotton fields are more likely to be negative. On the other hand, relationships to other more abundant prey species, particularly the overwhelmingly abundant cicadellids, could be expected to be positive.

Such correlations have been sought in the data describing the populations of all arthropods in cotton fields throughout two growing seasons under several insecticide strategies, as described in the previous chapters on predator abundance and seasonality.

6.4.1 Materials and Methods

The methods and treatments were the same as those described for the arthropod abundance survey in Chapter 5. The details of the suction sampling methods and the insecticide treatments are presented in Chapter 3.

'Midkin' in 1992/93 was the most regularly sampled site, with the most replicates, and is therefore regarded as the most rigorous data set. Within this site and year, the Bigvac data set was probably subject to the least sampling problems (Chapter 4.4). Therefore, if predatory relationships could be identified by spatial analysis, they were expected to be clearest using these data. However, it is also possible that the reduced insecticide use of the other data sets allowed larger populations of prey to have become initially established and therefore have exhibited larger and more detectable effects of predators.

The approach taken here is to suggest that if presumptive predators of *Helicoverpa* spp. exhibit enough preference, or are sufficiently effective, a correlation due to aggregation of predators or depletion of prey could be expected to exist and may be detectable using the data from these field surveys. Furthermore, with sufficient preference towards, or impact on *Helicoverpa* spp., the strength of the correlation for a particular predator might suggest its potential to contribute to biological control.

This analysis of the insect survey scanned the data for predatory insect species or functional groups whose abundance appeared to be spatially correlated with the abundance of *Helicoverpa* spp. and other prey species. These spatial correlations, tested by regression analysis, were designed to answer this question:

> *On a particular day in a particular field, did the abundance of one insect species correlate with the abundance of another in the same sample?*

On any sampling date at 'Midkin' in 1992/3, 16 samples of 10 meters were collected from each field (treatment), so each regression consisted of 16 points. At 'Midkin' in 1993/4 there were 8 samples per treatment per sampling date. The organic treatment and the unsprayed cotton only used 4 per sampling date.

Minitab® (Version 6.0, 1994), a statistical computer package (Ryan *et al.* 1992), was used for the analysis.

The precise commands used were:

```
MTB  > glm Cx = Cd  Ct  Cd*Ct  Cy;
SUBC> covariate Cy.
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where Cx = column containing insect species 1 (predator counts)
Cy = column containing insect species 2 (prey counts)
Cd = column containing sampling dates
Ct = column containing treatments

The use of the Generalised Linear Model (GLM) in this way is equivalent to an analysis of covariance, in which the covariate is fitted only after the potentially confounding effects of treatment and date of sampling have been removed. A significant result in this respect reflects the significance of the Cy term, not the significance of the entire model. The count data was also subjected to a square root transformation to disassociate the mean and variance before the analysis was conducted.

An Example

Results from the analysis of variance for the spatial correlation between *Geocoris* spp. and *Helicoverpa* spp. larvae at 'Midkin' in 1992/3 are presented below:

Source	df	Seq SS	Adj SS	Adj MS	F	P
Julian day	23	21.91	22.31	0.97	5.78	0.000
Field	1	8.33	8.2526	8.25	49.19	0.000
Julian day*Field	23	22.12	22.94	0.99	5.94	0.000
Helicoverpa larvae	1	0.89	0.89	0.89	5.32	0.021
Error	739	123.98	123.98	0.16		
Total	787	177.24				
Term	Coeff	Stdev	t-value	P		
Constant	0.277	0.034	8.14	0.000		
Helicoverpa larvae	-0.046	0.020	-2.31	0.021		

The conclusion from this analysis was that there were significant differences in the abundance of *Geocoris* spp. between fields and between sampling dates, and there was a significant interaction between these factors. This result might be expected in view of the different insecticides used in the two fields. The analysis also demonstrates that, after these factors are taken into consideration, a statistically significant proportion of the variance in *Geocoris* spp. numbers can also be accounted for by the correlation with *Helicoverpa* spp. larvae. However the amount of variance explained by the correlation is only a very small proportion of the total variance.

6.4.2 Results and Discussion

The statistical significance of the correlations obtained by the technique described above are summarised in Table 6.1. The most obvious result is that the predatory species in general were more abundant where cicadellid nymphs (the most abundant alternative prey) were more abundant. There was a general lack of correlations between predatory species and *Helicoverpa* spp. in the insecticide treated fields (soft-option, conventional and organic treatments). However there are negative correlations in the unsprayed treatment between the most abundant predatory species and *Helicoverpa* spp. The numbers of predators in the unsprayed plots were considerably higher than in the insecticide treated cotton and the *Helicoverpa* spp. were also more abundant (Figures 6.3 to 6.6). Possibly, these spatial correlations were present in the other insecticide regimes but the levels of abundance were too low for them to be detected. Table 6.1 presents correlations within each suction sample but the statistical analysis using this method can be expected to give a greater error term (because it including within plot errors) than the analysis using the means from each plot. This suggests that the power of the test might be greatly improved by using the average of the four samples from each plot. In this case, the analysis question becomes:

Is the abundance of Helicoverpa larvae in a plot (0.5 hectares) spatially correlated with the level of a particular species or group of predators in that plot once the effects of time and field have been removed?

The unsprayed and organically treated plots had to be excluded from this procedure because only one plot was sampled within each treatment making comparisons between plots impossible. The results of these analyses are presented in Table 6.2.

At 'Midkin' in 1992/3, there were several statistically significant negative correlations between predators and *Helicoverpa* spp. larvae. This contrasts with the lack of such correlations on the 10m sample scale (Table 6.1). However, the detection of positive correlations between cicadellid numbers and predators remained similar at both spatial scales. This is consistent with the suggestion that predators concentrate where there are abundant prey (cicadellids), and as a consequence the *Helicoverpa* spp. larvae in these regions are reduced. Of particular note are the negative correlations between *Helicoverpa* spp. larvae and *Geocoris* spp. ($P < 0.001$), *Orius* spp. ($P < 0.05$), and large Salticidae ($P < 0.05$).

The detection of spatial correlations, even with high levels of statistical significance, does not confirm predatory linkages because many concomitant factors such as common habitat requirements may cause strong spatial correlation, even in the absence of any predatory relationship. They can, however, direct attention to the most likely candidate predators for further study. The basic assumption here is that if a strong biological relationship exists some kind of pattern should emerge. There remains, however, a possibility that strong and effective predatory relationships can be missed by this analysis. Reasons a real predatory relationship may not have been detected include:

i) Insufficiently precise sampling methods. The effects may only be resolved at greater pest densities, with more samples or with more efficient sampling methods.

ii) Highly variable mortality factors, such as unfavourable wind, humidity or temperature, may overwhelm the effects of predators.

A possible criticism of the correlation analysis is that the arthropods, particularly *Helicoverpa* spp., were poorly sampled by the suction methods. The Bigvac method predominantly collected the very small to small *Helicoverpa* spp. larvae but these were found to correlate reasonably well with that of the visual samples of these life stages (Figure 6.7, R = 0.45). The strength of this correlation suggests that the lack of a correlation between the predators and *Helicoverpa* spp. in the preceding analysis is not a sampling artefact and focuses attention on cicadellids rather than *Helicoverpa* spp., as the major influence on predator aggregative numerical responses.

The samples were collected on each sampling date between 8-00 am and 12-00 noon. This may be expected to coincide with the searching and feeding times of many of the predator groups. However some spiders, particularly, *Oxyopes* spp. and Salticidae, appeared to be more active at night (Chapter 4.5). Their position relative to the *Helicoverpa* spp. larvae may vary with diurnal period. Lack of spatial correlation during the 8 am to 12 midday sampling period may therefore be misleading. It is possible that sampling during the night might have related the position of the spiders to the *Helicoverpa* spp. which may give a different impression about the importance of this group of predators.

		PREY										
Ref #	PREDATORS	*Helicoverpa* Larvae				Cicadellid Nymphs				Thrips	Mites	Total
	Sites	M92	M93	Org	US	M92	M93	Org	US	M92	M92	M92
1	*Coccinella* spp. *(Ad)*				-**	+***	+***			+**		+***
2	*Diomus notescens (Ad)*							+*				+*
3	*Dicranolaius bellulus (Ad)*				-*	+**			+*	+*		+***
4	*Geocoris* spp. *(Ad)*	-*			-*	+***				+**		+***
5	*Germalus* sp. *(Ad)*				-*	+*				+*		+**
6	*Nabis kinbergii (Ad)*					+***						+***
7	*Oechalia schellenbergii (Ad)*											
8	*Orius* spp. *(Ad)*						+*			+***	+*	+*
9	*Campylomma* spp. *(Ad)*					+***				+**		+***
10	*Creontiades dilutus (Ad)*					+***						+***
11	*Mallada signata (Larvae)*								+*			
12	Formicidae *(Ad)*						-*		+*			
13	Salticidae											
14	Oxyopidae					+**	+**					+**
15	Total Large Spiders (> 1mm HC)					+***	+**		+***			+***
16	Total Small Spiders (< 1mm HC)		+*	+*		+***	+***		+***	+***		+***
17	Total Spiders					+***	+***	+*	+***	+***		+***
18	Total Predators (21 minus 9,10 & 17)					+***	+***			+***		+***
19	Total Predators (21 minus 9, 10 & 16)					+***	+**		+*	+***		+***
20	Total Predators (21 minus 9 and 10)		+*			+***	+***			+***		+***
21	Total Predators (minus 16)		+*			+***	+***			+***		+***
22	Total Predators (1 to 12 & 17)		+**			+***	+***			+***		+***
23	Total Arthropod minus (Helicoverpa spp.)					NA	NA	NA	NA	NA	NA	NA

Table 6.1 Sign and statistical significance of Spatial Correlations of Arthropod abundance between individual samples from Australian Cotton Fields. (M92 = 'Midkin' 1992/93, M93 = 'Midkin' 1993/94, Org = Organic plots at 'Alcheringa' & 'Wilby' and US = Combined insecticide free sites).
(+ = positive correlation, - = negative correlation: * , ** & *** = 5%, 1% and 0.1% levels of statistical significance respectively)

		PREY						
Ref	PREDATORS	*Helicoverpa*		Cicadellid Nymphs		Thrips	Mites	Total Prey
		M92	M93	M92	M93	M92	M92	M92
1	*Coccinella* spp. *(Ad)*			+*	+**			
2	*Diomus notescens (Ad)*							
3	*Dicranolaius bellulus (Ad)*							
4	*Geocoris* spp. *(Ad)*	-***		+***		+*		+**
5	*Germalus* sp. *(Ad)*							
6	*Nabis kinbergii (Ad)*			+***				
7	*Oechalia schellenbergii (Ad)*							
8	*Orius* spp. *(Ad)*	-*		+*		+***		+**
9	*Campylomma* spp. *(Ad)*			+***		+**		+**
10	*Creontiades dilutus (Ad)*			+***				+**
11	*Mallada signata (Larvae)*							
12	Formicidae *(Ad)*							
13	Salticidae	-*						
14	Oxyopidae							
15	Total Large Spiders (> 1mm HC)				+*			
16	Total Small Spiders (< 1mm HC)			+**		+***		
17	Total Spiders			+***		+***		+*
18	Total Predators (21 minus 9, 10 & 17)	-*		+***		+***		+***
19	Total Predators (21 minus 9, 10 & 16)	-*		+***		+***		+***
20	Total Predators (21 minus 9 & 10)		+*	+***		+***		+***
21	Total Predators (minus 17)	-*		+***		+***		+***
22	Total Predators (1 to 12 & 17)			+***		+***		+***
23	Total Arthropod minus (Helicoverpa spp.)	-*		NA	NA	NA	NA	NA

Table 6.2 Sign and statistical significance of Spatial Correlations between the abundance of arthropods combined as plot means. M92 = 'Midkin' 1992/93, M93 = 'Midkin' 1993/94. + = positive correlation, - = negative correlation: * , ** & *** = 5%, 1% and 0.1% levels of statistical significance respectively. Note that the unsprayed and organic treatments are removed from this table because only one plot was surveyed at each site making comparisons between plots impossible.

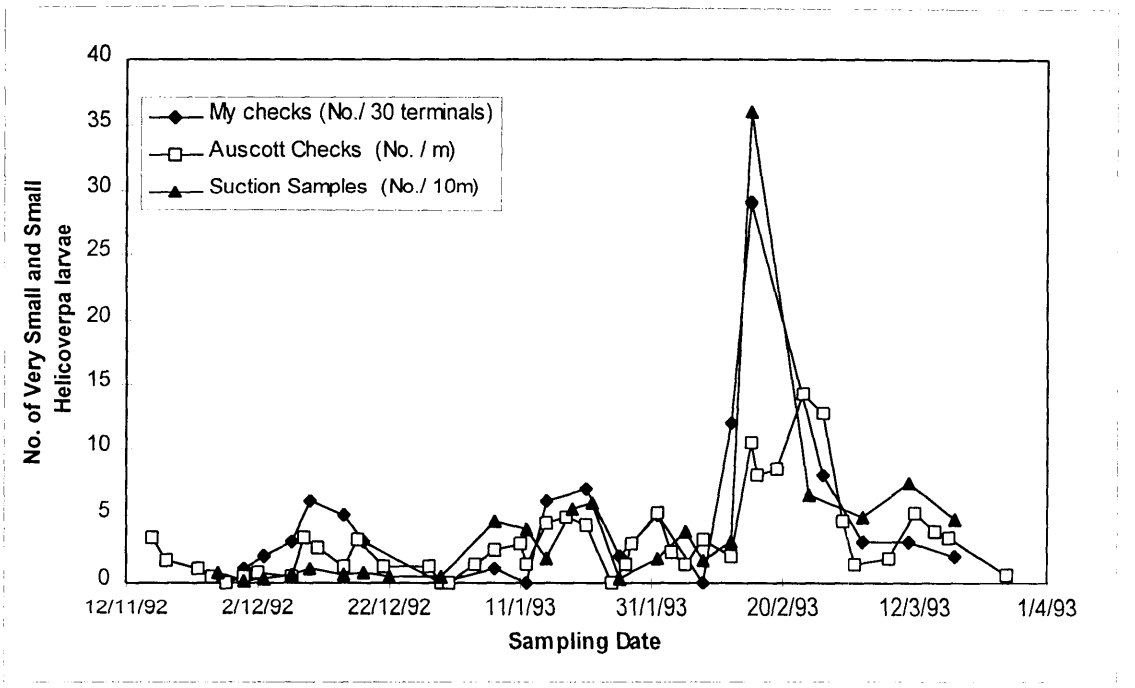

Figure 6.7 Comparison of the visual and suction samples of *Helicoverpa* spp. (very small and small larvae) in the soft option field over the 1992/93 cotton growing season. Auscott Counts are the records from visual sampling conducted by the crop scouts hired by Auscott in this season and were not necessarily made in the plots corresponding to the suction samples. Siratac counts and the suction samples were collected by the author. The Siratac counts are records of the *Helicoverpa* larvae recorded during visual sampling of 30 terminals from each plots. The correllation coefficient of 0.45 (R) was calculated using only the authors data. Note that the suction samples before the 22/12/92 were collected using the Elecvac which had a lower wind speed and did not appear to catch *Helicoverpa.*

6.4.3 Time Series Analysis.

Simple correlation analysis compares abundance at the same time. However, one could expect that the presence of a high level of predators at one time leads to a reduced level of pest at a later time. Therefore time series analysis was explored. Time series analyses are essentially tests for correlation, run sequentially as one series of species counts is shifted one sample interval earlier with each test. Relationships which appear negatively correlated if sampling dates are synchronised become positive with phase shifting if predation is a predominant factor.

If a parasitoid-host relationship is analysed in this way the positive correlation will occur with a delay of approximately one developmental period of the parasitoid because a peak in prey abundance would tend to produce the most parasites one development period

afterwards. If the predatory relationship is strong enough the time shift may detect the presence of a delayed density dependent effect, as discussed in Section 6.3.

The data sets were not particularly amenable to this approach because the sampling dates were not exactly regular. There were often difficulties with gaining access to the field at the most appropriate times, due to wet weather or the dangers to the operator from insecticide applications. However, the majority of the 1992/3 data set at 'Midkin' was considered regular enough to attempt time-series analysis. The validity of this approach was first tested by applying it to a known parasite-host relationship within the 'Midkin' 1992/3 data set (soft options); that of brown leafhopper eggs (*Orosius argentiferus*), parasitised by a wasp from the family Trichogrammatidae (*Aphelinoidea* sp.). Both of these species were highly abundant and readily sampled by the suction methods from the soft-option plots during this survey. The strong correlation expected between these species was clearly exhibited in the time series analysis (Figure 6.8) which included a biologically reasonable delay corresponding to the development period for Trichogrammatidae of 7-10 days (Parra *et al.* 1990). This set a benchmark against which the predatory relationships could be compared. Note however, that this approach was expected to be very conservative because the strength of even a major predator-prey links may not have been nearly so strongly exhibited as that for a parasitoid/host relationship, because of the much greater host specificity of parasitoids.

The time series analyses failed to show any clear relationships between *Helicoverpa* spp. and the predatory species within a biologically meaningful time shift. The time-series approach therefore failed to indicate any delayed effects of predators, whether density dependent or otherwise.

The evidence presented in this section suggests that some predatory arthropods might reduce the abundance of *Helicoverpa* spp. larvae. This conclusion is based on correlations within individual suction samples or plots. However these did not explain a large amount of the variation in any case and the impact was probably not substantial. *Geocoris* spp. showed the strongest negative correlation with *Helicoverpa* spp. larvae, but even in this case the percentage of the variance explained by the correlation was low. Unfortunately therefore, these negative correlations at best indicate a possible contribution to *Helicoverpa* spp. mortality rather than show that *Helicoverpa* spp. are controlled because numbers exceeding economic thresholds were present.

No. of Shifts	Correlation Coefficient	CORRELATION COEFFICENT -1.0 -0.8 -0.6 -0.4 -0.2 0.0 0.2 0.4 0.6 0.8 1.0	
-14	0.069	XXX	
-13	0.045	XX	
-12	0.024	XX	
-11	0.005	X	
-10	-0.014	X	
-9	-0.075	XXX	
-8	-0.161	XXXXX	
-7	-0.182	XXXXXX	
-6	-0.326	XXXXXXXXX	
-5	-0.412	XXXXXXXXXXX	
-4	-0.332	XXXXXXXXX	
-3	-0.246	XXXXXXX	
-2	-0.014	X	
-1	0.258	XXXXXXX	
0	0.334	XXXXXXXXX	← **A**
1	0.606	XXXXXXXXXXXXXXXX	↕
2	0.730	XXXXXXXXXXXXXXXXXX	← **B**
3	0.441	XXXXXXXXXXX	
4	0.251	XXXXXXX	
5	0.257	XXXXXXX	
6	-0.050	XX	
7	-0.299	XXXXXXXX	
8	-0.295	XXXXXXXX	
9	-0.039	XX	
10	-0.331	XXXXXXXXX	
11	-0.252	XXXXXXX	
12	-0.030	XX	
13	-0.012	X	
14	-0.066	XXX	

Figure 6.8 The time series analysis of *Aphalinoidea* spp. (egg parasitoid) to *Orosius argentatus* (cicadellid) from samples collected at Midkin 1992/3 from the soft-option field. '**A**' is the correlation at time 'zero' and '**B**' is the correlation two sampling intervals later. Showing that a delay of about 7 days (sampling interval was 3 to 4 days) produced the strongest correlation (0.730). This is a biologically sensible time delay from the peak abundance of *O. argentatus* adults (i.e. most eggs) to the peak in abundance of *Aphalinoidea* spp. adults (egg parasitoids) because trichogrammatids require about a week to develop, suggesting that this predatory relationship is real.

The general conclusion from spatial analysis is that there is no evidence that any particular species or group of predators had a major impact upon *Helicoverpa* spp. abundance. The predatory arthropods inhabiting cotton were therefore considered to be generalists, as previously reported (Room, 1979a). There was, however, a consistent pattern of significant positive correlations of predators with the abundance of cicadellids. Cicadellids were by far the most consistently abundant prey group, so the pattern of the correlations is consistent with the view that generalist predators aggregate or are arrested by a general abundance of prey, and not any particular prey species. This does not preclude the possibility that predators can provide a useful level of pest mortality, but suggests that their contribution will be difficult to detect or predict amongst other causes of mortality.

6.5 Gradients of Arthropod Abundance in Cotton Fields

6.5.1 Introduction

Cotton fields in Australia are large areas, usually in the order of 50 to 150 hectares, and are commonly bounded by a variety of other agricultural or natural habitats. Local habitats might be the initial sources of arthropods which colonise cotton crops. With the changing suitability of plants as food and shelter in these habitats as the season progresses, these areas may act as sources or sinks for predators. It might therefore be expected that, at least initially, density gradients will be produced as populations of herbivores and predators become established in the crops. The fields used for the general arthropod survey (Chapter 5) were bounded by areas which could be considered as sources or sinks of predatory species.

The regular flood irrigation might also be expected to cause gradients by carrying insects towards the 'tail drain' end of the field, or by creating regions of varying plant quality via differential watering. Also the rows (resembling hedges) may present a barrier to movement and colonisation across the field, especially before the canopy becomes continuous between rows.

The discovery of density gradients of predators in cotton fields might have important implications for the design of effective sampling methods, and for the inclusion of predator

source areas adjacent to fields in IPM strategies. This section describes comparisons between the number of arthropods caught in plots from the various sides of the cotton fields, to indicate whether density gradients appeared on a large scale in relation to the presence of bushland or other neighbouring crops.

6.5.2 Methods

At 'Midkin' in 1992/3, the samples for the general arthropod survey were collected from four 0.5 hectare plots, one in each corner of the soft option treated treatment fields (Figure 6.9). Four replicate samples were collected from each plot on each sample date, using the methods explained in Chapter 3. The corner of each plot closest to the corner of the field was 100 meters from the side and end of the field, imposing a 100 meter border to avoid edge effects. Both fields were bordered on the northern side by Carole Creek, and on the southern side by the Gingham Road. The western end of each field was flanked by a raised irrigation channel, the 'head ditch', from which the irrigation water was siphoned into the field rows. The 'tail drain' ran along the eastern end of each field. This channel returns excess irrigation water to the irrigation supply (Figure 6.9).

Comparisons between the numbers of arthropods caught in plots from the various sides of the fields could indicate whether density gradients were appearing on a large scale across the crop. Taking samples on a regular basis, especially early in the season or after insecticide applications, could potentially detect colonisation from particular directions and imply possible sources. The presence of the bushland-creek system (Carole Creek) along the side of each field allowed exploration of the possibility that predators were colonising the crops from this area. Sweep net collections of arthropods along the banks of Carole Creek while the cotton was only seedlings (September 1992), discovered virtually all the species of predatory arthropods which were later found in the cotton crop.

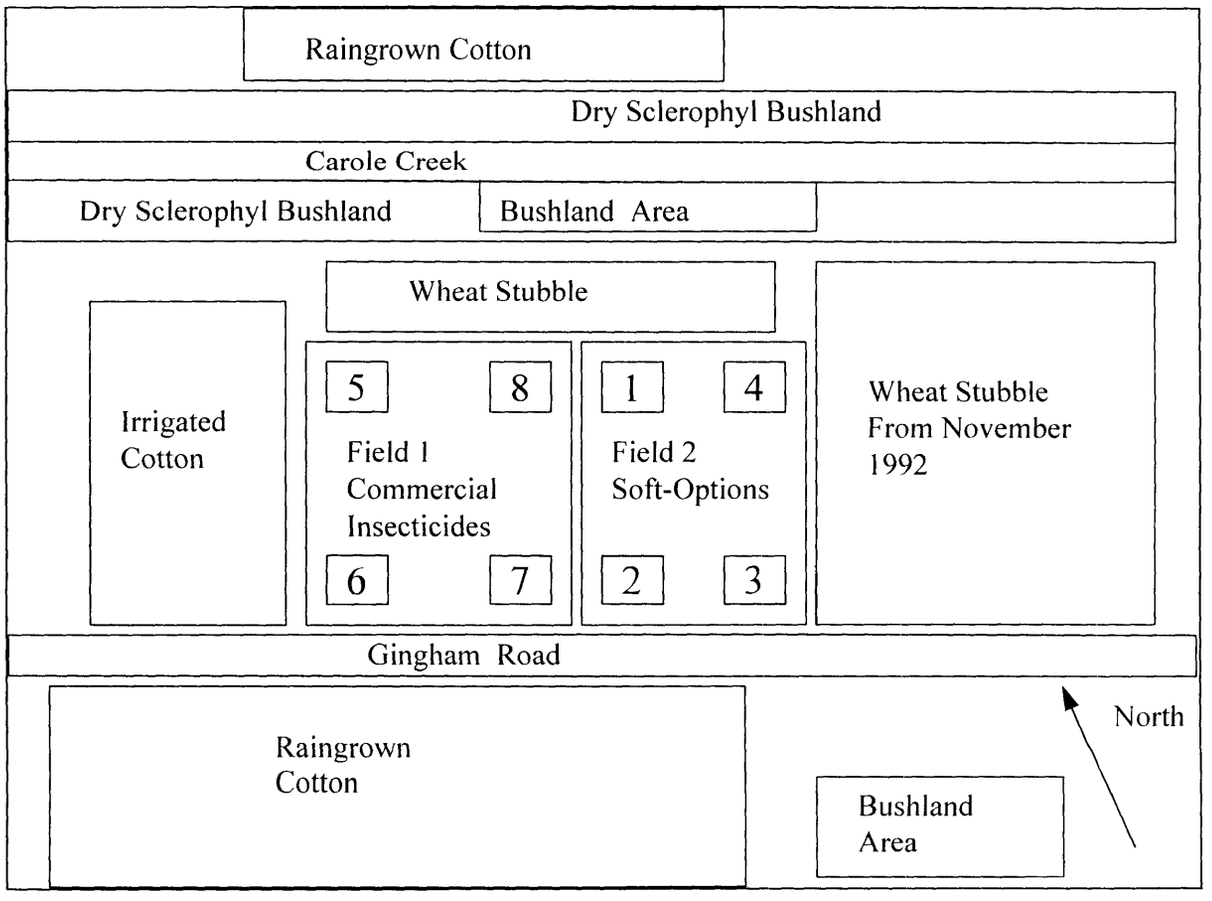

Figure 6.9 Schematic layout of field and surrounding habitats in 1992/93 at Midkin. The numbers mark the 0.5 hectare plots where four replicate suction samples were collected every three to four days throughout the majority of the cotton growing season in 1992/3. The conventional field was 100 hectares (1km square) whereas the soft-option field was 75 hectares. The orientation of the crop rows was west/east in both fields (irrigation water flowed from west to east from head ditch to tail drain).

6.5.3 Results and Discussion

Very few of the arthropods displayed any indication of density gradients from one side of the field to the other (Figures 6.10 to 6.25). Only Thysanoptera and *Coccinella transversalis* displayed a marked difference in their abundance such that colonisation from any particular direction might be suggested. Thysanoptera appeared to be associated with the creek side later in the season whereas *C. transversalis* were in greater densities in the tail drain plots early in the season. The latter might be related to differential watering across the field which may create different humidity or plant quality gradients, both potentially capable of altering the distribution of insects. Generally, however there were no large differences between the numbers of arthropods caught in the different plots when the plots were grouped by field position and there were no early season density gradients which might indicate colonisation from any particular direction. The possible explanations for this result are that colonisation

occurred in one or more of the following ways: i) Evenly from all directions; ii) Too quickly for this sampling interval (three days) to detect; iii) On such a large scale that supply from immediately adjacent bushland areas is of little significance to arthropod densities in nearby fields.

On the broad scale, this study suggests that the location of sampling sites for the general monitoring of predator populations in a field does not need to account for the location of potential predator sources or sinks surrounding those fields, at least over the final two thirds of the season when *Helicoverpa* spp. is considered most threatening and the most rigorous sampling occurs. This is not to suggest that management of vegetation surrounding fields might not increase the number of predators within a crop, but that the distribution of those predators at the scale of a field appears to be broadly uniform. However, the general condition of the bushland areas throughout the cotton season during this study was dry and therefore the number of predators inhabiting this region could be expected to be low in comparison with wetter seasons.

Nearby irrigated crops, which have growing seasons which closely precede or overlap with the beginning of the cotton season, may be much more important as predator source areas. This study suggests that the dry sclerophyll bushland communities near creeks (which are common throughout the cotton growing areas of NSW) supply only low numbers of arthropods to adjacent cropping systems during the cotton season. However the importance of a low level of input, particularly early in the season, must be considered. The detection of gradients towards the center of the field was not possible using this layout because the plots were equidistant from the center. Byerly *et al.* (1978) also found no differences in predator distribution within fields, except for spiders. They investigated the distribution in concentric bands from the center to the edges, but their fields were possibly too small for conclusive practical interpretation (0.13 hectares). On the other hand, Duffield and Aebischer (1994) found that predators, mostly Coleoptera (Carabidae and Staphylinidae) and Araneae (Linyphiidae) progressively recolonised winter wheat fields in England from the outer edges inwards, after insecticide use. They suggested that this pattern reflected the very limited use of flight amongst these species. That a more uniform pattern of recolonisation was found in Australian fields might therefore suggest that the Australian species make larger movements during establishment, probably making greater use of flight.

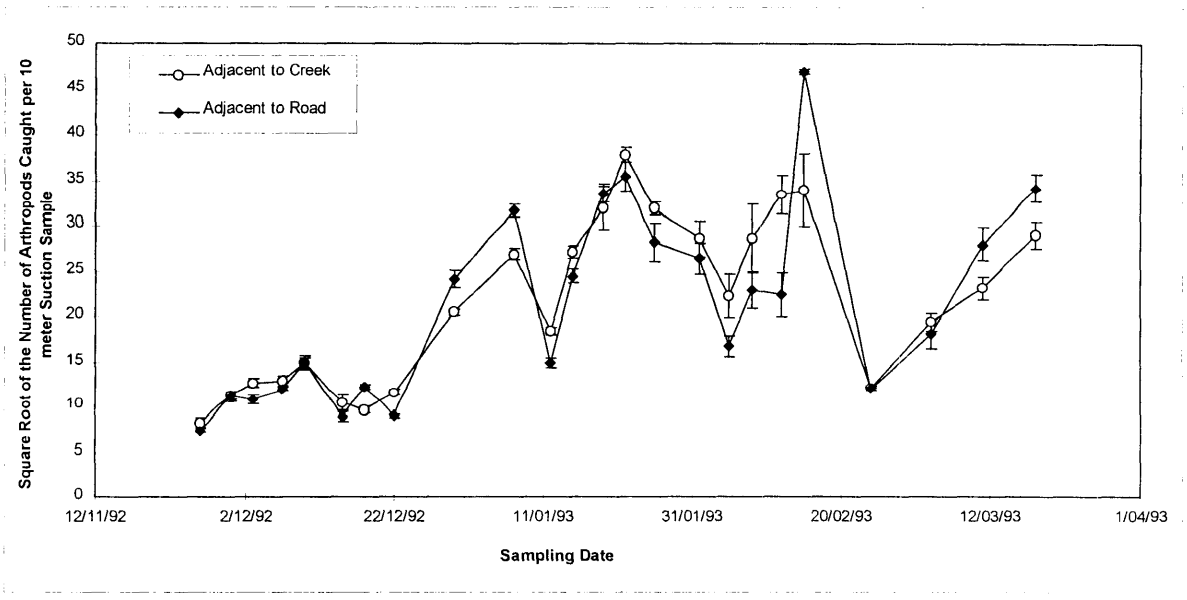

Figure 6.10 Total Arthropods, Midkin 1992/3: The abundance of total arthropods recorded by 10m suction samples from cotton fields 2 treated with soft-option insecticides. Adjacent to Carole Creek (-O-) or to the Gingham road (--◆--) at Midkin over the 1992/3 cotton growing season. The error bars represent the Standard error of the means (n=8). The Elecvac was used up to and including the 22-12-92; the Bigvac was used from then on including 22-12-92.

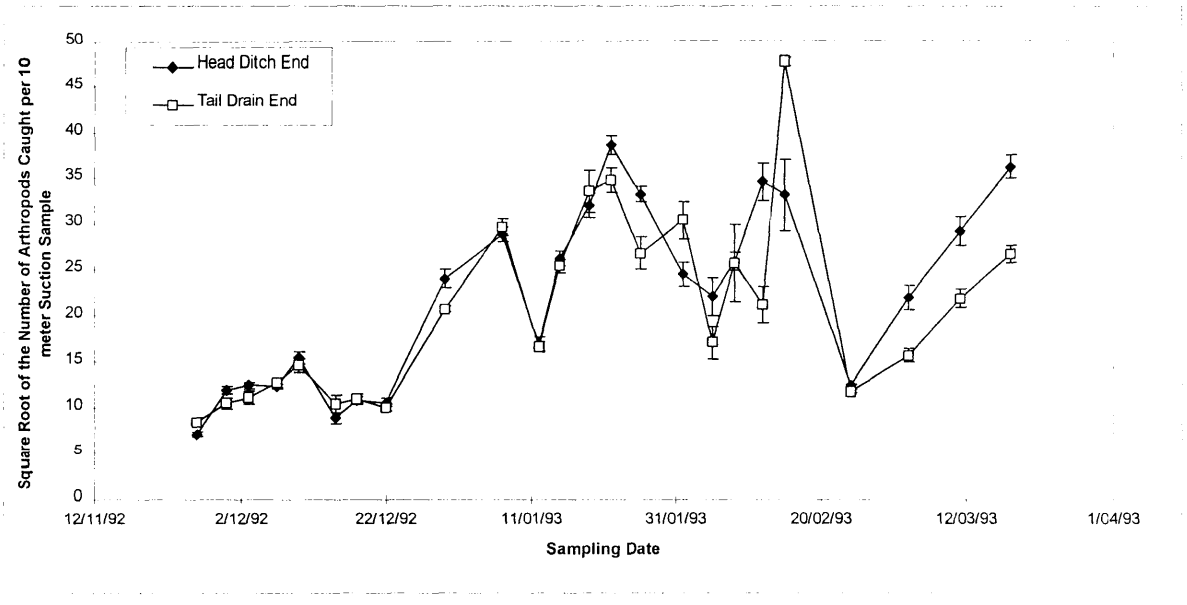

Figure 6.11 Total Arthropods, Midkin 1992/3: The abundance of total arthropods recorded by 10m suction samples from cotton fields 2 treated with soft-option insecticides. Adjacent to the tail drain (-O-) or to the head ditch (--◆--) at Midkin over the 1992/3 cotton growing season. The error bars represent the Standard error of the means (n=8). The Elecvac was used up to and including the 22-12-92; the Bigvac was used from then on including 22-12-92.

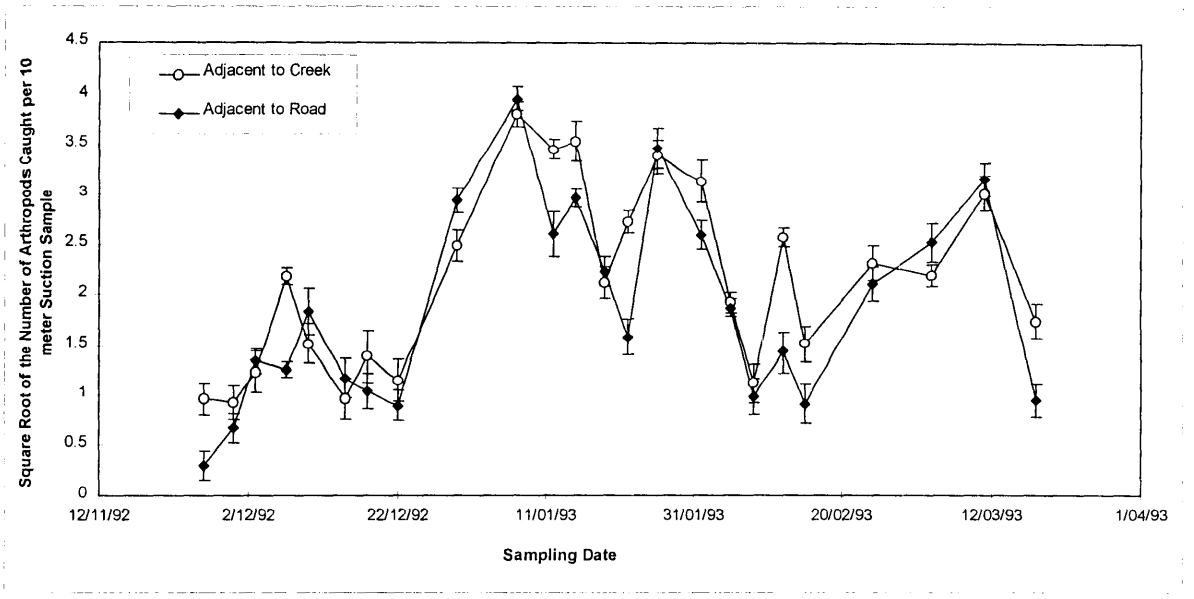

Figure 6.12 Total Predators, Midkin 1992/3: (not including *Campylomma* spp., *Creontiades* spp. or small spiders). The abundance of arthropod predators recorded by 10m suction samples from cotton fields 2 treated with soft-option insecticides. Adjacent to Carole Creek (-O-) or to the Gingham road (-◆-) at Midkin over the 1992/3 cotton growing season. The error bars represent the Standard error of the means (n=8). The Elecvac was used up to and including the 22-12-92; the Bigvac was used from then on including 22-12-92.

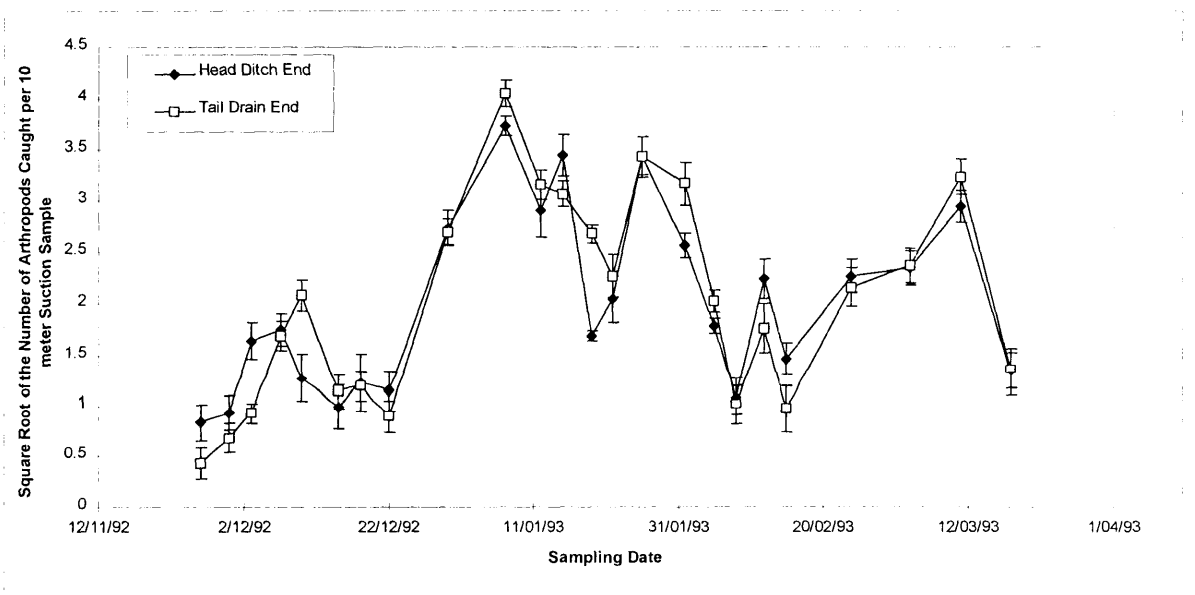

Figure 6.13 Total Predators, Midkin 1992/3: (not including *Campylomma* spp., *Creontiades* spp. or small spiders). The abundance of arthropod predators recorded by 10m suction samples from cotton fields 2 treated with soft-option insecticides. Adjacent to the tail drain (-O-) or to the head ditch (-◆-) at Midkin over the 1992/3 cotton growing season. The error bars represent the Standard error of the means (n=8). The Elecvac was used up to and including the 22-12-92; the Bigvac was used from then on including 22-12-92.

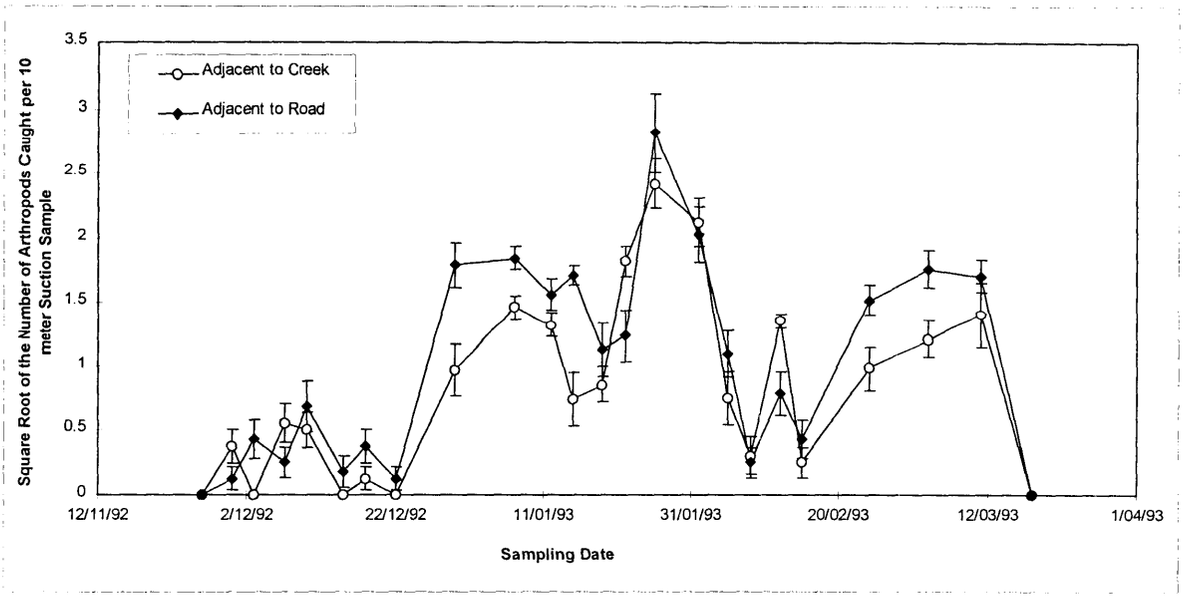

Figure 6.14 ***Dicranolaius bellulus*, Midkin 1992/3:** The abundance of *Dicranolaius bellulus* recorded by 10m suction samples from cotton fields 2 treated with soft-option insecticides. Adjacent to Carole Creek (-O-) or to the Gingham road (--◆--) at Midkin over the 1992/3 cotton growing season. The error bars represent the Standard error of the means (n=8). The Elecvac was used up to and including the 22-12-92; the Bigvac was used from then on including 22-12-92.

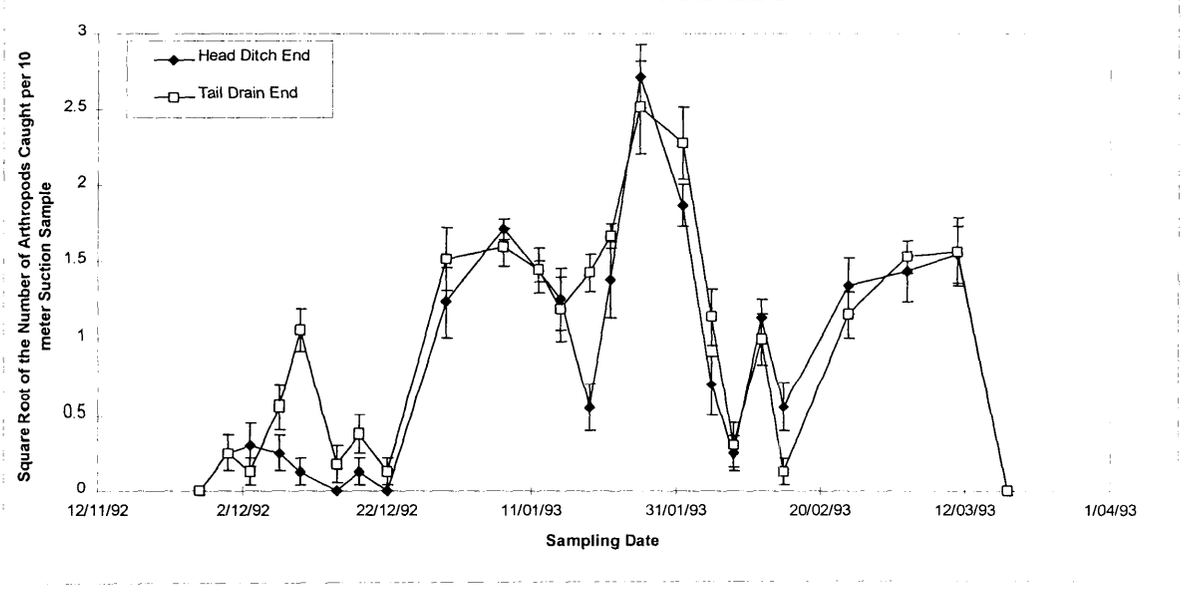

Figure 6.15 ***Dicranoliaus bellulus*, Midkin 1992/3:** The abundance of *Dicranoliaus bellulus* recorded by 10m suction samples from cotton fields 2 treated with soft-option insecticides. Adjacent to the tail drain (-O-) or to the head ditch (--◆--) at Midkin over the 1992/3 cotton growing season. The error bars represent the Standard error of the means (n=8). The Elecvac was used up to and including the 22-12-92; the Bigvac was used from then on including 22-12-92.

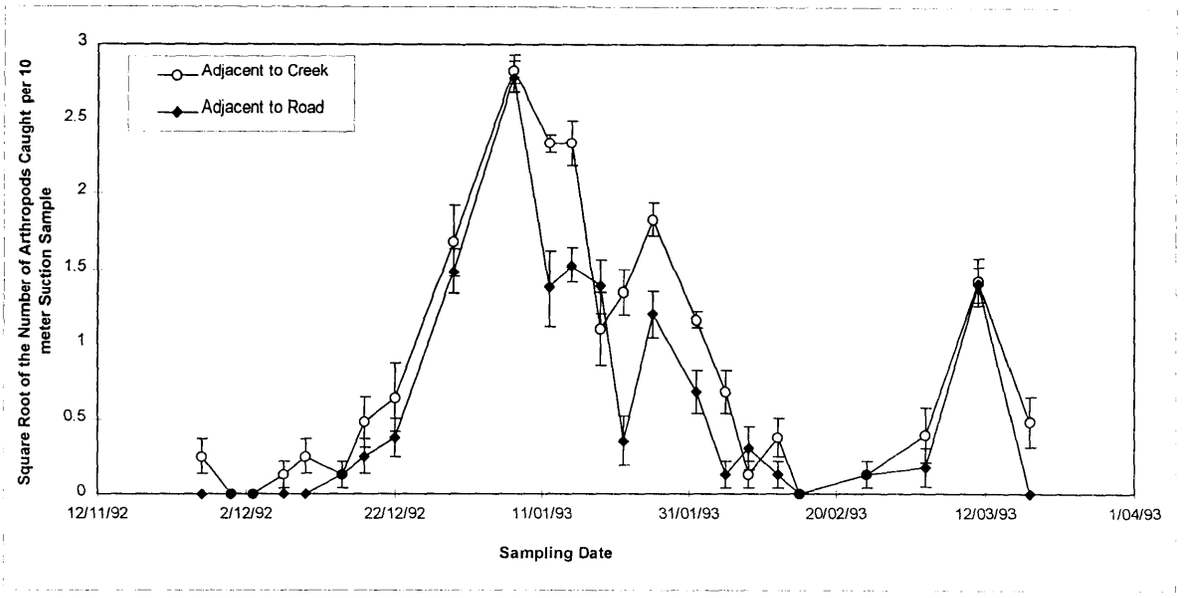

Figure 6.16 ***Nabis kinbergii*, Midkin 1992/3:** The abundance of *Nabis kinbergii* recorded by 10m suction samples from cotton fields 2 treated with soft-option insecticides. Adjacent to Carole Creek (-O-) or to the Gingham road (--◆--) at Midkin over the 1992/3 cotton growing season. The error bars represent the Standard error of the means (n=8). The Elecvac was used up to and including the 22-12-92; the Bigvac was used from then on including 22-12-92.

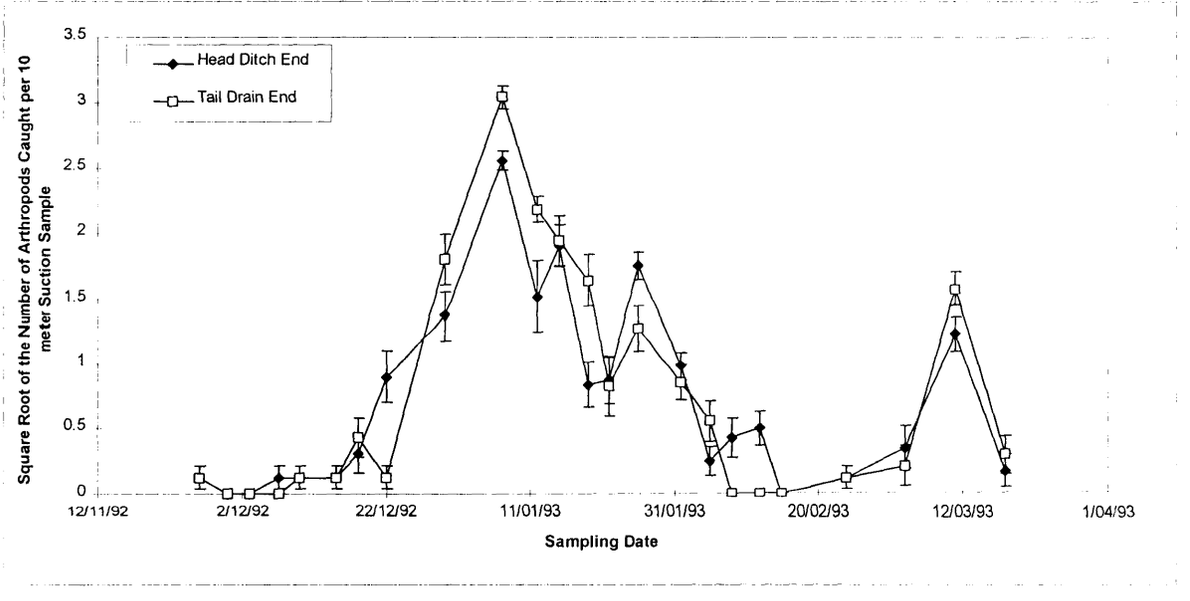

Figure 6.17 ***Nabis kinbergii*, Midkin 1992/3:** The abundance of *Nabis kinbergii* recorded by 10m suction samples from cotton fields 2 treated with soft-option insecticides. Adjacent to the tail drain (-O-) or to the head ditch (--◆--) at Midkin over the 1992/3 cotton growing season. The error bars represent the Standard error of the means (n=8). The Elecvac was used up to and including the 22-12-92; the Bigvac was used from then on including 22-12-92.

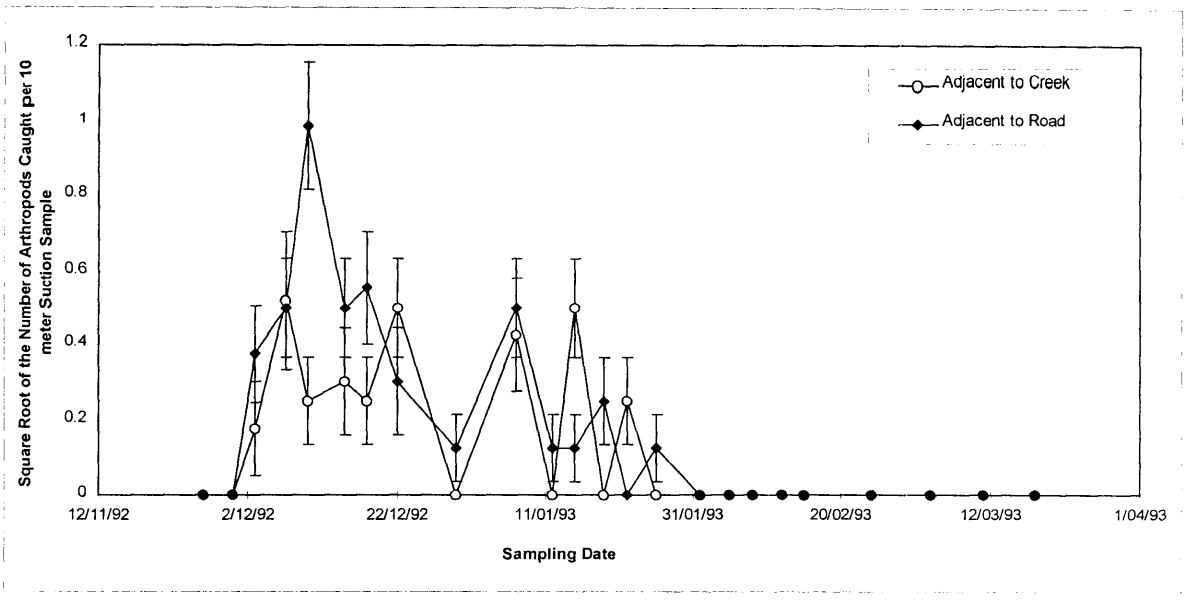

Figure 6.18 ***Coccinella transversalis*, Midkin 1992/3:** The abundance of *Coccinella transversalis* recorded by 10m suction samples from cotton fields 2 treated with soft-option insecticides. Adjacent to Carole Creek (—o—) or to the Gingham road (--◆--) at Midkin over the 1992/3 cotton growing season. The error bars represent the Standard error of the means (n=8). The Elecvac was used up to and including the 22-12-92; the Bigvac was used from then on including 22-12-92.

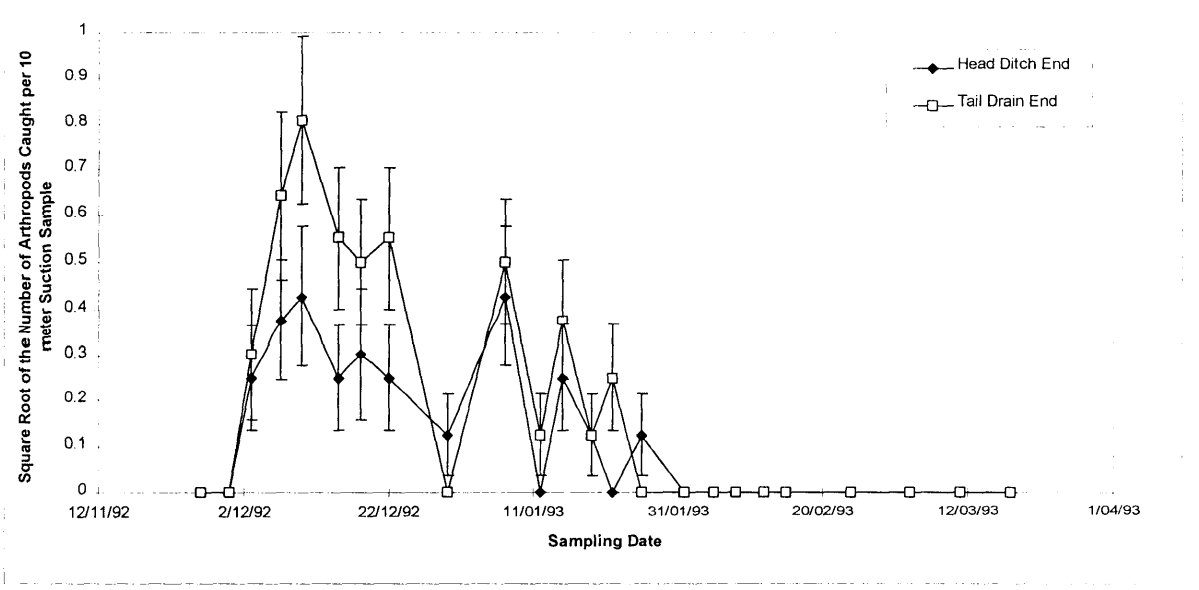

Figure 6.19 ***Coccinella transversalis*, Midkin 1992/3:** The abundance of *Coccinella transversalis* recorded by 10m suction samples from cotton fields 2 treated with soft-option insecticides. Adjacent to the tail drain (—o—) or to the head ditch (--◆--) at Midkin over the 1992/3 cotton growing season. The error bars represent the Standard error of the means (n=8). The Elecvac was used up to and including the 22-12-92; the Bigvac was used from then on including 22-12-92.

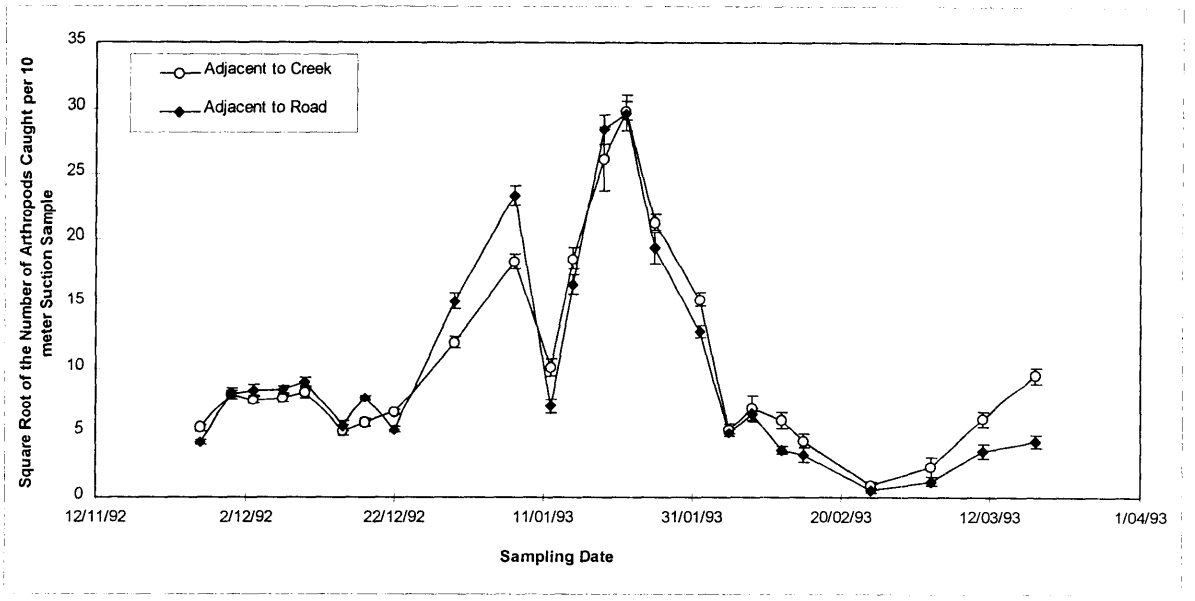

Figure 6.20 Cicadellid nymphs, Midkin 1992/3: The abundance of cicadellid nymphs recorded by 10m suction samples from cotton fields 2 treated with soft-option insecticides. Adjacent to Carole creek (-O-) or to the Gingham road (--◆--) at Midkin over the 1992/3 cotton growing season. The error bars represent the Standard error of the means (n=8). The Elecvac was used up to and including the 22-12-92; the Bigvac was used from then on including 22-12-92.

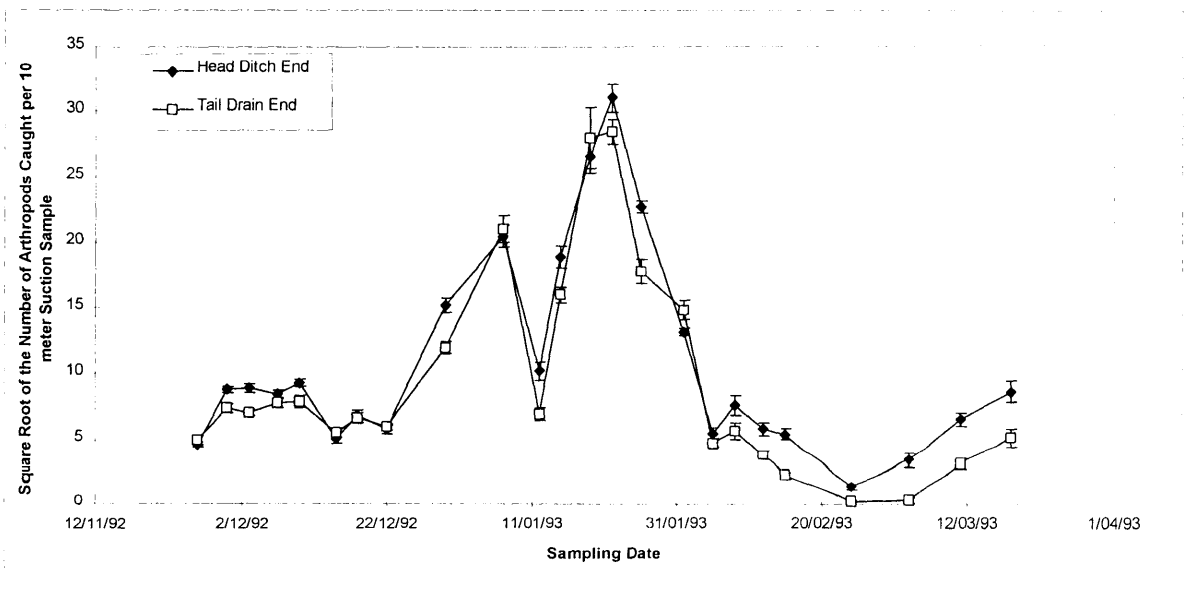

Figure 6.21 Cicadellid nymphs, Midkin 1992/3: The abundance of Cicadellid nymphs recorded by 10m suction samples from cotton fields 2 treated with soft-option insecticides. Adjacent to the tail drain (-O-) or to the head ditch (--◆--) at Midkin over the 1992/3 cotton growing season. The error bars represent the Standard error of the means (n=8). The Elecvac was used up to and including the 22-12-92; the Bigvac was used from then on including 22-12-92.

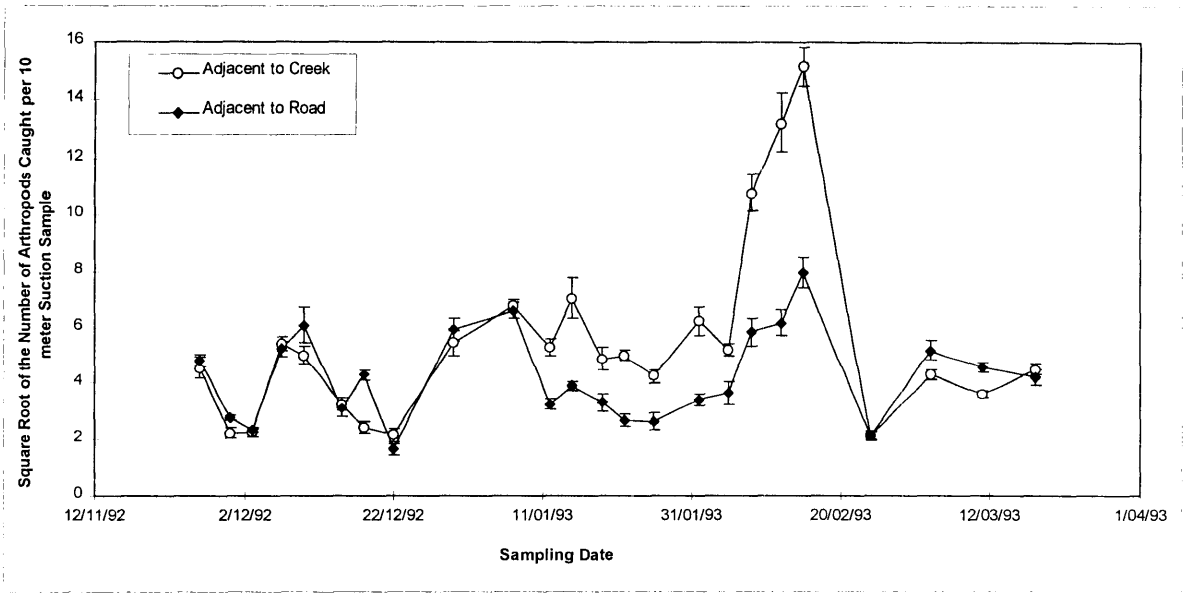

Figure 6.22 Thysanoptera, Midkin 1992/3: The abundance of Thysanoptera recorded by 10m suction samples from cotton fields 2 treated with soft-option insecticides. Adjacent to Carole creek (-O-) or to the Gingham road (--◆--) at Midkin over the 1992/3 cotton growing season. The error bars represent the Standard error of the means (n=8). The Elecvac was used up to and including the 22-12-92; the Bigvac was used from then on including 22-12-92.

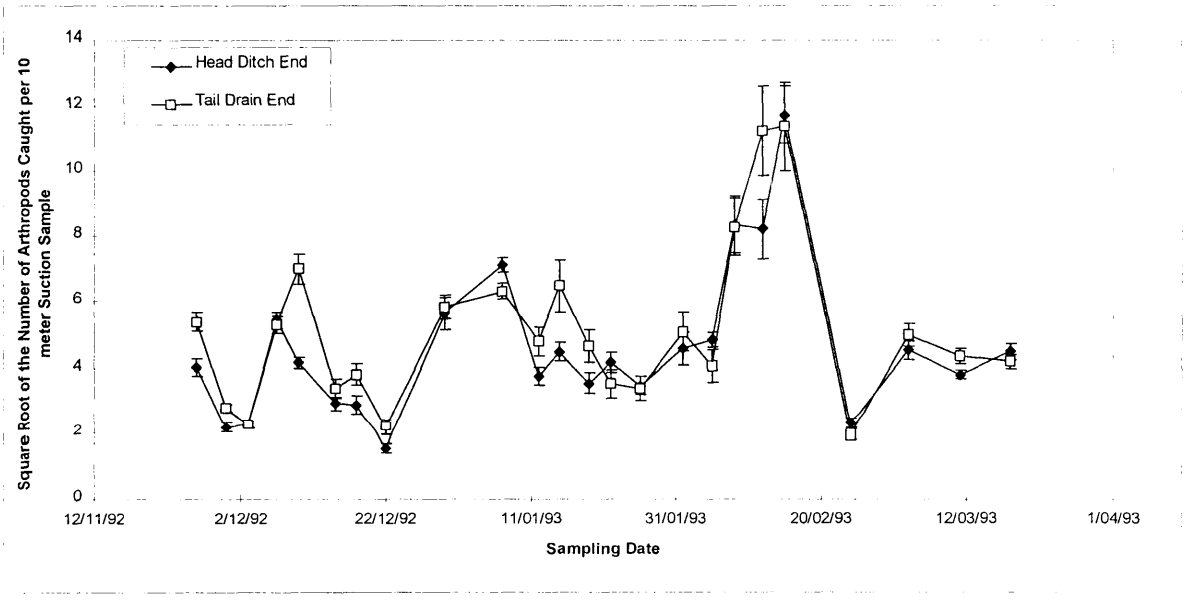

Figure 6.23 Thysanoptera, Midkin 1992/3: The abundance of Thysanoptera recorded by 10m suction samples from cotton fields 2 treated with soft-option insecticides. Adjacent to the tail drain (-O-) or to the head ditch (--◆--) at Midkin over the 1992/3 cotton growing season. The error bars represent the Standard error of the means (n=8). The Elecvac was used up to and including the 22-12-92; the Bigvac was used from then on including 22-12-92.

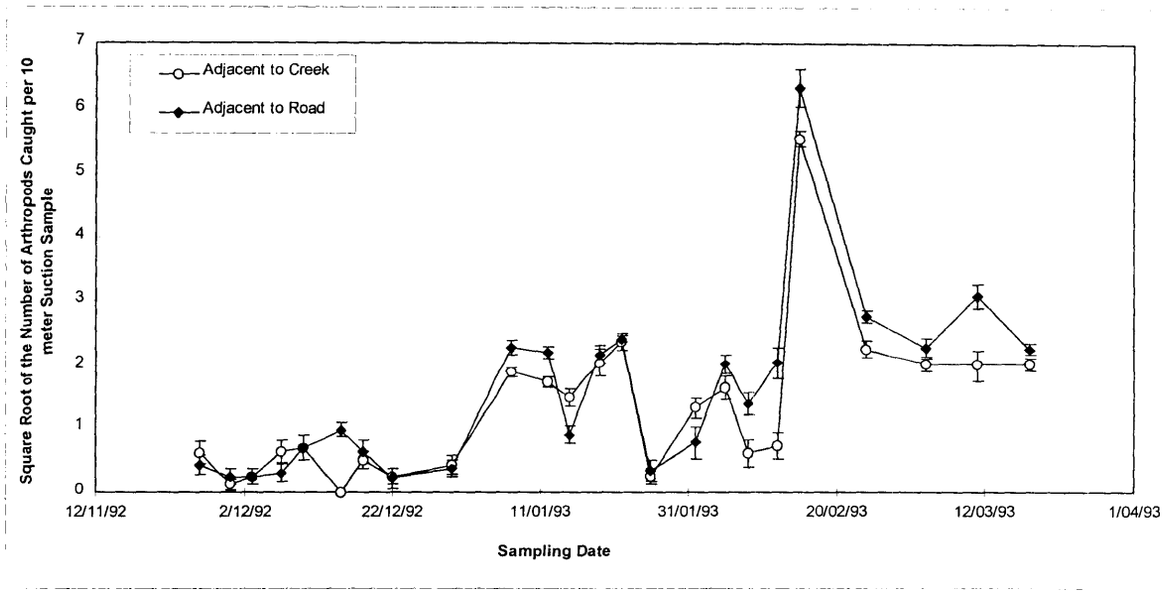

Figure 6.24 ***Helicoverpa*** **larvae, Midkin 1992/3:** The abundance of *Helicoverpa* larvae recorded by 10m suction samples from cotton fields 2 treated with soft-option insecticides. Adjacent to Carole creek (-O-) or to the Gingham road (--◆--) at Midkin over the 1992/3 cotton growing season. The error bars represent the Standard error of the means (n=8). The Elecvac was used up to and including the 22-12-92; the Bigvac was used from then on including 22-12-92.

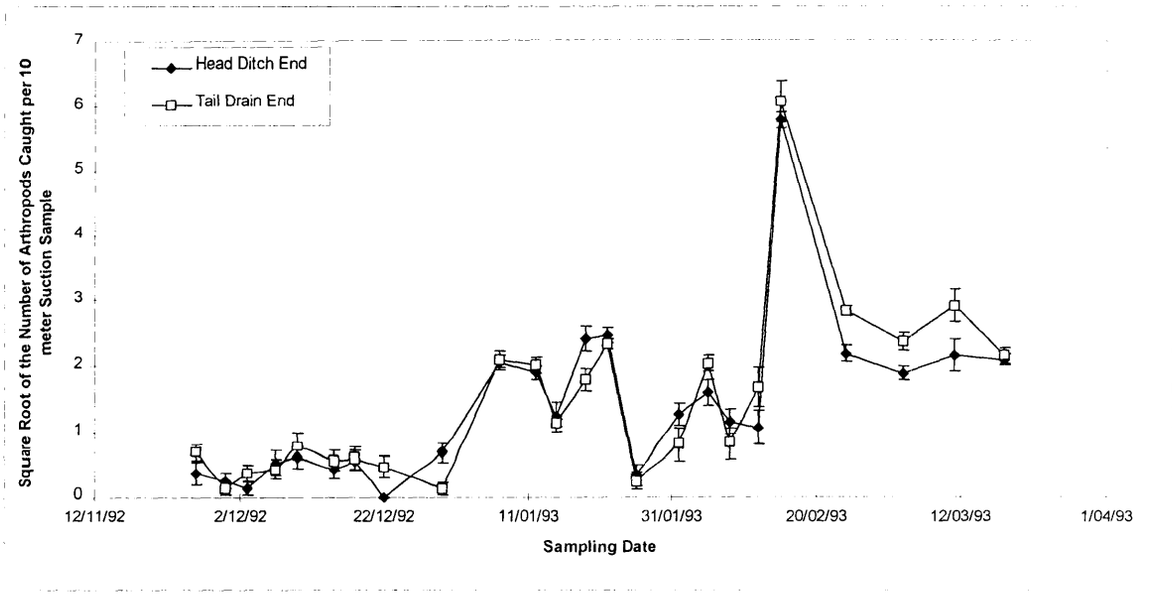

Figure 6.25 ***Helicoverpa*** **larvae, Midkin 1992/3:** The abundance of *Helicoverpa* larvae recorded by 10m suction samples from cotton fields 2 treated with soft-option insecticides. Adjacent to the tail drain (-O-) or to the head ditch (--◆--) at Midkin over the 1992/3 cotton growing season. The error bars represent the Standard error of the means (n=8). The Elecvac was used up to and including the 22-12-92; the Bigvac was used from then on including 22-12-92.

Chapter 7

Experimental Section

7. Predation by Generalist Predators on *Helicoverpa* spp. in Laboratory and Field Cage Experiments

7.1 Introduction

To include the impact of predators in pest management decisions it is necessary to identify which predator species are effective and how many individuals are needed to contain a given pest population. It is often suggested that the ratio of predator abundance to pest abundance, monitored during crop scouting, may indicate whether intervention with other forms of pest control (particularly insecticides) is necessary (King and Coleman 1989, Fitt 1994 and Sugonyaev 1994). Ratios suggested in United States cotton growing regions are in the order of 1 or 2 predators to each *Helicoverpa* spp. egg or larva. However, ratios such as these are dynamic, that is, they change at different times throughout the growing season, taking into account crop stage and other conditions found to affect predator efficiency, such as predator types and abundance of alternative prey (King 1986). For Australian cotton growers to consider using such ratios, accurate and repeatable measures of predation, for the Australian species of predators and pests, will be required. The following experiments were designed to measure the predation rates of some of the Australian predators over a range of *Helicoverpa* spp. densities to provide this information. *Dicranolaius bellulus, Coccinella transversalis, Mallada signata* and *Nabis kinbergii* were selected for these experiments because they were abundant and available (they had figured prominently in survey suction samples), were relatively large (could be readily observed and sampled) and had exhibited high rates of predation in petri dish consumption trials conducted by Room (1979a).

Given the practical difficulties with quantifying the impact of predators in real situations, laboratory studies are often employed. This approach seeks to discover intrinsic attributes of consumptive behaviour such as; i) maximum prey consumption rates, ii) prey preferences, iii) functional responses, iv) reproductive numerical responses and v) other

biological parameters such as longevity and fecundity on various types of prey. These parameters are used to indicate predatory behaviour or estimate predatory performance in the field. The laboratory has advantages of allowing control over many variables such as temperature, humidity and lighting. Originally these types of studies were planned for several of the Australian predator species in this thesis, however the initial laboratory trials returned predation rates which appeared exaggerated relative to field observations. Therefore the laboratory approach was curtailed in favour of more realistic experiments using field cages.

Large field cages, enclosing several whole plants, have been widely used for predation studies with cotton insects (van den Bosch 1969, Lingren *et al.* 1968, Ridgway and Jones 1968 and 1969, Lopez *et al.* 1976, Hutchison and Pitre 1983, Stark and Whitford 1987, Thead *et al.* 1987, Knutson and Gilstrap 1989 and van den Berg and Cock 1993 and 1995). These present a search arena and environment nearer to that of the field than the laboratory trials, but the cage is not without influence. The cage environment tends to reduce the extremes of temperature, light and wind speed (Hutchison and Pitre 1983, Titmarsh 1992), all capable of affecting the survival or behaviour of the predators or prey. Cage confinement may also inhibit the freedom of the predator to display normal searching behaviour, particularly if a larger area is required than catered for by the cage. Nonetheless, three field cage experiments were conducted to provide estimates of predation by *Dicranolaius bellulus*, *Coccinella transversalis*, *Mallada signata* (larvae) and *Nabis kinbergii* on *Helicoverpa* spp. eggs or young larvae over various prey densities.

The arthropod survey had shown that *Helicoverpa* spp. contributed only a very small proportion of the available prey for the generalist predators within a cotton crop (Chapter 6). The presence of alternative prey or plant food has long been recognised as having the potential to affect the impact of predators on any particular target species (Murdoch *et al.* 1985, Chesson 1989). Therefore the effect of alternative prey, in this case cotton aphids (*Aphis gossypii*), on the predation rates of three predators was also explored.

Finally, because so little was known about *D. bellulus*, it was decided to observe the development of this beetle from eggs collected during the laboratory consumption trial. The life cycle and the procedures used to rear this species under laboratory conditions are presented in Appendix 7.3.

7.2 Laboratory Consumption Trial of *Helicoverpa* spp. Eggs by Adult *Dicranolaius bellulus*.

7.2.1 Introduction

Room (1979a) demonstrated in petri dish styled consumption trials that *Dicranolaius bellulus* readily fed on *Helicoverpa armigera* and *H. punctigera* eggs. Field observations have also indicated that *D. bellulus* is commonly entomophagous in cotton crops (D.A.H. Murray, pers. comm. 1993). However, Schicha (1974) found mostly pollen and anther tissue, along with some chitin, in the alimentary canal of specimens collected from flowering rice crops in the Murrumbidgee Irrigation Area of southern NSW. Although this indicates some predation, the species was, at least in those conditions, predominantly phytophagous. *D. bellulus* was one of the most abundant predators in Australian cotton fields under the reduced and conventional insecticide strategies imposed during the arthropod population surveys conducted during for this thesis (Chapter 5). This may have been due in part to a tolerance exhibited for the commonly used insecticide, endosulfan (Cox 1981). Despite the obvious potential of these features, little is known of the life cycle and predatory impact of *D. bellulus*.

As mentioned in the introduction, studies have suggested that the availability of alternative prey may affect the rate of predation on a target prey or may add supplemental nutrients which increase fecundity (Murdoch *et al.* 1985, Chesson 1989). These possibilities were explored by including pollen as an alternative food source in some treatments. The aim of this experiment was to quantify the impact of *D. bellulus* on *H. punctigera* eggs in laboratory trials, with and without access to alternative food.

7.2.2 Materials and Methods

Insect Source

Adult *D. bellulus* were collected from cotton crops at 'Midkin' in February 1993 and held for two weeks in mass cultures to allow mating. The culture containers were perspex cylinders (30 cm diameter x 50 cm high) with metal bases (baking tins) and organza lids for ventilation. Each culture contained approximately 200 beetles of mixed sexes. Every two days, 3 grams of bee pollen pellets (People's Choice®, Bee Pollen, 143 Phynne Road,

Morningside Qld. 4170) were crushed and presented to the culture in a 10cm petri dish. The beetles readily fed on the bee pollen but there was no attempt made to establish whether this was a complete or satisfactory diet beyond observing acceptable survival rates (>90%) over the holding period of two weeks. Moisture was provided daily as wetted dental wicks placed in a second petri dish. Handling beetles was facilitated by tending during the cool of the morning or by cooling at 4°C until beetles were subdued. No obvious mortality was observed from this procedure.

Treatments

Adult *D. bellulus* of both sexes were collected from mass cultures and placed singly into petri dishes. These were held at 25°C (± 0.5°C) and 16hr/8hr (light/dark) throughout the experimental period. The beetles were given moisture via wetted dental wicks. Petri dishes which contained females also received a tightly folded (concertina-like) and stapled piece of filter paper as an oviposition site. Eight females and four males were randomly allocated to each of three treatments:

1) Offered two crushed pellets of bee-pollen daily.
2) Offered newly laid *H. punctigera* eggs daily or
3) Both 1 and 2.

The greater number of female replicates were included to increase the likelihood of oviposition observations.

Treatments which received *H. punctigera* eggs (2 and 3) were given between 30 and 40 newly laid (previous nights oviposition) *H. punctigera* eggs on the first day. Subsequently, they received a greater number of eggs on the next day than they had fed upon on the previous day. The *H. punctigera* eggs were collected daily at 8-00 am from a stock culture at the University of New England produced using the methods of Teakle and Jensen (1985). The paper towelling onto which the eggs had been laid was cut into sections, each having the required number of eggs. These were presented to the adult *D. bellulus* by 10-00 am each day for 12 consecutive days. Eggs were examined and counted under a binocular microscope before and after presentation to ensure that they were normal in appearance and well hydrated so that any subsequent damage to eggs could be attributed to the beetles. In virtually all cases a record of predation meant that the egg was completely removed. Records were made daily,

between 8.00 am and 10.00 am, of egg consumption by each beetle (the number of eggs offered minus eggs remaining) and the number of beetle eggs oviposited by each female.

7.2.3 Results and Discussion:

Consumption of Helicoverpa Eggs

As illustrated in Figure 7.1, each *D. bellulus* consumed on average 6.3 *H. punctigera* eggs/day over the 12 day period. Laying females consumed 10.3 eggs/beetle/day on average. This appeared to be greater than non laying females which took 5.7 eggs/beetle/day, but the difference was not statistically significant (t = 0.15) (Table 7.1). Egg consumption by males (mean = 4.6 eggs/beetle/day) and non laying females were also not significantly different (t = 0.43). Room (1979a) also identified *D. bellulus* as a predator of *Helicoverpa* spp. and reported consumption rates of 6.4 to 7.9 *Helicoverpa* spp. eggs/predator/day in petri dish consumption trials.

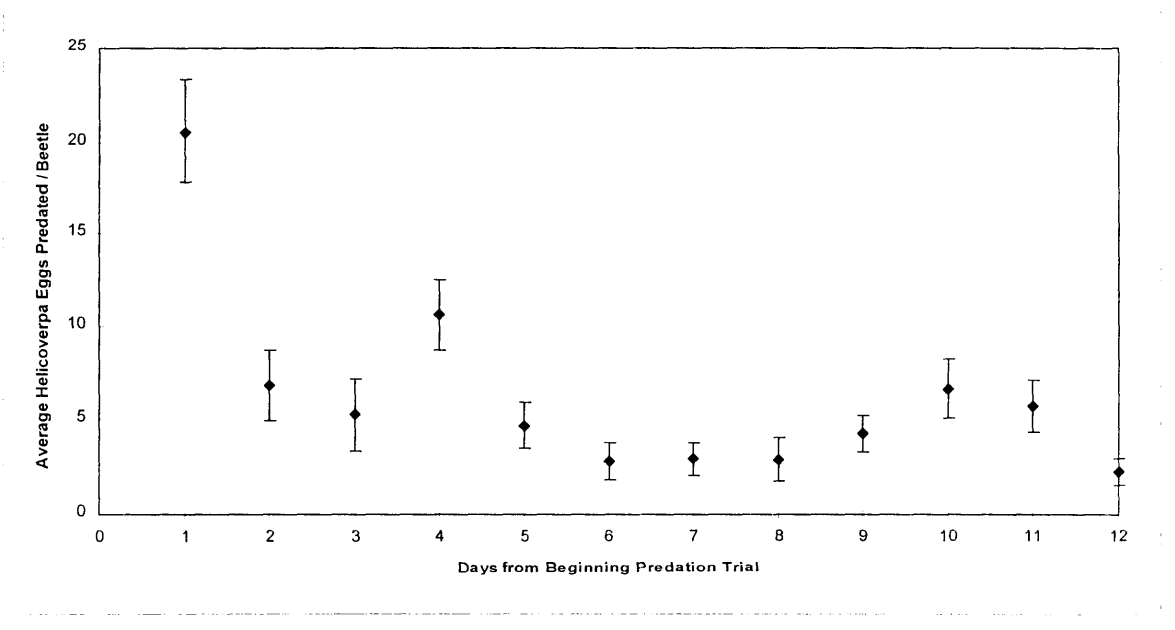

Figure 7.1 The average daily consumption of *Helicoverpa punctigera* eggs by each *Dicranolaius bellulus* in petri dishes. Bars represent ± the standard error of the mean (n = 24).

Females offered both eggs and pollen laid similar numbers of beetle eggs as those fed only pollen (29.5 and 21.5 respectively, t = 0.44) but there were only six females which laid

any eggs, four from the pollen and egg treatment. Only one female laid when fed *H. punctigera*. eggs alone.

***Dicranolaius bellulus* Grouping**	***Average* Egg consumption (*H. punctigera* eggs/beetle/day)**
Overall Average	6.3
Comparison 1	
Laying Females	10.3 a
Nonlaying Females	5.7 ab
Males	4.6 b
Comparison 2	
Offered Eggs & Pollen	6.8 a
Offered only Eggs	5.8 a

Table 7.1 Consumption of newly laid *Helicoverpa punctigera* eggs by *Dicranolaius bellulus* in petri dish styled consumption trials. Entries in the same comparison, followed by the same letters are not significantly different at $P < 0.05$ (Students t-test).

These egg consumption rates are likely to exceed those normally experienced in the field, because at this rate the prevailing populations of *D. bellulus* alone (usually 0.5 to 1 per metre but ranging up to 3.5 per metre; Chapter 5) would remove virtually all of the *Helicoverpa* spp. eggs commonly present (usually in the vicinity of 2 to 10 eggs per metre). Since there is little evidence from the field that this actually occurs, these rates probably reflect the ease with which the prey could be found over the small area of a petri dish. Several researchers working on other predatory species have found large discrepancies between estimates of prey consumption rates in petri dishes compared to larger and more complex arenas (Lingren *et al.* 1968, Butler and May 1971, O'Neil 1989 and Awan 1990).

The high rate of consumption on the first day (21 eggs per beetle) and the relatively high rates over the following three days of this experiment probably reflect a state of starvation in the beetles in the mass cultures, even though pollen was continuously available (Figure 7.1). It is common practice to starve test insects for a fixed period of 24 to 48 hours before laboratory trials to bring them to a similar state of starvation and therefore reduce variability between individuals. The results here suggest that if consumption is only recorded for a short period after this treatment, consumption rates might be grossly exaggerated compared to a longer term average rate. This would overestimate the potential impact of the predator.

Ignoring the initial 4 days of this trial suggests that the average per capita consumption for these beetles would lie in the range of 2 to 6 *H. punctigera* eggs per day in petri-dish styled feeding trials.

7.3 Field Cage Experiment 1: The Predation of *H. punctigera* Eggs by Adult *D. bellulus*.

7.3.1 Introduction and Aim

Considering the shortcomings of the laboratory consumption trials of the previous section, efforts were directed toward measuring the predation rate of *Dicranolaius bellulus* on *Helicoverpa* spp. eggs under various predator/prey densities in a much more realistic search arena. This section describes experiments conducted in large field cages.

7.3.2 Materials and Methods

Cage Design

Each cage had a 1 metre square base, a height of 2 metres and enclosed a 1 metre length of cotton row (10 plants) in terylene woven fabric (mesh size of 0.2 x 0.2 mm square holes, 9 holes/ sq mm). The floor of each cage was also made of the terylene mesh and was suspended at the level of the plant stems just below the lowest branches. The two mesh panels forming the floor were joined between the plant stems with Velcro® and completely sealed around the stems using a non-silicone sealant. A run of sealant was also placed along the top of the Velcro® join to make sure of this seal (Figure 7.2). A full description of the field cage design is presented in Appendix 7.4 with photographs of the cages, floor seals and use of the Velcro® door slits on plates 6 to 8.

Cage Effects

The cage fabric excluded 46% of the light measured as photosynthetically active radiation (PAR) when the fabric was held in a horizontal plane to the path of the light (using a Licor® Li-170 photometer, Lambda Instruments Corporation, Lincoln, Nebraska, USA. 68504). The temperature difference between the external and internal cotton canopy was generally small but could reach up to 8°C (Appendix 7.1). The larger differences occurred at the diurnal temperature extremes especially during the afternoon when the shading of the cage probably had the greatest influence on internal temperatures. Night temperatures were also cooler in the cages but only by about 1°C.

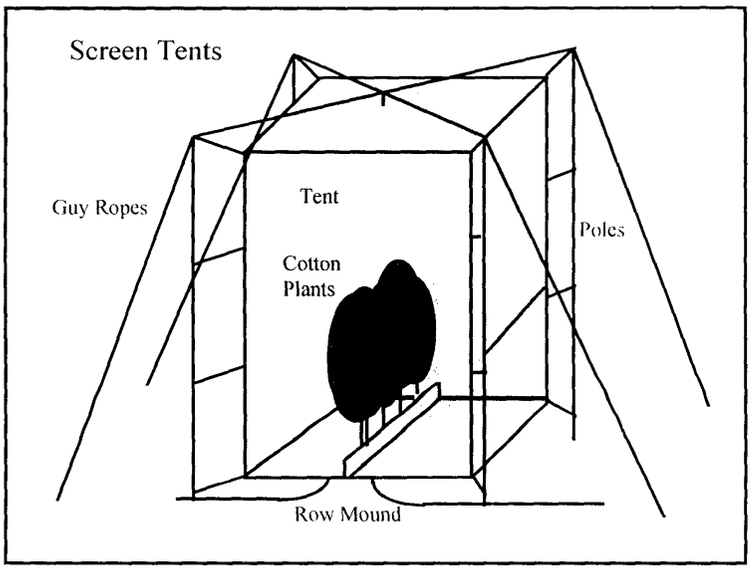

Figure 7.2 A field cage erected over cotton plants showing the suspended floor and how it sealed around the stems of the cotton plants to totally exclude other insects than those intended for evaluation. Photographs of the field cages are presented in Appendix 7.4.

A hotwire anemometer was used to investigate the difference in windspeed by comparing readings taken with the probe held inside the cage and then transferred quickly to a similar canopy position outside. This method was not very satisfactory because of the highly variable air currents around the canopy. However, it tended to indicate that the internal windspeed was only 10% of the external measurement.

The *H. punctigera* eggs were laid within the field cages by field collected moths. The moths were caught up to 3 days prior to their use using mercury vapour light traps operated in the same region where the cage experiments were conducted ('Midkin', Chapter 3). The moths were kept in laboratory cages (50cm long x 30cm wide x 20cm high cardboard boxes with gauze covers) with access to 5% honey solution. 200 moths were collected to ensure enough pairs (48) were available for the day of the trial.

Predators were collected in the field using sweep nets and suction samplers one day prior to their release into the field cages, and kept *en masse* in plastic containers (15 cm dia x 12 cm height) with access to water in dental wicks. Field collected material was expected to provide predators of realistic age distribution and level of hunger. The beetles were sexed and sorted into plastic tubs corresponding to the 5 beetle/cage or 30 beetle/cage treatments during the cool of the early morning whilst relatively docile. The sex ratio of 3 females to 2 males was determined by availability.

Cage Preparation

All cages were treated with a synthetic pyrethrin (RAID®, S. J. Johnson & Son Pty. Ltd., 16 Epping Road, Lane Cove NSW 2066 Australia; active ingredients: 4.05 g/kg tetramethrin, 0.90g/kg allethrin 20:80, and 0.90g/kg d-phenothrin) to remove resident arthropods which might influence the measurement of the test insects. This was applied from domestic 350g pressure packs by directing the nozzle towards the plants for 10 seconds and along the cage seams including the roof, where insects and particularly spiders might hide, for another 10 seconds. Two follow-up applications occurred at three day intervals to remove arthropods which may have hatched after the first insecticide application. These treatments ceased four days before the introduction of the test insects.

Two or four pairs of *H. punctigera* moths were introduced to lay on the caged cotton plants and were expected to produce a broad range of egg (prey) densities; this was generally successful. The following morning the moths were removed and the oviposited eggs counted. Some eggs were discovered on the cage material. These were easily seen and were removed by dislodging with a large 15cm wide paint brush and collected from the floor of the affected cages with a small electric vacuum cleaner. The presence and position of each egg laid on the

plants was marked with a blue tick on the plant surface adjacent to the egg, using a fine permanent marker pen. At 6 pm that evening the predatory beetles (*D. bellulus*) were introduced into the cages in one of three predator densities; zero, five and thirty beetles per cage (i.e. per metre of cotton row). The beetles were left for that night, one day and the next night to feed. The following morning the eggs which remained were counted. Hatching was observed in the afternoon of that day but no hatching or identifiably hatched eggs were encountered during the counting period (Figure 7.3).

Experimental Design

Twenty four field cages were erected in a widely spaced grid pattern within an area of unsprayed, rain grown cotton (var. Siokra L23) at 'Midkin' (Figure 7.4). At this stage in the season the cotton plants were vegetative and approximately 40 cm high. Adding two or four *H. punctigera* pairs to the cages for one night produced egg densities ranging from 4 to 170 per cage (i.e. per row metre of cotton). These egg densities were ranked and divided into high or low prey density categories, regardless of how many moths produced them. The beetle treatments were randomly allocated, producing four control cages (zero beetles), 4 x 5 beetles cages and 4 x 30 beetles cages in each of the two egg density categories.

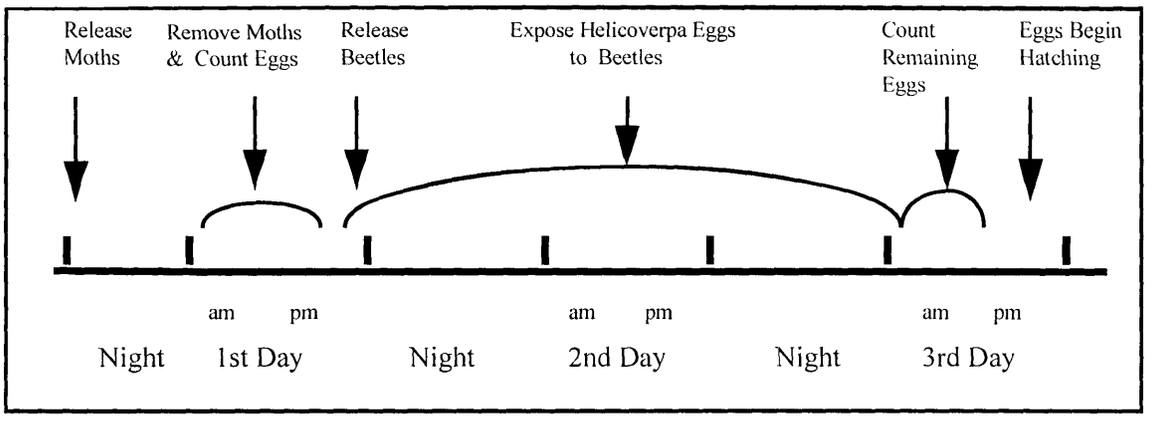

Figure 7.3 The sequence of activities during the first cage experiment which exposed various densities of naturally laid *Helicoverpa punctigera* eggs to two densities of adult *Dicranolaius bellulus*.

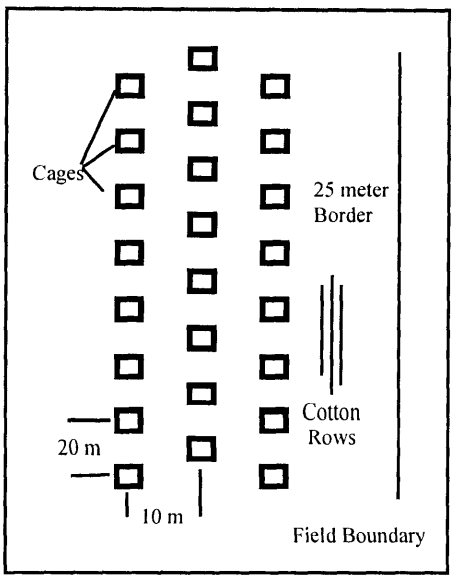

Figure 7.4 The plan of the field cages for experiment 1 in unsprayed cotton at Midkin, December 1993. A 25 metre border separated the cages from the side of the field. There was at least 100 metres of cotton crop in any other direction. Cages were spaced out to reduce the shading and wind breaking effects between adjacent cages.

7.3.3 Results and Discussion

Average Egg Consumption per Cage

Figure 7.5 shows the number of missing *Helicoverpa* eggs in each cage after the predation period, plotted against the number of eggs initially found and marked. Comparison of the slopes of the regression lines between the three treatments was used to investigate the extent of predation. Linear regressions forced through the origin indicated that the level of mortality was 15% (R^2 = 0.86) in control cages, 22% (R^2 = 0.91) in the treatment with 5 beetles / cage and 46% (R^2 = 0.80) when 30 beetles / cage were present. That is, the best estimates of the increase in mortality are 7% with 5 beetles per metre, and 31% when 30 beetles were present.

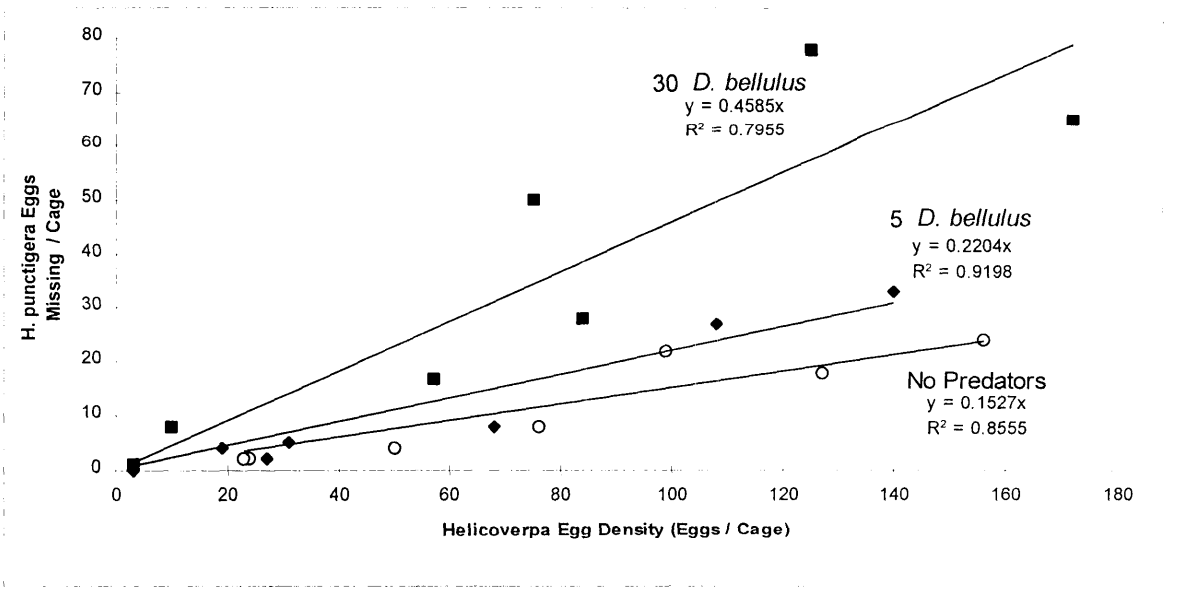

Figure 7.5 The number of missing *Helicoverpa punctigera* eggs per cage (i.e. missing eggs per metre) in field cages after exposure to zero (o), 5 (♦) and 30 (▪) *Dicranolaius bellulus* adults per cage for 36 hours.

Average Egg Consumption per Beetle

Average egg consumption per beetle was calculated by subtracting the 15% rate of mortality, estimated using the control cages, from the mortality in cages where beetles were present, and then dividing by the number of beetles placed in those cages. The low variance in the background mortality from the control cages facilitated this procedure, however negative results occurred where background mortality was below average (Figure 7.6). This measurement only considers the additional mortality due to predation. Therefore the actual number predated could have been higher than these estimates because in the cages containing beetles many of the eggs missing as background mortality may have been predated before other factors occurred. Therefore the maximum additional mortality or apparent predation recorded per beetle during this trial was about 2.3 eggs/day for both the 5 and 30 beetles per cage treatments, and it occurred at high prey densities. Low predation rates of less than 0.2 eggs per day occurred where prey densities were less than approximately 60 eggs per metre. This indicates that unless egg densities are much greater than those usually encountered in the field, these predators are unlikely to take many eggs. The lower figure of 0.2 prey per predator per day at normal field densities of prey is similar to estimates of predation of 0.4 per day made for many generalist predators studied in field situations at realistic densities on a variety of target prey (O'Neil 1989). Furthermore the similar estimates of maximum predation per beetle for

both predator densities (5 or 30 beetles per row m) indicates that intraspecific interference did not occur over this range of predator density, which extends well beyond those commonly observed.

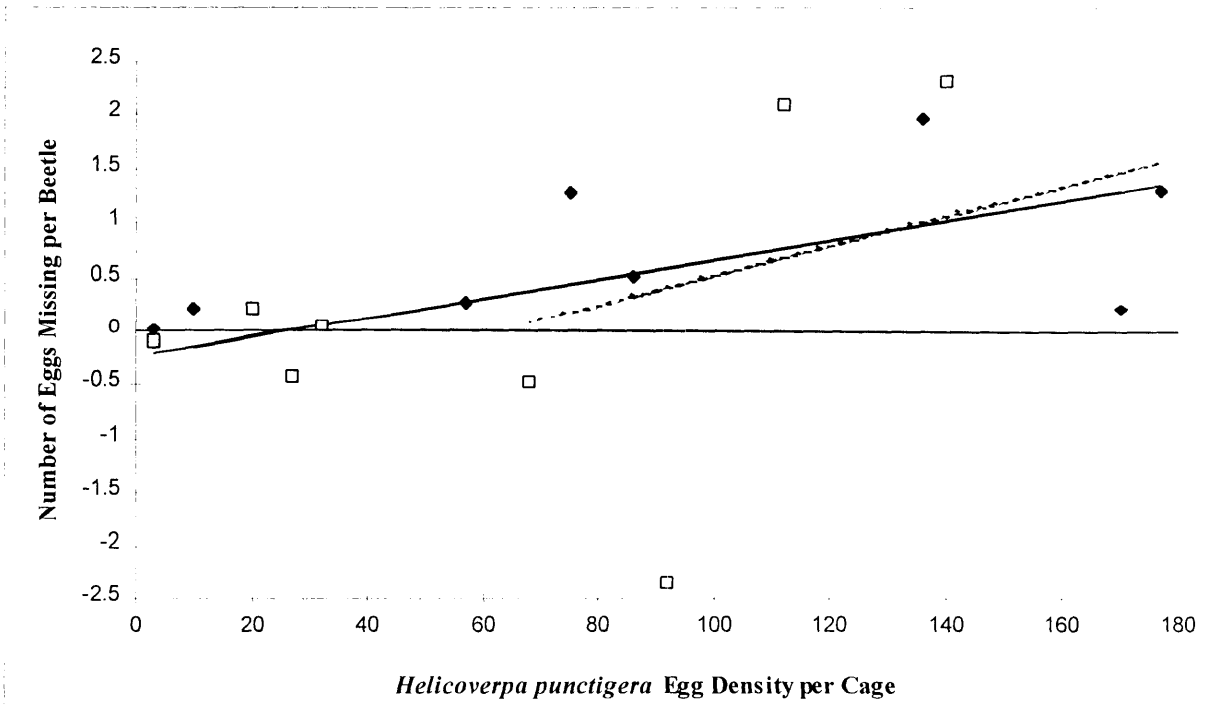

Figure 7.6 Per capita rate of predation by *D. bellulus* offered various densities of *H. punctigera* eggs in field cages at Midkin 1993. 5 (▫) and 30 (▪) *Dicranolaius bellulus* per cage. The solid regression line (——) includes all the data points in this analysis ($p=0.08$, $R^2 = 0.45$). The dashed regression line (-------) only includes *Helicoverpa punctigera* egg densities of greater than 60 eggs per cage ($p=0.33$, $R^2 = 0.37$).

Figure 7.6 provides an indication of the functional response of *D. bellulus* under field conditions within a realistic searching arena. The main features of this functional response are a tendency for greater predation rates to occur at higher densities of the prey, a maximum average predation rate of 2.3 eggs per day per beetle and a very low rate of predation until prey densities reach greater than about 60 eggs per metre.

The solid regression line in Figure 7.6 uses all the data points on the chart and indicates that the apparent increase in prey consumption per beetle over prey density was not statistically significant ($p = 0.08$, $R^2 = 0.45$), but there were few data points and high variance. The pattern of the scatter plot suggests that there are possibly two populations of points, one below 60 eggs

per cage (per metre) and one above 60 eggs per cage, so a single linear model might not be the best description of these data. Fitting two lines (Figure 7.6), one to each of these groups of points improved the fit below 60 eggs per metre but showed no significant increase with egg density either below or above this level of eggs (up to 60 eggs per metre: $p = 0.61$, $R^2 = 0.23$ and above 60 eggs per metre: $p=0.33$, $R^2 = 0.37$).

The mean egg consumption of 0.038 eggs per beetle up to 60 eggs per metre suggests that *D. bellulus* are essentially unable to find eggs until the prey densities exceed 60 eggs per metre. The fact that this range of *H. punctigera* egg densities conservatively covers the prey levels encountered in the field suggests that the potential of this predator to contribute to the control of *H. punctigera* is low. Densities of *D. bellulus* were commonly 0.5 to 1 per metre but up to 3.5 per metre in absolute terms on cotton treated with soft-options or some conventional insecticides (Chapter 5). Unsprayed plots reached densities of 7.5 beetles per metre but even at this level only 0.285 *H. punctigera* eggs per metre per day would be consumed by this species at the rates recorded during this cage study.

The maximum predation rate of 2.3 eggs per metre only occurred in the presence of unrealistically high egg densities and even then was much lower than the average of 6.3 eggs per day recorded for this species in laboratory trials. If this approximates the highest predation rate possible by this species on cotton plants, even in the absence of alternative prey and in the presence of exaggerated *H. punctigera* egg densities, it again indicates that the potential impact on eggs is very low.

The low predation rates recorded in this study are consistent with reports by Hutchison and Pitre (1983) who found a type I functional response for *Geocoris punctipes* on *Heliothis virescens* (F.) eggs using field cages in Mississippi cotton over a realistic range of field prey densities (2 to 5 eggs per metre). Each predator consumed around 0.11 to 0.24 eggs per day. At the highest density used in this trial of 2 to 3 predators per metre, which was used to represent a relatively high but 'normal' predator density, the maximum prey consumption was 0.46 per metre when only *G. punctipes* adults and *H. virescens* eggs were present.

In the present study, the data were not comprehensive enough to identify the functional response of *D. bellulus* on *H. punctigera* eggs in field cages. However the most likely position and relevance of the critical points of the response might be indicated. If the maximum rate of 2.3 eggs per predator represents the plateau region of a type II or type III functional response

the limitations to predation rate are clearly not due to handling time. *D. bellulus* were observed to require only 20 seconds to consume a *Helicoverpa* egg during the laboratory trials. Searching efficiency or decisions to 'search or sit' may therefore be more important in large searching arenas. Also, if these data have an underlying type III functional response the inflection of the curve would appear to occur between 60 and 80 eggs per metre and therefore be irrelevant to the maintenance of this prey to below thresholds in the vicinity of 5 eggs per metre. Therefore the relevance of an accelerated predation rate, implying negative feedback for controlling *Helicoverpa* spp. outbreaks at greater that 60 eggs per metre is of limited practical interest while current yield expectations and economic thresholds persist.

It has been suggested that predators locate newly laid eggs with greater efficiency than older ones (McDanial and Sterling 1979, Hutchison and Pitre 1983). The counting processes in the present experiment prevented the presentation of newly laid eggs to the predators. Hutchison and Pitre (1983) artificially placed and marked much lower numbers of eggs in their cages (6 to 18 per cage) and thereby avoided the time required by the researchers to search the plant canopy for naturally laid eggs. Predators could therefore be introduced prior to or within minutes of seeding the cages. The reliance by Hutchison and Pitre (1983) on wetted eggs to re-adhere to the cotton plants after being removed from the artificial oviposition material (polyester) was a cause for concern so their method was not employed during the present study. Allowing females to choose oviposition sites and lay naturally within the cages also avoided the possibility that the artificial method might place eggs in unrepresentative positions or lose important cues used by the predators to locate the eggs.

Although ample time (4 days) was allowed for the lethal effects of the insecticides to disappear before the test insects were introduced, the possibility of sublethal influences of insecticide residues on the behaviour of the predators or prey cannot be dismissed entirely. Ideally the cages could be hand picked and or vacuumed to produce insect free plants. Simply augmenting existing populations of predators might also avoid this problem and retain the influence of alternative predators and prey. Unfortunately there was not enough time or labour available to explore these options.

7.4 Field Cage Experiment 2: The Predation of *H. punctigera* larvae by *Dicranolaius bellulus* adults and *Mallada signata* larvae.

7.4.1 Introduction

In many cases the generalist predators which inhabit cotton fields are capable of attacking early instar *Helicoverpa* spp. larvae (Room 1979a). An appreciable removal of this stage would be beneficial to pest control before further damage is inflicted by the more rapidly growing later instars. The aim of this trial was to measure the predation rates of *Dicranolaius bellulus* adults and *Mallada signata* (Chrysopidae) larvae on *Helicoverpa* spp. larvae over their first week of development, at various prey densities in a realistic search arena.

7.4.2 Materials and Methods

The methods were similar to those for Field Cage Experiment 1. However by this stage in the season the cotton was 65 cm tall and had reached mid-flowering. The cages were placed over new sections of row and the plants cleared of resident arthropods using pyrethins (as described in Section 7.2.2) three times at three day intervals until four days prior to the introduction of *Helicoverpa* spp. larvae. Field captured *H. punctigera* were allowed to mate and lay in insectary containers. Four thousand eggs were placed on artificial diet (Teakle and Jensen 1985) in 200 x 20 ml clear plastic containers (i.e. 20 eggs/container) to ensure enough larvae were available. On the morning of hatching the neonate larvae were individually transferred to the caged cotton plants using a small, water-moistened paint brush. The larvae were distributed evenly across the top third of the plants in shaded positions near new growth or within terminals, and allowed one night to locate feeding sites. These positions were selected because they were observed by Wilson and Waite (1982) to be the sites most frequently chosen by field-hatching larvae. Twenty four cages were prepared, each with one of four *H. punctigera* densities; 20, 40, 60 or 120 larvae/cage. This produced six replicates of the four *H. punctigera* densities. Three predator treatments were allocated to each density; controls (no predators), 30 *D. bellulus* and 30 first instar *M. signata* larvae. Therefore there were two replicates of every possible prey/predator density combination.

The following morning (8-30 am) the predators were introduced to the cages by sprinkling them onto the plant canopy from an open container above the canopy. The predators were either 30 lacewing larvae *(Mallada signata:* insectary reared by Biological Alternatives at Stahmann Farms Inc., (MFS 2058, Pallamallawa, New South Wales 2399.) or *D. bellulus* (field collected). The cages were left for six days to allow the surviving *H. punctigera* time to develop to a size which could be confidently found during destructive plant searches. These searches consisted of cutting the plants into 10cm long sections within the cage and systematically searching each section before discarding them from the cage. All flowers and fruiting structures were split and the cage thoroughly scanned to complete the search. The larvae were between 10 and 15 mm long at this stage of development (ca. third instars).

7.4.3 Results and Discussion

Predation of *H. punctigera* larvae increased linearly with prey densities from 20 to 120 larvae per row metre of cotton over the six day period of exposure to the predators. 30 beetles per metre increased the disappearance of *H. punctigera* larvae to 62% from a background mortality of 40%. *M. signata* larvae introduced as 1st instars produced a similar impact. Therefore the average additional mortality of *Helicoverpa* larvae was 22% greater when 30 beetles or 30 lacewing larvae were present. The mortality was linear for both predatory species (i.e. a type I functional response) which suggests that the predators were not limited at these prey densities by satiation or handling time (Figure 7.7). Although petri dish trials by Room (1979a) indicated that each *D. bellulus* could consume 1.7 to 2.1 larvae each per day, the maximum rate in these trials was 0.11 larvae per beetle per day. As in the case of predation on eggs (Section 7.2), the effects of size and complexity of the search arena on the behaviour of the predators probably explains these differences.

The per capita rates of consumption by these predators were; for *D. bellulus* 0.13 to 0.80 prey / individual over the five days or 0.03 to 0.16 prey / individual / day; and for *M. signata* 0.14 to 0.87 prey / individual over the five days or 0.03 to 0.17 prey / individual / day. Therefore even at these exaggerated prey densities, relative to those normally encountered in the field, the predators exhibited very low rates of consumption. This suggests that these predators are not contributing very much to the removal of *Helicoverpa* spp. in cotton crops. Could it be that the predators we have so far looked at are simply ineffective ones?

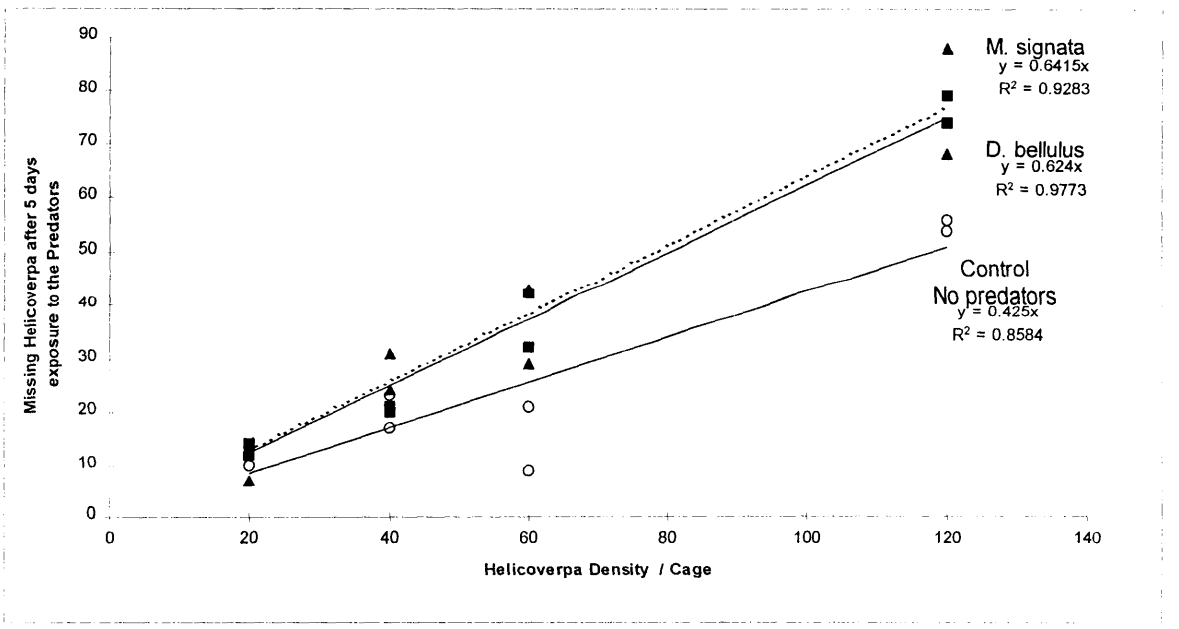

Figure 7.7 The rate of disappearance of *Helicoverpa punctigera* larvae from field cages seeded with various densities of first instar larvae and exposed to zero predators (o), 30 *Dicranolaius bellulus* (♦) or 30 first instar *Mallada signata* (▪) for 6 days.

The selection of *D. bellulus* for experimentation was based on its relatively large size and abundance, and its performance in Petri dish studies (Section 7.1). Although these characters are general indicators of overall predatory capacity they may not necessarily correlate well with predation on *Helicoverpa* spp. in the field. Therefore, since these field cage studies have provided no evidence to the contrary, it is likely that *D. bellulus* is a relatively inefficient predator of *Helicoverpa* spp.

On the other hand *M. signata* was originally thought to be a good candidate for an efficient predator. This was thought to be demonstrated practically, if indirectly, by the successful cage trials of Ridgway and Jones (1968) using the American counterpart, and theoretically because they were less likely to use 'sit and wait' tactics for survival (Wiedenmann and O'Neil 1992, discussed in the literature review Chapter 1, Section 1.5). The fact that similar predation rates were exhibited by both these predators in the present field cage studies suggests that low per capita rates of predation may be quite general. If this is so the total abundance of predators becomes more important to the predatory mortality of *Helicoverpa* spp. than variations in the efficiency of predatory species. The predator densities used in these trials were generally around five times those recorded under conventional or soft option

strategies (Chapter 5), and even so were found to be insufficient for control. The potential to exceed these levels of predator abundance by augmentation, conservation or manipulation remains unanswered, but it seems clear that very substantial increases will be needed.

7.5 Field Cage Experiment 3: Predation of *Helicoverpa punctigera* Larvae by Endemic Predators in the Presence of Alternative Prey

7.5.1 Introduction

As discussed in Chapters 2 and 6, the presence of alternative prey has the potential to markedly modify the predation rate on a target prey species. Therefore the aim of this section was to estimate the rate of predation on *Helicoverpa* spp. by three of the most commonly collected endemic predatory arthropods in Australian cotton in the presence of alternative prey.

7.5.2 Materials and Methods

This experiment was conducted using the same methods as Field Cage Experiment 2 (Section 7.3), with the following differences: two additional predators were included, various levels of alternative prey (cotton aphids, *Aphis gossypii*) were present and the cotton plants had reached a later stage of development (boll burst).

The cages were seeded with neonate *H. punctigera* larvae as in Experiment 2, followed by the same methods of predator release and survival counts of the *H. punctigera* larvae. In addition, scoring of the aphids to estimate the population changes in the alternative prey was carried out. The aphids were scored by recording the percentage infestation of plant terminal shoots per cage at the beginning and end of the experiment. Two further predatory species were included in this trial: *Coccinella transversalis* (Coleoptera, Coccinellidae) at a density of 30 per cage and *Nabis kinbergii* (Hemiptera, Nabidae) at 15 per cage. All the predators were field collected, kept in plastic containers for one day and offered only moisture via cotton rolls before introduction to the cages. *N. kinbergii* were held singly because they readily attacked and killed each other when placed together. The other species were kept *en masse*.

7.5.3 Results and Discussion

There was no discernible additional predation of *H. punctigera* larvae by any of the predator treatments above the background mortality of 70% recorded for the control cages (Figure 7.8). However, the aphid populations were reduced by 15 and 10% by *C. transversalis* and *D. bellulus* respectively (Figure 7.9). The statistical analysis of aphid abundance is presented in Appendix 7.2.

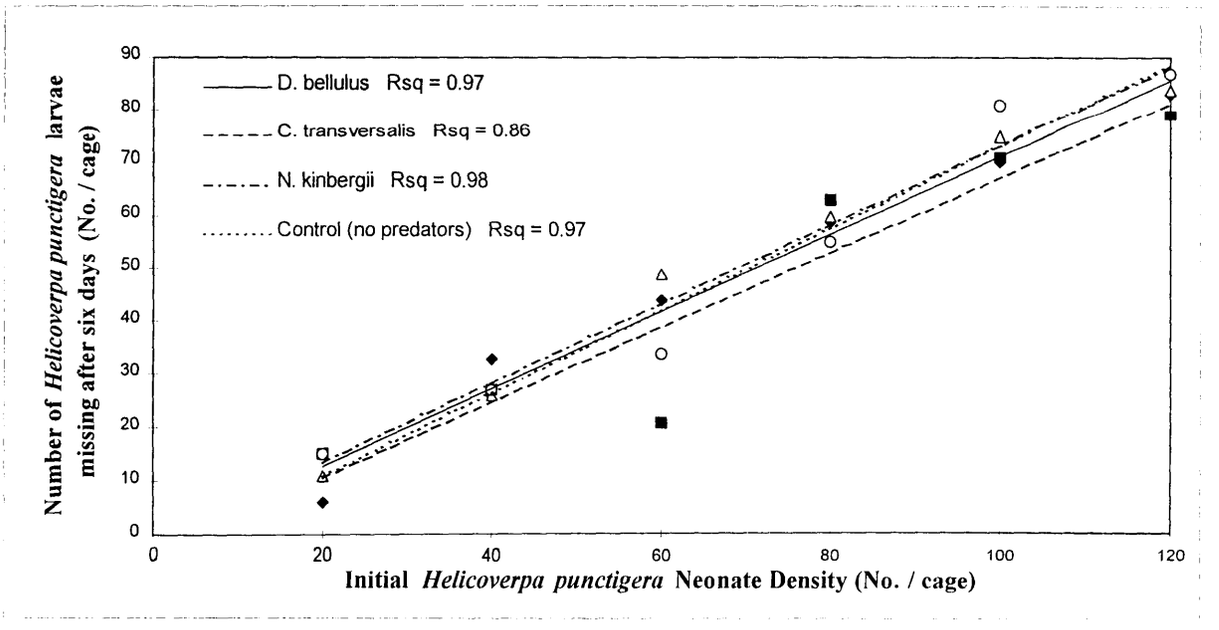

Figure 7.8 The number of *Helicoverpa punctigera* larvae missing from each field cage after six days exposure to 30 *Dicranolaius bellulus* (♦), 30 *Coccinella transversalis* (■), 15 *Nabis kinbergii* (Δ) or with no predators (control, o) after initially seeding cages with 20, 40, 60 or 120 neonate *H. punctigera.*

There was low variability in *Helicoverpa* sp. mortality overall treatments. High background mortality is commonly observed in trials on predation of lepidoptera, especially during the egg to 2nd instar stages (Titmarsh 1992). The reasons for such a high background mortality during this trial might include poor weather or an unsuitable plant stage. The lack of detectable additional mortality, beyond natural mortality, in the presence of alternative prey, suggests that the predators may have switched to the more abundant aphids. This may limit the value of predators because aphids are frequently present in maturing cotton crops at the time

when the crop is most vulnerable to the effects of *Helicoverpa* spp. Furthermore, it is sometimes suggested that if the impact of predators is generally low they would be most useful when their contribution bolsters a substantial mortality by other factors. The results in this section imply that occasions of high background mortality may often coincide with conditions of low additional predatory impact.

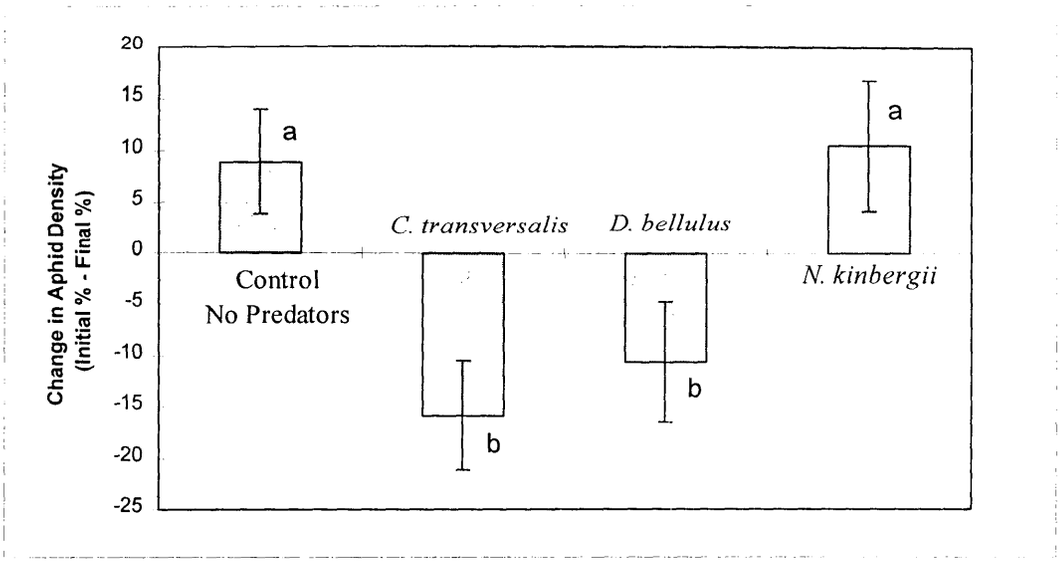

Figure 7.9 The change in alternative prey density (*Aphis gossypii)* in the four predator treatments over the six days of the third cage trial. The error bars are the standard error of the means (n=6). Treatments marked with the same letter were not significantly different from each other at the 5% level (LSD).

7.5.4 Overall Conclusions for the Field Cage Series of Experiments

The discrepancy between the field estimates and the petri-dish trials (Section 7.1) indicates that handling time is unlikely to be the rate limiting factor under field conditions, and re-establishes the importance of information on searching behaviour in understanding predation rates. In field cages *D. bellulus* consumed up to 2.3 *Helicoverpa* spp. eggs per beetle per day, but this was at unrealistically high prey densities (60 to 170 eggs per metre). At realistic densities of less than 20 eggs per metre the rate was less than 0.2 eggs per beetle per day. The general similarity of these results with those of Hutchinson and Pitre (1983) with *G. punctipes,* and Wiedenmann and O'Neil (1992) with *Podisus maculiventris* (Say) suggests that useful

generalisations may be possible for describing the prey consumption rates of the generalist predators found in cotton. The discovery that predatory species in Australia exhibit similar patterns of prey consumption to those commonly found in annual crops in America suggests that these predators behave according to their requirements for survival under low prey densities, not according to their potential prey consumption (O'Neil 1989). This may better explain predator behaviour and the real limits to predatory impact, rather than the more remote interpretation of laboratory derived functional response analysis.

Alternative prey, crop size and the age structure of the predator or prey population have the potential to greatly affect the predation rate on a particular target pest. Understanding these in realistic conditions will be important to appropriately including predation in pest management decisions. For example, if crop growth effectively dilutes prey density to levels where predators are less likely to find them, some function of crop size may be required for modifying predator/prey ratios.

Different sized *Helicoverpa* spp. larvae are prey to a different range of predator species or size of predator. Awan (1985) demonstrated that the anti-predator ploys of *Helicoverpa* larvae in response to *Oechalia schellenbergii* varied from the desperate escape tactics of first instars, which roll and fall from the plant, to the aggressive retaliation by the 5th instars which were rarely taken by the predator. Clearly a large portion of the predators commonly found in cotton fields become ineffective against later instar larvae; for example *Orius* spp., *Geocoris* spp. *Coccinella* spp. and *Dicranolaius* sp. The discovery of large larvae should modify a predator prey ratio based on predator efficiency against early instars.

The low but variable linear responses exhibited throughout these trials in field situations suggests that we need to understand the factors affecting the impact of predators better before reliable predator to pest ratios can be developed to improve the use of predators in Australian cotton. Most likely, practical implementation of such ratios will preceed accurate assessment, so caution is required. The experiment described in Section 7.4.3 indicates that if the *Helicoverpa* spp. threshold is deemed to be around 2 larvae per metre a simple predator to prey ratio of 2:1 will not reduce a population of 10 larvae per metre below this figure. Note that when 15 *Helicoverpa* spp. larvae per metre were present, the presence of 30 *D. bellulus* per metre still allowed 6.5 to survive. This is well above threshold.

The high variation in 'background' mortality between the experiments suggests that changes in environmental or plant factors also have a large affect on the level of the pest mortality. The lack of apparent predation in Experiment 3 demonstrates that although predation may be occurring it is sometimes small compared to overall mortality. Cold conditions may have caused both higher pest mortality and a reduced effort by the predators (Appendix 5.5). The reduction in aphids in this instance, however implies that predatory activity was not curtailed but switched. If switching was the reason for reduced *Helicoverpa* spp. predation then the uniform effect across the various aphid densities implies that all these densities were able to totally distract these predators (Figure 7.9).

Similar cage trials in South Africa by van den Berg and Cock (1993) found appreciable effects of predation (65% by ants and Anthicorids, *Orius* sp.) on *H. armigera*. These studies had very high levels of predators by Australian standards (> 60 Anthicorids and/or ants per metre). The presence of ants also corresponds to reports of effective predation against *Helicoverpa* spp. in the USA (McDaniel and Sterling 1979). These results were from raingrown crops where the habitat of many ant species is not disrupted by irrigations. McDaniel and Sterling (1979) attributed high rates of *Helicoverpa/Heliothis* spp. egg and larval mortality to the red imported fire ant (*Solenopsis invicta* Buren). More recent studies by van den Berg and Cock (1995) in open field treatments, using insecticide exclusion and sticky barriers, found background mortalities of 96.4% to 99.7%. These levels of background mortality precluded the measurement of any additional predatory effect. This result is similar to the third cage trial reported in Section 7.4 of this thesis.

Overall, predators have been shown to take significant numbers of prey (pests) in field cage trials if high densities of predators are present and background mortality of the pest is low. However, for poorly understood reasons, these rates are highly variable. The possible explanations for this are: the presence and abundance of alternative prey; variable environmental conditions; differences in the predator development stage or expression of various plant defences.

What is clear, however, is that if these cage trials reflect events in the open field then endemic levels of the Australian predators tested offer only relatively small and unpredictable contributions to the mortality of *Helicoverpa* spp., given our present level of understanding and our present levels of acceptable damage.

As the impact of generalist predators over realistic prey densities appears to be linear, the only avenue to impose control on abundant pests is to encourage greater numbers of predators. There remains the prospect that many more predators will appear with the introduction of transgenic cottons and the concomitant reductions in the use of insecticides. However, the discovery by O'Neil (1989) that predators trade off predatory effort for persistence suggests that there may be avenues for manipulating predatory behaviour to better effect. For example, manipulating predators with chemical cues to remain in a searching mode well beyond the limits of their instincts would appear beneficial.

Chapter 8

Experimental Section

8. Serological Methods for Assessing the Predation of *Helicoverpa* spp. by *Dicranolaius bellulus*

Perspective

In the light of the difficulties posed by many methods of studying predation which have been highlighted in previous chapters of this thesis, there is a clear need for a method of determining predator impact which does not rely on dubious extrapolations from laboratory studies to the field, or on techniques which might interfere with the environment or behaviour of predators. Serological detection of predation potentially offers such a technique.

This chapter describes some initial studies aimed at investigating some of the methods which might be used for a serological study of predator impact. These were started late in the course of the project, so only preliminary experiments of the laboratory techniques which might be used were completed. This research is being continued by another researcher at the University of New England (M-L. Johnson, pers. comm. 1996).

8.1 Detecting Prey in the Gut Contents of Predators

8.1.1 Introduction

Techniques for detecting the presence of a particular prey within suspected predators were recently reviewed by Sunderland (1988) and are broadly divided into two categories:
i) Artificially placed markers, and ii) Naturally occurring markers.

Using artificial markers involves mark and recapture techniques. The prey are collected and marked (possibly with a dye) and then released into the field. The dye is assumed to be ingested during predation so that with subsequent sampling of the predators, the individuals

which have fed upon the marked prey can be identified by the colour of their gut contents. Early predation studies have used liquid or powdered dyes. For example, Hawkes (1972) successfully used fluorescent dye to show lepidopteran (*Tyria jacobaeae* (L.)) egg predation by *Forficula auricularia* (L.) (Dermaptera). The colour or taste of the dye may affect prey acceptability and, with juvenile stages, the dyes can be lost during moults (Room 1977). The most sophisticated of the artificial marker techniques is radiotracing (Room 1977, McDaniel and Sterling 1979 and 1982, and Thead *et al.* 1987), which utilises radioisotopes to label prey.

Naturally occurring marker techniques take advantage of natural characters of the prey which persist for long enough in the predator gut to be recorded. The proportion of a predatory population which have recently fed on a target species can be identified without prior disruption to the conditions of the site or treatment. This avoids many of the disruptive activities inherent in assessing predator impact by most of the methods discussed in the previous chapters. The most direct technique of this kind is to sample predators and search their gut for identifiable pieces of prey, usually durable sections of exoskeleton (Sunderland *et al.* 1987). Many prey, however, cannot be identified this way because the predators (especially spiders and true bugs) take fluid meals or macerate the prey into unidentifiable fragments. The more sophisticated natural marker methods which can overcome this problem involve identifying the prey remains at the molecular level. Serological methods have dominated this approach although potentially DNA marker techniques could be used (Sunderland *et al.* 1987, Stuart and Greenstone 1990). The former are also referred to as immunological methods or immunoassays.

8.1.2 Radiotracers

This method involves labelling prey with radioisotopes, usually with ^{32}P, and releasing them in the field. Subsequently predators are collected and those which register radioactivity have gained radioactive elements from the prey and are assumed to have fed on them. The radioactivity is quantitatively detected using Geiger counters or scintillation/disintegration counters. Allowance must be made for the ongoing physical decay of the radioactive source using half life calculations (McDaniel and Sterling 1979). Portable Geiger instruments can also be used to locate radioactive prey in the field, including *Helicoverpa* spp. eggs which may

have been dislodged from cotton leaves rather than removed by predators (McDaniel and Sterling 1979). Several authors have established methods for dosing female *Helicoverpa* spp. adults with ^{32}P in order to produce radioactive eggs or young larvae (Room 1977, McDaniel *et al.* 1978). Detectable doses which do not significantly alter the insects life history have been established along with the average expected dose transferred to eggs by the female, either fed or injected with specific quantities of radioisotopes.

Some authors claim that radiotracer methods give a quantitative estimate of the number of prey taken by a predator (McDaniel and Sterling 1979, Gravena and Sterling 1983). However, several difficulties, commonly associated with mark-recapture techniques, question this:

- whether the labelled prey is distributed, behaves and survives realistically in the field.
- whether secondary predation can be distinguished from primary predation.
- whether predators ingest a constant proportion of the radioactivity with each meal.
- whether enough prey are labelled and predators collected for statistical confidence.
- whether prey items are shared (particularly amongst social insects such as ants)

Attention has focused on naturally occurring markers to avoid the need to collect, mark and realistically distribute prey. Immunological assays achieve this by identifying specific naturally occurring proteins.

8.1.3 Immunological Assays

These methods have been available in one form or another for about 40 years and early work has been reviewed by Boreham and Ohiagu (1978). However, major improvements to the specificity of these tests have recently been achieved with the introduction of techniques to produce monoclonal antibodies (Greenstone 1996).

Essentially the immunological methods take advantage of the mammalian immune response. Antibodies can be produced which specifically attach to particular insect proteins. For example, if a small amount of haemolymph from a *Helicoverpa armigera* larva is injected into a mouse, the lymphocytes of the mouse will generate a wide array of antibodies, each

capable of binding to particular sites on the *H. armigera* proteins (antigens). Some of these antibodies will bind to proteins which are present in many insects, but others may be totally specific to *H. armigera,* possibly even to the particular sex or life stage of that insect.

Many early attempts at immunoassays were confounded by the production of this general array of antibodies. Antiserum collected from inoculated mammals contained antibodies which were produced by many lymphocytes and therefore called polyclonal. Often, the polyclonal antiserum was not specific enough because at least one of the antibodies in the mixture bound to non-specific proteins or sites (Greenstone 1996).

It is possible to isolate and clone a particular lymphocyte, and collect the particular antibody that it is producing. These are called monoclonal antibodies and, because they are being made separately, can be selected for their specificity. This step has solved many of the specificity problems associated with the early immunoassays, however it may also raise unusual challenges because rare molecules which have quite unusual secondary characteristics may be selected. This focuses the problems of specificity on screening for an effective antibody amongst the many produced by the different lymphocytes (Kemeny 1991).

The antibody/antigen binding must produce a measurable effect so that the presence of the antigen (eg. *Helicoverpa* spp. protein) can be determined. The red-cell agglutination reactions are an example of where the antibody/antigen linkage produces a visible effect. There are many ways of developing this 'signal'. Some simply produce a visible colour change to give presence/absence results. Others are more quantitative, using spectrophotometry to measure the extent of colour change within a solution. Usually another antibody which binds to the mammalian 'end' of the specific monoclonal antibody is used. It possesses a pre-established link to an enzyme which is required to convert a colourless substrate to a coloured product. The general name give to this procedure is ELISA (Enzyme-Linked ImmunoSorbent Assay) (Kemeny 1991).

Given that an antibody which specifically binds to *Helicoverpa* spp. is located, tests must be carried out to determine whether the reaction will occur in the gut contents or completely homogenised material of the suspected predator. Further tests are needed to establish the size of the meal which can be detected for each predator and how quickly the detectable proteins are degraded during normal digestion rates of the insect. Complications which can arise are:

1) The antigenic site is readily digested by the predator so that the residence time for detection is too short. Lovei *et al.* (1990) and Sopp and Sunderland (1989) point out many of the difficulties associated with the decay of the antigen in the gut of the predators. Significant variation can occur due to species of predator or prey and concomitant dietary components or metabolic rate. This implies that the life stage of the insect, its water intake or dietary mix could all effect the residence of detectible levels of antigen in the gut contents.

2) There may be proteins within the predator which bind the antibodies. This problem of lack of specificity has often been overcome by developing monoclonal antibodies, but any of the so-called specific bonds used in the colour development steps could potentially be disrupted by compounds of predator origin.

3) The preparation of the predators, eg. freezing, thawing, life stage etc. may alter the specificity of the reactants.

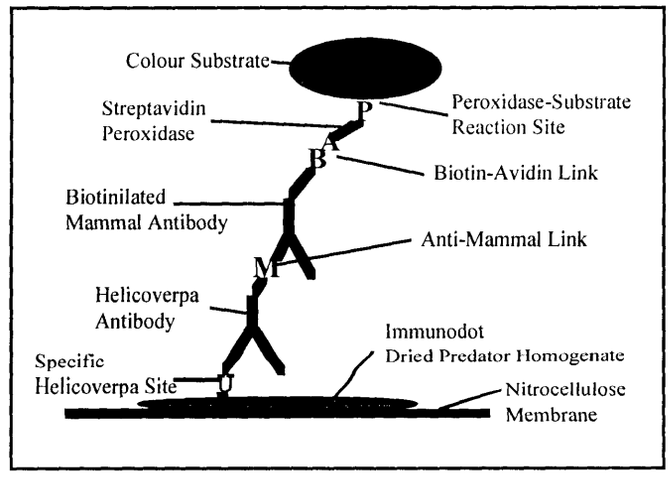

Figure 9.1 A schematic diagram of the ELISA sequence of reactions to identify the presence of specific proteins in the homogenised components of a suspected predator. Each component of the reaction chain has specific binding sites which when added in the correct sequence generate a colour reaction. If the antibody has bound to the *Helicoverpa* sp. protein at 'U' each sequentially added component binds to form a chain with peroxidase providing the colour reaction. If not the components fail to form the chain because each is washed away during the washing steps, leaving no peroxidase for the colour change.

Quantifying Predation Using Serological Methods

Although serological techniques are capable of determining the amount of antigen within a sample to a high degree of accuracy, quantification of the prey ingested by a predator remains an elusive goal (Greenstone 1996). The most obvious unknowns which affect the level of antigen found in predator gut contents are meal size and time from feeding but several other confounding factors may also be important, depending on the aims of the study (Lovei *et al.* 1990, Greenstone and Hunt 1993 and Sunderland 1996).

The contribution of a predator to pest reduction requires the measurement of predation which is additional to other causes of mortality. Serological tests do not distinguish between feeding on moribund prey or carrion, and living prey. Furthermore they do not indicate wounding or wasteful killing (i.e. attacks which fail to result in a meal) but nevertheless lead to the death of the prey. Partial consumption, when only a fraction of the antigen available in a prey item is consumed or pre-oral digestion, the digestion of prey before ingestion (typical of spiders and true bugs) also dislocate the correlation between antigen assay and the number of prey items killed or consumed (Sunderland 1996). The results therefore indicate the presence or absence of predation, not the amount.

In situations where the assumption can be made that a positive indication of predation represents a very low number of predatory events; either because the prey abundance is very low or because one prey item is consumed very slowly, statistical estimates of predation rates are possible from such presence-absence records, but this assumption is often incorrect (Greenstone 1996). Predation on *Helicoverpa* spp., however, may accommodate this assumption because the prey occurs in relatively low densities and is consumed quickly by most predators. Therefore the serological approach was explored in this study.

The experimental sections of this chapter follow a course of experiments which were designed to develop serological techniques to detect *Helicoverpa* spp. predation by *Dicranolaius bellulus*.

8.2 Serological Experiments

8.2.1 Introduction

Dr. Stephen Trowell of the CSIRO (Commonwealth Scientific and Industrial Research Organisation) at Canberra (ACT) had produced a monoclonal antibody (marketed in the LepTon™ Kit by Abbott Australasia Pty. Ltd., P.O. Box 304, North Ryde, NSW. 2113) to distinguish between *H. armigera* and *H. punctigera* in the field using egg or larval squashes (Trowell *et al.* 1994). Many antibodies of various specificity had been produced during the development of the Lepton™ kit and this presented an opportunity to search a range of these antibodies for one which could detect *Helicoverpa* spp. in the gut contents of suspected predators using ELISA techniques. Testing began using *Dicranolaius bellulus* and the first step was to devise a convenient ELISA using a range of concentrations of the antibodies supplied by the CSIRO on individuals which were known to have fed or not to have fed on *Helicoverpa* spp. eggs.

8.2.2 General Methods

The methods were those presented for the simplified immunodot assay published by Greenstone and Trowell (1994).

Briefly the steps involved in the assay were:

1) Prepare a liquid sample (homogenate) of the suspected antigen source (predator or prey) by macerating the complete insect in a 1 ml glass mortar and pestle in 0.3 ml of a buffer solution. Where the antigen source is very small (for example a single insect egg) or if a small concentration of antigen is expected, the source can be directly smeared onto the membrane test sheet.

2) The homogenates are kept cool (4^0C) until 0.01 ml portions are spotted onto a sheet of nitrocellulose membrane using a micro pipette and allowed to dry. The contents of the sample bind to the membrane and each dot forms the site of the ELISA reactions.

3) The membrane is immersed into a series of solutions each followed by an incubation and a washing step in strict sequence.

These steps are:

a) Add a solution containing the *Helicoverpa* spp. antibody.

i.e. Anti-B (PabB rabbit polyclonal) or Anti-70 (Mab70.5 mouse monoclonal) which binds only to dots containing antigen).

Incubate for approximately 30 minutes at 36^0C.

Wash by rinsing in clean tap water.

b) Add a solution containing biotinilated anti-rabbit or anti-mouse antibody. (This only binds to dots containing the *Helicoverpa* spp. antibody and incorporates a biotin molecule).

Incubate for approximately 30 minutes at 36^0C.

Wash by rinsing in clean tap water.

c) Add Streptavidin peroxidase (This has an avidin site which strongly bonds to biotin, i.e. only those dots which have retained anti-mammal antibody).

Incubate for approximately 30 minutes at 36^0C.

Wash by rinsing in clean tap water.

d) Add colour reaction substrate (colour develops only where peroxidase is bound).

Observe at room temperature for colour development (incubation may be required).

In general, increasing incubation periods or increasing the concentrations of any of the reactants can increase the strength of the colour development. However, the aim of the method is to produce a distinctive difference between the predators which have fed or not on the prey. Therefore it is important to amplify the specific indication of *Helicoverpa* spp. antigen (the signal) while decreasing the non-specific colour development (the noise). The signal to noise ratio is altered by pretreatments with compounds which suppress endogenous peroxidases (phenylhydrazine or hydrogen peroxide) or by coating non-specific proteinacious binding sites with a blend of proteins of non-insect origin (blotto solution eg. reconstituted powdered milk).

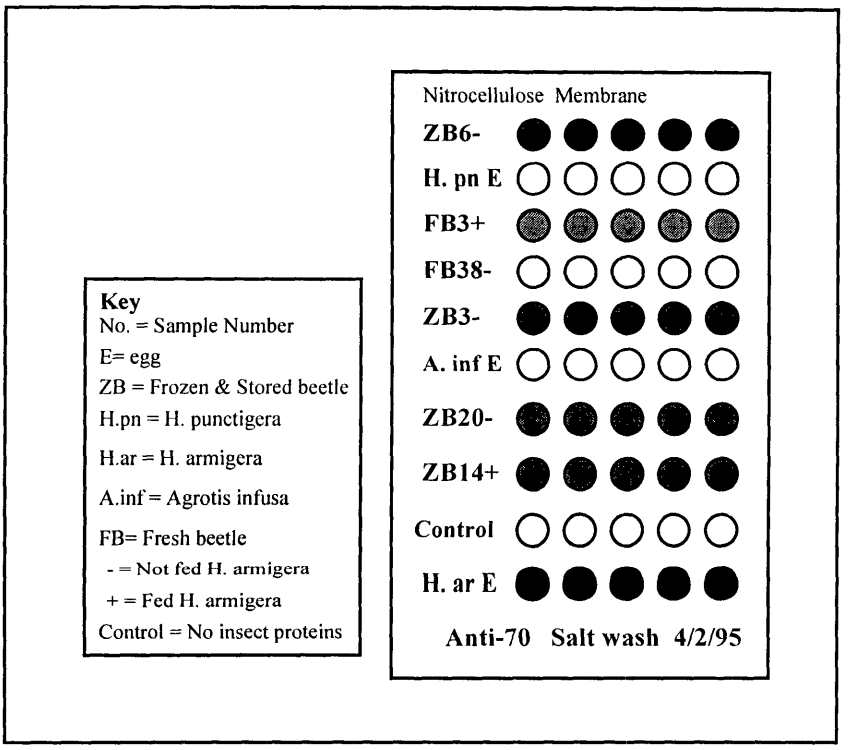

Figure 9.2 A typical layout of the nitrocellulose membrane used in the ELISA procedures and a simulated colour result. Each dot is a 0.01ml test droplet which exhibits a colour response of an intensity relative of the concentration of the *Helicoverpa* spp. antigen or cross reactive compounds present in that sample.

8.3 Experiment 1: Using Antibody-B to Detect *Helicoverpa armigera* in the Gut contents of *Dicranolaius bellulus*

8.3.1 Aim

The aim of this experiments was to select a promising antibody for predator analysis from the those offered by the CSIRO, and establish the details of an ELISA protocol particularly for predator analysis. This experiment tested Anti-B, a polyclonal antibody expected to be specific for *Helicoverpa* spp. in general.

Tests were also included to identify the concentration of antibody which registered the most distinctive positive. Phenylhydrazene and/or hydrogen peroxide, for removing non-specific colour development, were also included to investigate the potential for improving the contrast between the positive results and non-specific reactions.

8.3.2 Methods

The ELISA methods are described in the general methods section and include a series of dilutions of anti-B (no antibody (control), 1:1000, 1:5000 and 1:10 000). These assessments were also conducted with and without phenylhydrazine and/or hydrogen peroxide.

Antigen Sources and Treatments

Living field collected *D. bellulus* were placed in 500ml plastic containers (20 per pot) and offered moisture via wetted cotton rolls but otherwise starved for two days prior to the allocation of treatments. On the day of the assay beetles were collected from these containers and placed into petri dishes containing newly laid *H. punctigera* eggs (laid on the previous night in laboratory cultures) and constantly observed until an egg was consumed. If a beetle did not feed in five minutes it was replaced with another. Eventually four treatments were established:

1) +B, Beetles fed on 1 egg and immediately homogenised in 0.3 ml buffer.

2) - B, Beetles not fed on eggs, homogenised in 0.3 ml buffer.

3) E, Single *H. armigera* egg homogenised in 0.1 ml buffer.

4) CE, Single *H. armigera* egg squashed directly onto the membrane.

Endogenous Peroxidase Test

A brief test was conducted to establish the level of endogenous peroxidases in the predator homogenates. If present a strong colour reaction will occur regardless of the presence of the specific antigens, thus invalidating the test. For this test the colour development steps were followed without any antibody additions.

H. armigera eggs showed no coloration but the +B and -B treatments showed an equal but slight colouration. This degree of colour was not expected to interfere with the prospects of using this test. Therefore the amount of endogenous peroxidase was considered acceptable to continue using this series of colour development reactions.

Detection of H. armigera antigen

There was a clear distinction between +B and -B treatments but this was slight and only visible at the 1:5000 antibody dilution. The *H. armigera* egg treatments were easily distinguishable from the beetle treatments. The strongest colour development (a red dot) was for the smeared egg but the homogenised egg was also a distinct positive.

The addition of phenylhydrazine did remove some background colouration but did not appear to help accentuate the distinction between the beetle treatments. A second replicate of this trial, including a set of treatments in which both phenylhydrazine and hydrogen peroxide were removed, showed a much stronger background colour development but did not appreciably alter the distinction between the treatments. Again the 1:5000 dilution of antibody showed the strongest distinction between the +B and -B treatments.

The conclusions from this section were:

1) Anti-B showed good potential for distinguishing between *D. bellulus* which had or had not recently predated on fresh *H. armigera* eggs.

2) The inclusion of phenylhydrazine and peroxide is debatably beneficial for improving the signal to noise ratio therefore these substances were retained in the following methods.

3) The best antibody dilution of those tested was 1:5000

The following experiments continued with Anti-B because of its immediate potential.

8.4 Experiment 2. Using Antibody-B to Detect *Helicoverpa* spp. in Field Collected *Dicranolaius bellulus*

8.4.1 Introduction

Many predators had been sampled and stored in liquid air from the previous cotton season. Therefore it was necessary to test if this treatment had affected the samples. The following experiment was conducted using the general methods outlined above. The previous experiment had shown the potential to identify *H. armigera* in the gut contents of recently fed

D. bellulus. The availability of other noctuid species being cultured at the University of New England also allowed limited exploration of the specificity of Anti-B on other species.

8.4.2 Methods

Preparation of Predators

Adult *D. bellulus* were taken from a maintenance culture (originally field collected from the Australian Cotton Research Institute, Narrabri, NSW. in January 1995). Approximately 60 beetles of mixed sexes were collected and subjected to two days starvation in the 500 ml plastic containers with access to wetted cotton rolls for moisture. Thirty eight beetles were placed singly in petri dishes and offered one *H. armigera* egg on a small piece of paper towelling. Moisture was also available in the form of wetted cotton rolls. The predators were checked at regular intervals for feeding and the time which elapsed before they were stored was recorded. The predators were placed individually into polypropylene centrifuge tubes (1 ml) and immersed in liquid air for snap freezing and storage. This produced fed and unfed beetles stored at 0.5, 1, 6, 12, 24 and 48 hours after feeding.

On the day of the assay homogenates were prepared from predators individually removed from the liquid air. Homogenates of *H. punctigera* and *Agrotis infusa* (Boisduval) (Noctuidae) eggs and *H. armigera* larvae were also tested.

8.4.3 Results and Conclusions

All the noctuid samples gave an equally strong colour development. This showed that Anti-B not only recognised *Helicoverpa* spp. antigens but also those of *Agrotis infusa*. This raises suspicion that the antigens recognised by Anti-B may be quite general throughout the noctuid family. This aspect may not necessarily affect the use of this antibody for study of predators on cotton because species other than *Helicoverpa* spp. are often only a small proportion of the noctuid presence and in any case may represent useful predation because all damage the crop. A more serious concern is that Anti-B may also recognise insects in different families or even different orders, though this has not been investigated in this thesis.

Another disturbing result was that all the beetle samples (fed and non-fed, stored early or late) exhibited an equally distinctive colour development although lighter than the noctuid dots. With reference to the previous experiment this suggested that the storage procedure disrupted the test and meant that Anti-B could not be used without discovering the reason for this problem.

8.5 Experiment 3: Using Antibody-70 to Detect *Helicoverpa armigera* in the gut Contents of *Dicranolaius bellulus*.

8.5.1 Introduction and Aim

Considering the extra specificity of monoclonal antibodies, it was thought that Anti-70 may avoid the problems which occurred with Anti-B. Therefore it was decided to begin trials with Anti-70 before searching for the underlying causes of the effect of storage on beetle samples.

8.5.2 Methods

The general methods were employed again with the same set of samples used in the Anti-B; Experiment 2.

8.5.3 Results and Conclusions

The same results were exhibited for Anti-70 as Anti-B. However, as expected, *H. punctigera* and *A. infusa* did not show any colour development. *H. armigera* spots were clearly the strongest colour, which agreed with suggestions that Anti-70 was specific for *H. armigera* within the Noctuidae. The beetle dots were again relatively even in intensity but definitely lighter than the *H. armigera* samples.

8.6 Experiment 4: Testing for Endogenous Colour Reactions

8.6.1 Introduction and Aim

The fact that the increased specificity of this antibody did not reduce the colour development of the frozen and stored beetle samples suggested a problem with the internal specificity of the colour development reactions. Therefore the next experiment in this series was designed to identify which steps were failing.

8.6.2 Methods

Experiment 3 using Anti-70 was repeated twice but with each run of the experiment one of the antibody reactants was removed:

1) Minus *Helicoverpa armigera* eggs.
2) Minus the anti-mammal antibody.

8.6.3 Results and Discussion

All these tests should have produced a clear membrane except for the minor colouration caused by small amounts of endogenous peroxidases. In fact all these trials produced colour development to the degree noted for the complete reaction (i.e. a positive). This showed that only Streptavidin peroxidase and the colour substrate was needed to give a strong response. Therefore the Streptavidin was finding a binding site within the beetle contents regardless of whether antibodies were present or not. As the Streptavidin peroxidase is designed to bind through a biotin-avidin link, the avidin molecules of the peroxidase were probably binding to biotin, or something like it, within the beetle. The biotin-avidin link has one of the strongest affinities known, therefore it may have been possible to remove the Streptavidin peroxidase from false sites (non antibody linkages) before adding the colour substrate by a more severe washing step.

8.7 Experiment 5: Biotin-like Binding Sites in Frozen Beetle Samples

8.7.1 Aim & Methods

Experiment 4 was repeated but included fresh fed and unfed beetle samples to conclusively establish that storage was causing different test results. Two washing treatments were also compared; i) using distilled water and ii) using a 2M salt solution (in Tween (0.5%) in PBS). This experiment was also conducted using Anti-B to confirm the consistency of results between antibodies.

8.7.2 Results and Overall Conclusions

The more severe washing procedures made no difference to the intensity of the frozen samples immunodots. Also the non-fed, fresh beetle samples produced only very light immunodots (negatives). Therefore, *D. bellulus* presents biotin, or a similar site of similar affinity, when stored in liquid air, which invalidates the use of the biotin-avidin link in the ELISA colour development steps. Therefore this ELISA protocol is not suitable for studies with *D.bellulus* unless different storage procedures are used. An alternative might be to immediately prepare homogenates or smears on the nitrocellulose membrane from fresh samples and store them dry at 4^0C as with the LepTon™ Kit procedures (Trowell *et al.* 1994). Otherwise, one could use ELISA reagents which do not rely on the biotin-avidin link.

8.8 The potential for Serological Methods for Assessing Predatory Impact on *Helicoverpa* spp.

This study has only investigated one species. Therefore it is possible that the storage problem does not occur for other species, and the technique may be more useful for them. Alternatively, it may be that more detailed studies could determine the biochemical nature of the problem, and indicate ways of overcoming it. Using ELISA techniques which do not rely on the biotin-avidin link would be a logical progression from these preliminary studies. If the difficulties encountered with storing samples can be overcome the prospects of devising an ELISA technique which can identify *Helicoverpa spp.* or *H.armigera* in the gut contents of predators appear to be quite good since predation could be identified in fresh samples.

Further work would be required to establish whether the antibodies are of acceptable specificity because only a low number of predators and prey species were investigated in these experiments. Considering that the overall aim of this study was to identify the proportion of predators feeding on both *Helicoverpa* spp. in the field, Anti-B is probably too non-specific since it recognises other noctuids and perhaps other insects. However, if it is shown to be specific to noctuids, this may be useful since *Helicoverpa* spp. species are usually the dominant Noctuids in cotton. Anti-70 is too specific to determine overall predation on *Helicoverpa* spp. since it only recognises *H. armigera.* However, being a more serious pest in cotton than *H. punctigera* (owing to greater problems with insecticide resistance), studies aimed at quantifying predation on this species alone would still be of considerable value.

Chapter 9
Conclusions

9. Conclusions and Relevance to *Helicoverpa* spp. Management in Australian Cotton

9.1 Suction Sampling

Suction sampling methods were developed and compared in Chapter 4. The Bigvac method of suction sampling, developed for this study, provided useful population density estimates of the predatory arthropod species generally found in Australian cotton fields. This was an improvement over the usual methods of using D-vacs because it included a more comprehensive coverage of the crop canopy. Methods which sampled from the top of the canopy caught an increasingly smaller proportion of the predatory guild as the size of the plants increased, and were prone to errors introduced by the intracanopy movements of these arthropods. The consistently high proportion of the 'absolute' number of particular species of predator collected in the first pass of the Bigvac suggests that this may be a better method for future monitoring programs on cotton. The largest and most consistent samples were collected between 8 am and 12 noon, indicating that this would be the best time for sampling.

9.2 Classical Predation Measurements

The seasonal abundance records in Chapter 5 clearly show that *Helicoverpa* spp. contribute only a very small proportion (1 to 3%) to the available prey in cotton crops. Furthermore, the spatial correlation analysis of predator and prey populations (Chapter 6) failed to identify predators which are likely to specialise on *Helicoverpa* spp. Therefore this pest would be expected to have very little influence over the reproductive or other numerical responses of the group of generalist predators encountered in Australian cotton fields.

In this situation the classical measurements of functional and reproductive numerical responses would be unsuitable for estimating predatory impact because they rely heavily on

specific predatory relationships and do not take into account the effects of searching behaviour, alternative prey and climate. The discrepancies between the egg consumption rates of *Dicranolaius bellulus* in the laboratory verses field cages support these criticisms (Chapter 7).

9.3 Serological Analysis

The successful distinction between fresh samples of fed and unfed *D. bellulus* indicate that the prospects for devising ELISA techniques for identifying *Helicoverpa* spp. predators and possibly quantifying predation in crops are good. Although storing samples in liquid air confounded the these attempts to devise a protocol for *Dicranolaius bellulus*, the use of other biochemical procedures might overcome this problem, possibly through the use of a series of ELISA reactions which does not rely on the biotin-avidin link. However the question of prey specificity will require further studies. The antibodies tested here were either too specific to identify both *H. armigera* and *H. punctigera* (Anti-70) or too general (Anti-B) to identify only *Helicoverpa spp.* However, if these levels of specificity are useful, or an antibody of relevant specificity can be found, predation of *Helicoverpa* spp. appears to be amenable to assumptions which allow for statistical quantitative assessment using serological methods.

9.4 Predator Abundance

The abundance of predators can be increased with a reduced frequency of insecticide applications or by using softer insecticide options (Chapter 5). However some insecticides presumed at the start of this study to be softer on beneficial arthropods were shown to be clearly not very soft. In particular thiodicarb and chlorfluazuron were more harmful to the numbers of some beneficial species than expected. In contrast endosulfan, generally considered to be a broad spectrum insecticide, was relatively soft.

A common pattern of predator abundance was a decline in mid-season. The use of insecticides at the regional level appeared to have a marked effect on the number of predators in fields not directly treated with insecticides. This can probably be attributed to the loss of predators from local source areas or the possibility that predators roam far enough to contact

treated fields. The seasonal abundance records from isolated unsprayed plots showed that most species of predator could be present for most of the cotton growing season at higher densities if insecticides were not used. However even these levels were low compared with many cotton growing areas in the USA.

With the advent of target-specific pest control measures, such as Bt spray formulations or transgenic Bt plant varieties, the number of insecticide applications may decrease considerably, especially throughout the early season. With this, we may realise, for the first time in decades, the potential abundance of predators in Australian cotton fields.

9.5 Predator Impact

Low rates of predation were measured under the relatively realistic conditions of field cages. *Dicranolaius bellulus* adults and *Mallada signata* larvae contributed additional mortality when the background mortality of *Helicoverpa* spp. larvae was low (about 15%). No evidence of additional *Helicoverpa* spp. mortality was exhibited by *D. bellulus* (adults), *Nabis kinbergii* (adults) or *Coccinella transversalis* (adults) when background mortality was 70% and alternative prey were available. When these low rates were extrapolated to the prevailing field populations of these predators it is likely that their impact was insufficient to contribute much to the control of this pest while economic thresholds remain around current levels (5 eggs to 2 larvae per meter of cotton row).

These conclusions are restricted to the predators selected for consumption trials. There may have been other, more efficient, *Helicoverpa* spp. predators for which impact studies were not attempted in this thesis. However the spatial correlations (Chapter 6) suggested that limited prey consumption rates were common for a wide selection of predatory species and for all predators combined. These correlations showed that generalist predators are more abundant where there is more general prey but did not provide evidence that in these regions the abundance of *Helicoverpa* spp. was likely to be lower. These correlations also suggested that *Geocoris* spp. was perhaps the most efficient predator because it exhibited the strongest negative correlation with *Helicoverpa* spp. larvae. However, none of the predatory correlations accounted for much of the variance of *Helicoverpa* spp., suggesting that none of the predators exhibited a strong preference for, or impact on, this pest. Perhaps the greatest predatory impact

exhibited in this study was at 'Alcheringa' (unsprayed plot) where the predators appeared responsible for halving the *Helicoverpa* spp. abundance. Even in this case, it was clear that the predators still allowed much greater numbers of *Helicoverpa* spp. to survive than is currently acceptable for effective control (Section 6.3).

9.6 Predators in Integrated *Helicoverpa* spp. Management

The view of *Helicoverpa* spp. as prey, portrayed in thesis, is one of a few individuals scattered amongst abundant alternative prey. This, together with the conclusion that no particular species of predator commonly found in Australian cotton fields specialises on *Helicoverpa* spp., suggests that acts of predation are largely simple stochastic events. Therefore the amount or type of alternative prey could be expected to have a strong influence over the abundance, prey choice and survival behaviour of these generalist predators, which would in turn have a strong effect on the number of *Helicoverpa* spp. attacked. Furthermore, the often large and highly variable levels of background mortality of *Helicoverpa* spp. suggest that other factors can have an overwhelming influence on the development of damaging populations in cotton. These factors may explain the highly variable measures of predation recorded between experiments in this thesis. It may also help explain the variable results attributed to predators in the field, and the differing opinions as to the value of predators held among both cotton entomologists and cotton growers.

Any appraisal of the prospects for including arthropod predators in *Helicoverpa* spp. management decisions must reflect the lack of definitive measurements showing that they are effective, and the high variability found with natural mortality overall. Although all known methods for measuring predatory impact are flawed to some extent, as discussed in the literature review (Chapter 1), the corroboration of different techniques, as in this thesis, is more convincing. For *Helicoverpa* spp. on cotton as it is currently grown in Australia, a controlling effect would have to be a large one. The fact that such a large effect was never detected, either during the cage trials with realistic numbers of predators, or at some time in one of the six crops surveyed, strongly suggests that predation was not commonly important. The fact that exaggerated levels of predators were used in some of the field cage treatments to little effect (Chapter 7), reinforces this and further questions the view that in the absence of insecticides

Helicoverpa spp. would automatically come under effective control by increasing numbers of predators.

The highly variable rates of predation recorded between experiments in this study also raise concerns about the predictability and therefore the possibility of utilising their impact on *Helicoverpa* spp. Since predator to prey ratios are being considered for *Helicoverpa* spp. management in Australia, greater understanding of the factors which cause this variability will be required.

With such unpredictability it is difficult to take advantage of this mortality because there is no means of inflicting high mortality on a population of *Helicoverpa* spp. if an influx escapes control. The incompatibility of natural enemies and insecticides presents a major barrier to harnessing the contribution of predators for *Helicoverpa* spp. control regardless of the size of their impact. Current insecticides are most effective if applied when eggs are hatching, which means insecticides are used pre-emptively, that is, before a damaging population has been seen to develop. Therefore the inclusion of predators in Australian pest management decisions has so far been confined to the organic farming niche or in borderline decisions, near threshold levels, where the risk of loss is small.

9.7 The Future

Although the work described in this thesis indicates that the impact of predators is unreliable and usually small, their contribution to *Helicoverpa* spp. mortality is worthy of inclusion in IPM programs. This is particularly so since there are other pests of cotton such as mites (Tetranychidae) and possibly aphids (*Aphis gossypii*) which can be controlled by predators, and usually emerge only as secondary pests in response to predator destruction by insecticides. Programs could incorporate practices which are compatible with predators, either via conservation or augmentation to maximise their impact. Mensah & Harris (1995) have approached this by testing compounds which attract, arrest or alter predator feeding behaviour, or increase predator sources. These compounds have also been able to deter the oviposition of *Helicoverpa* spp (Mensah 1996).

Other predator-compatible measures are resistant plant varieties or more target-specific insecticide chemistry. Such options are becoming available with the introduction of transgenic Bt cotton varieties, and the new insecticide spinosad (Tracer®) which is extremely soft on beneficial arthropods (Murray & Lloyd 1997). In this new environment, that is, one in which predators are more abundant, secondary pests may be more important and *Helicoverpa* spp. are being reduced by measures which are compatible with natural enemies, cotton growers can be expected to be far more willing to include predator/prey ratios in their pest management decisions and therefore benefit from whatever pest mortality they may contribute.
