Chapter 7

Preliminary estimation of adult populations of *M. demolitor* in an unsprayed cotton field

Abstract

This trial investigated a capture-recapture technique in order to estimate the population of *M. demolitor* in a 5 ha. unsprayed cotton field. A method of marking *M. demolitor* adults in the field with water-based paint was successful. The computer program "CAPTURE" was used to estimate the population of male *M. demolitor* adults. An estimate of between 1503 and 2421 males was obtained for the 5 ha. unsprayed cotton block sampled. An attempt was made to relate this number to percent parasitism rates of the host, *Helicoverpa* spp. obtained from the same block at the same time.

Introduction

It is useful to obtain an estimate of the population of animals for such purposes as environmental inventories, environmental impact studies or determining quotas for harvest or culling purposes. Determination of the population of *M. demolitor* in a cotton field is useful for conservation reasons. For a biological release program to be successful, an assessment of the density of beneficial insects in the natural population must be made, so that an adequate number of beneficials can be released. If an estimate of the population of *M. demolitor* could be related to the amount of control over a pest species, then calculations of the number required for augmentative release can be made and the feasibility of augmentative release assessed.

Previous attempts at estimating the populations of key beneficial insects have been made. Keller and Chang (1995) studied *Cotesia rubecula* (Marshall) (Hymenoptera: Braconidae) a parasitoid of *Pieris rapae* L. (Lepidoptera: Noctuidae) using traps baited with live female wasps to capture-recapture males. Males were marked with a dot of enamel paint, which did not affect the survival of wasps. Keller and Chang (1995) found that trap catches correlated weakly with

population estimates. They conclude that if trapping intensity is sufficiently high, this method may provide an estimate of wasp activity.

The aim of this study was to estimate the population of adult *M. demolitor* present in an unsprayed cotton block. These data will be useful in the conservation of *M. demolitor* in future IPM systems and may be useful in assessing the feasibility of releasing laboratory insects for augmentation of field insects.

Materials and methods

This experiment was carried out in 1997, in a 5 ha. unsprayed block assigned as a refuge for an Ingard® block at Warra (see Appendix 4). Sampling was carried out over 4 consecutive days starting on 21 January and terminating on 24 January. Sampling was carried out for 3 hours approximately between 0930 and 1230.

Sweep-netting was carried out by two operators walking continuously for the 3 hours. This method is described on p. 55, except captured female wasps were released while males were collected and held in the shade till the end of the sampling period. Wasps which were identified as *M. demolitor* but not caught, were recorded as unknown. Males were then marked with a small dab of water-based paint on their thorax and released. Different colours were used for each day of sampling. Recapturing was achieved by noting the presence of a mark from any of the previous days.

Data were analysed using "CAPTURE" software (White, 1982). The manual for CAPTURE (White *et al.*, 1982) and an associated publication (Otis *et al.*, 1978) were used in interpreting the CAPTURE results.

Results

Only male wasps were marked and used in the population estimation, as there was considered to be an insufficient number of females. There may be variations in the sex ratio of

wasps due to differential sex allocation in preceding generations or due to migration of females or males. There were five times as many males as females at the start of the trial. Table 7-1 shows the number of males and females and unknowns that were captured during the trial. This table also shows the ratio of males to females, which showed a trend towards lower sex ratios over the trial period.

Table 7-1 Number of male and female *M. demolitor* caught over 4 consecutive days.

Sampling day	Males	Females	Unknown	Ratio males: females	% females captured
1	56	10	1	5.6:1	15
2	77	18	1	4.2:1	18.9
3	86	24	0	3.5:1	21.8
4	88	27	0	3.2:1	23.5

Table 7-2 shows the number of male *M. demolitor* captured, the number of male wasps that were marked and released, and the number of marked wasps that were recaptured on the subsequent days of the trial. The number of males that were marked was always less than the number caught, because a few died from heat or during the marking process. In total, 307 males were captured, 186 were marked, 260 were considered part of the analysis, 14 were captured twice and no wasps were captured more than twice.

Table 7-2. Capture/recapture of male *M. demolitor* over 4 consecutive days.

Sampling day	Captured	Marked	No. recaptured from day 1.	No. recaptured from day 2.	No. recaptured from day 3.	% of marked wasps recaptured
1	56	47	-	-	-	-
2	77	67	4	-	-	8.5
3	86	72	2	1	-	2.6
4	88	-	3	1	3	3.8
Total	307	186	9	2	3	

Table 7-3 shows population estimates for 5 models used by the computer program CAPTURE. Estimates for the population of *M. demolitor* in the 5 ha. unsprayed block of cotton varied between 503-2421 males. The low value can possibly be discarded due to the use of an unrealistic model (see below). These models are discussed below.

Table 7-3. Estimations of the population of *M. demolitor* using 5 models in the computer program CAPTURE. These models are discussed below.

Test	Population estimate	95% Confidence interval	Profile likelihood interval
constant probability of being captured, (model $M_{(o)}$).	1503	1010-2321	1180-3707
Time specific change in probability of capture, (model $M_{(t)}$)*.	1927	1217-3168	1234-3330
Time specific change in probability of capture, (model $M_{(t)}$)*.	1741	1110-2841	-
Individual heterogeneity in capture probabilities, (model $M_{(h)}$).	2421	1483-4080	-
Generalised removal model	503	450-572	-

* There were two $M_{(t)}$ models. See White *et al.*, (1982) for the first model and Chao (1989) for the second model.

Figure 7-1 compares, rather than statistically tests the relationship between percent parasitism of collected *Helicoverpa* spp. larvae and the numbers of male and female *M. demolitor* caught in sweep nets at the time of this trial. This figure shows that there was between 3 and 5 times as many male wasps caught in sweep nets as female wasps. Unfortunately, percent parasitism was not determined after this trial, and it was seen in the previous chapter that the relationship between percent parasitism and the number of wasps caught in sweep nets has a time lag effect. This will be discussed further in the discussion section. The percent parasitism data shown in Figure 7-1 were discussed in Chapter 6.

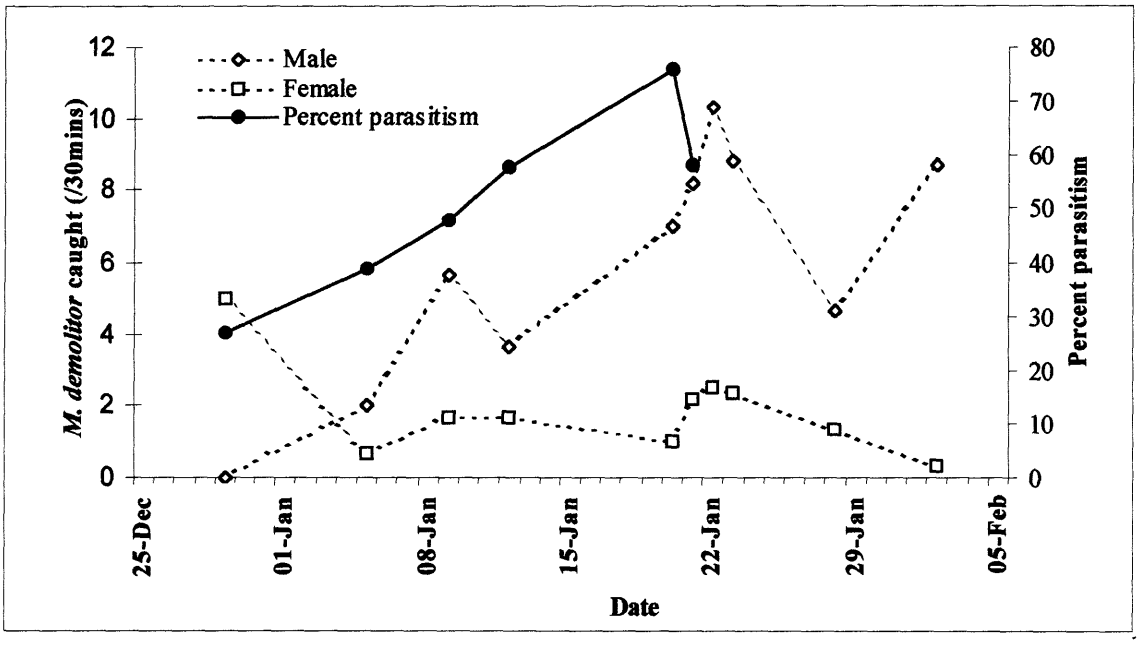

Figure 7-1. Number of male and female *M. demolitor* adults caught in sweep netting and percent parasitism of collected *Helicoverpa* spp. larvae from unsprayed refuge block at Warra during 1996/97. These data were presented in Chapter 6.

Discussion

Four assumptions are necessary in order to estimate a chosen population. These are that the population is closed, that the marked animals do not lose their marks, that all marks are correctly recorded and that each animal has a constant and equal probability of being captured on each trapping occasion (Otis *et al.*, 1978). It is of course impossible to ensure that these rules are met in their entirety. Some wasps die from predation, old age or the like, some will emerge from pupae as adults, some will immigrate and some will emigrate. It is of the utmost importance that any immigration or emigration of wasps is minimised. This was achieved by placing the trial plot on the edge of the field, and against an Ingard® block on one side. Therefore there would be little stimulation to leave the trial plot because of few hosts in a fallow paddock and fewer hosts in the Ingard® paddock. The program CAPTURE has been designed so that some of the above assumptions are relaxed and considered in the analysis of the data. It is important that the design of field techniques minimises any discrepancies in the data as no amount of sophisticated analysis will compensate for poor data collection techniques (Otis *et al.*, 1978).

It is an important assumption of any mark-release-capture study that the marked and unmarked insects have the same probability of being captured (Southwood, 1966). The marking technique may have increased the mortality of the marked males, although all measures were taken to reduce this. This is an unfortunate side-effect of this method. These data show that the chance of being recaptured did change over the trial period. This indicates that mortality did appear to increase over the trial period and wasps may have learnt to avoid capture or become "capture-friendly". Although Table 7-2 shows that these effects where minimal as the total number of marked males caught on each day was relatively constant to the total number of marked males over the trial. The catch efficiency increased from day 1 to day 2, however, it stayed constant over the last three days. This is probably an artifact due to the operators rapidly becoming more proficient at catching wasps.

The sex ratio of males to females decreased over the trial. The reason for this is unclear, although male mortality during catching and marking may have been a factor. Approximately ten wasps died on each marking occasion during the marking process. Table 7-1 shows that the number of males caught on each day stayed about the same, while the number of females caught increased over the trial period. The cause of this is unclear, although immigration is possibly responsible.

CAPTURE generates models based on certain assumptions about the animal being studied. Five models were included in this study. These models make an estimation of the population of the target animal based on certain assumptions. Model $M_{(o)}$ assumes that every animal has the same probability of being captured on every occasion. This model allows for no sources of variation in capture probabilities. Model $M_{(t)}$ allows for capture probabilities to vary with time, so that on each sampling occasion, there is a different probability of being captured. Therefore the past capture history of any animal does not influence its probability of being recaptured. As a result, any behavioral response will invalidate this model. Under this model, trap catches are expected to be erratic, with large catches on good days and low numbers caught under poor conditions. CAPTURE generates two time models. The $M_{(b)}$ model assumes that the probability of capture varies with any prior capture, due to the experience of being captured as pleasant or unpleasant, thereby increasing or decreasing the probability of further capture. This model could allow for any

increase in mortality of marked individuals due to the marking procedure. The $M_{(h)}$ model assumes that the probability of capture varies between individuals (with sex, age, level of activity, method of trapping etc). CAPTURE also generates models which are combinations of the above models, for example, the model $M_{(bh)}$ indicates that animals vary in capture probability as well as in capture experience. The model $M_{(th)}$ indicates that animals vary in capture probability and the chance of capture varies on each sampling occasion. The model $M_{(tb)}$ indicates that both time and behavioural responses affect capture probabilities, and model $M_{(tbh)}$ indicates that all three factors affect the probability of capture. In analysis of data, CAPTURE recommends which model to use, but this, as the model's authors admit, is not always accurate. For example, the last three models do not estimate the population, so if the program selects one of these models, the next best model must be used (White *et al.*, 1982).

Understanding of the statistical mathematics associated with the analysis of the data is not necessary in interpreting the data presented here. Indeed the whole idea of the program is that the biologist can leave the mathematics to the mathematician. From these data, CAPTURE recommended the $M_{(tbh)}$ model. However, there is no estimate of the population associated with this model. The next two models recommended by the program were the $M_{(o)}$ and $M_{(h)}$ models. The $M_{(o)}$ model is perhaps the best estimator of the *M. demolitor* population because environmental conditions were constantly very good throughout the trial and sampling occurred at the same time each day. It is doubtful that *M. demolitor* would have learned to avoid being captured or were capable of avoiding capture. The data certainly support this assumption. Although it is conceivable that the *M. demolitor* population may have varied by age, populations in the field have fairly discrete generations. Only males were counted, and it is assumed that all males were actively foraging for females, or possibly food, thereby reducing the effect of heterogeneity.

This study shows that the estimation of beneficial insect populations by capture-recapture is feasible. This study, although carried out on a small and highly mobile parasitoid, which was present in medium to low density, was not extremely difficult. The population density of *M. demolitor* males present in the 5 ha. block of unsprayed cotton was estimated to be between 1503 and 2421 with the lower figure being the best estimate coming from the $M_{(o)}$ model (ca. 300 male wasps/ha.). Although the actual number needs to be validated by further research, this study shows

that this method is useful in determining populations density of beneficials, especially highly mobile parasitoids. This technique may be useful in investigating the effects of insecticides in the field by determining an estimate of the population before and after insecticide application.

From this study it is possible to relate the population estimate from male wasps to the number of females and then relate this to the percent parasitism by *M. demolitor* of *Helicoverpa* spp. larvae in the field. The population of male wasps was 1503 and there were approximately 4.1 times as many males as females (see Table 7-1). Therefore there were approximately 365 females present in the 5 ha. unsprayed block of cotton. At the time of the study there was approximately 60% parasitism. This estimate could be improved if parasitism was determined after the time-lag between females present and parasitism. Therefore, in order to obtain 60% parasitism of pest larvae; approximately 365 females are needed for inundative release per 5 ha. This equates to approximately 73 females/ha. These calculations are very speculative and cannot be used to recommend that a release rate of 73 females/ha would always give 60% parasitism of *Helicoverpa* spp. in the field. However this study and the above calculations show that an estimate of parasite density can be determined and that this figure can be related to the rate of parasitism obtained in the field. This study indicates that the relative numbers of *M. demolitor* needed for significant rates of *Helicoverpa* spp. parasitism in the field appear to be quite low. This figure is very low compared to release rates of *M. croceipes* and *C. nigriceps* by Lewis and Gross (1989) and Lewis *et al.* (1972). These studies estimated that ca. 900-1500 female wasps caused approximately 80% parasitism of *H. virescens* (see p. 19). This indicates that augmentative releases may be economically cost effective. Further work is needed in order to validate the number of *M. demolitor* which must be released to cause adequate parasitism based on numbers and the sampling methods presented here.

Conclusions

This study describes a method of estimating *M. demolitor* adult populations in the field. Although problems were encounter and the results must be viewed with reservation and are considered preliminary only. This study showed that this method may be useful for studying highly mobile beneficals such as *M. demolitor*. The population of *M. demolitor* males present in the 5 ha.

block of unsprayed cotton was estimated to be between 1503 and 2421. It was found that relatively low numbers of *M. demolitor* females are contributing to high rates of parasitism of *Helicoverpa* spp. This method could be useful for investigating the effects of pesticide treatments, by making before and after estimations of the population present. This study shows that inundative release of *M. demolitor* may be economically feasible.

Chapter 8

Diurnal activity of *M. demolitor* adults in the cotton crop

Abstract

This trial aimed to investigate the diurnal activity of *M. demolitor* adults in the field by direct observations. It was found that there was no significant difference in activity of either males or females throughout the day. Male wasps were generally more common than females. Adult wasps were inactive before 0700 and after 1800 and pheromone catches showed a distinct diurnal pattern. These data may be important in recommending standardised sampling methods for *M. demolitor* in the field. The implications of this study for IPM are discussed.

Introduction

Most insects have a daily rhythm of activity, which is termed diurnal activity. If the diurnal activity of beneficials is studied and any periods of inactivity determined, then some disruptive insecticide sprays can be timed for periods of inactivity. This will reduce direct exposure to the insecticide. This approach has been taken by manufactures of insecticides who recommend that applications not be made when honey bees are actively foraging. Hendrix *et al.* (1997) found that if wasps avoided direct initial contact with spinosad, once dry, residues were harmless. This means that application timing is crucial for conservation of *M. demolitor* if spinosad is to be used.

The diurnal flight activity of *M. croceipes* has been studied. Powell and King (1984) found that males were most active during the morning, starting at 0630 with peak numbers occurring between 0800 and 1100. Females were also more active in the morning compared to the afternoon, but females were active throughout the day. Females became active one hour later than males.

Powell and King (1984) also studied the activity of *M. croceipes* males in response to sticky traps baited with virgin females. They found that the earliest catch was at 0830 and almost twice as many males were caught between 0830 and 0915 (20 males) than from 0915 to 1200 (11 males). No males were captured from 1200 to 1830.

This study aimed to examine the diurnal behavior of *M. demolitor* adults. With more detailed information on the behavior of adult *M. demolitor*, periods of inactivity may be determined. With these data, recommendations can be made to time insecticide applications during periods of minimum wasp activity. This may contribute to conservation of *M. demolitor* populations in the field. These data may also be helpful in recommending standardised sampling times for *M. demolitor*.

Materials and methods

Sampling dates

Sampling was carried out on 31 January 1996 at Warra in a conventionally sprayed block, 19 March 1996 at Dalby Agricultural College in an unsprayed block, 15 January 1997 at Warra refuge block and 4 April 1997 at Witu refuge block (see Appendix 4). On the 19 March 1996 and 15 January 1997 three observers took part. On 31 January 1996 and 4 March 1997 one observer took part. Sweep-netting was used on all occasions, supplemented with virgin female sticky traps on 19 March 1996.

Cotton was dryland, single and double skip (one or two rows of cotton remain unplanted in order to conserve soil moisture), on all occasions except at Dalby (19 March 1996) which was solid planted irrigated cotton.

Sampling methods

Direct observations (sweep netting)

Direct observations were carried using similar methods as described in Chapter 6 (p. 53). The samplers walked continuously for 45 minutes every hour up and down the rows of cotton. Walking speed was about 1.5 km/h, with about 200 metres of cotton sampled every 45 minutes. Sampling commenced at 0600 and terminated at 1800 in all studies except 15 January 1997, which terminated an hour later (1900). Results are displayed as the number of male and female *M.*

demolitor caught per observer per 45 minutes of searching. Often, a *M. demolitor* adult was identified but not caught and sexed, and on these occasions, wasps were noted as unknown.

Sticky traps baited with virgin female *M. demolitor*

Virgin female traps were similar to those described in Chapter 6 (p. 53). Sampling with sticky traps was carried out at Dalby Agricultural College on the 19 March 1996. A grid of 25 traps was set up in each treatment. Traps were spaced 40 m apart by 7 rows (about 7 m). Traps were placed just below the cotton canopy and examined hourly for the first day and then at 0600 each day for 3 days. Initially, 25 traps were used, but this number decreased as females died. The numbers of traps on each day were day 0= 25, day 1= 25, day 2= 20, and day 3= 13. Data were pooled across days, subject to a logistic transformation and analysed by regression analysis.

Results

Counts from direct observations of diurnal activity of male and female *M. demolitor* are shown in Figure 8-1, Figure 8-2, Figure 8-3 and Figure 8-4. Combined data from all studies are shown in Figure 8-5. This figure highlights the trends in the data. These data show that male *M. demolitor* were active shortly after sunrise. In all but one case (31 January 1996), activity commenced between 0700 and 0800. In all but one case (4 April 1997), males were more numerous than females. Adult activity was found to be not significantly different ($p > 0.05$) over the entire day.

Figure 8-6 shows the number of male *M. demolitor* caught each hour in the sticky traps baited with virgin female wasps. The first catch occurred between 0700 and 0800, with a peak between 0900 and 1000. Catches dramatically dropped between 1000 and 1100, and declined till 1800 when sampling was terminated. No males were caught overnight. Trap catches declined daily from 19 March 1996 till 22 March 1996 (Figure 8-7).

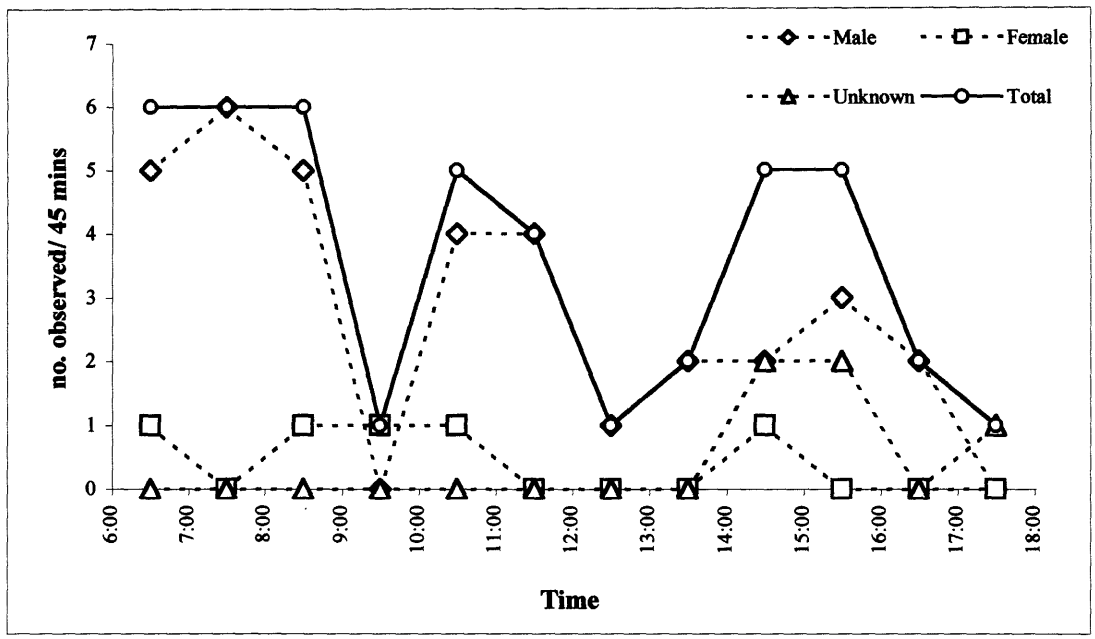

Figure 8-1. Diurnal activity of male and female *M. demolitor* in a conventionally sprayed block at Warra on the 31 January 96 (1 observer).

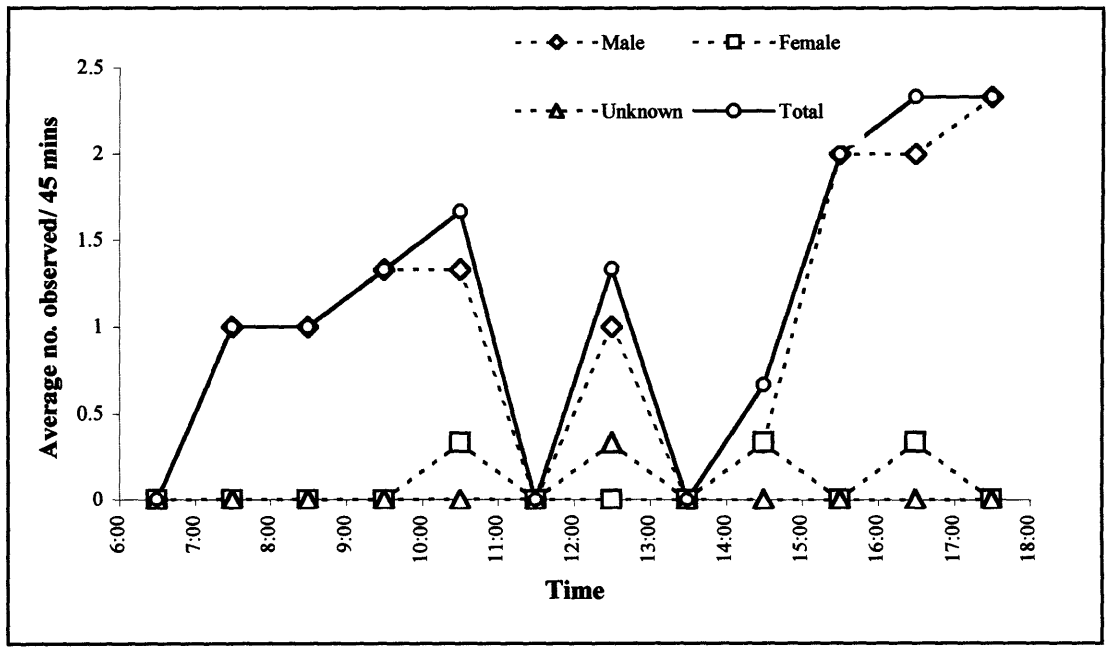

Figure 8-2. Diurnal activity of male and female *M. demolitor* in and unsprayed block at Dalby on the 19 March 96 (average of 3 observers).

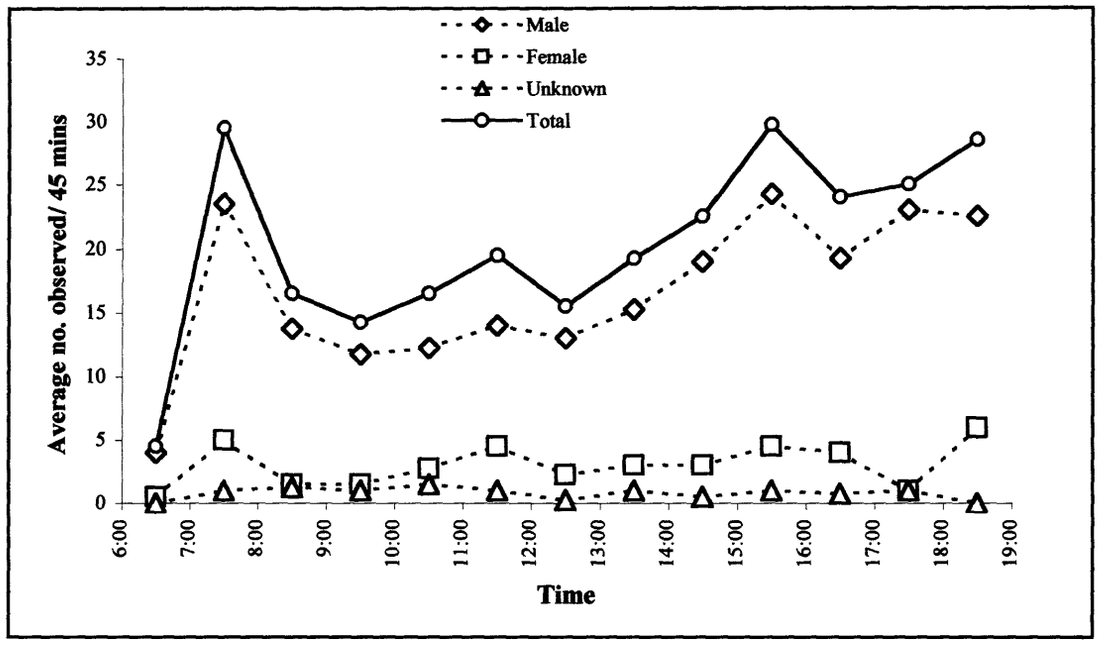

Figure 8-3. Diurnal activity of male and female *M. demolitor* in an unsprayed block at Warra refuge on the 15 January 97 (average of 3 observers).

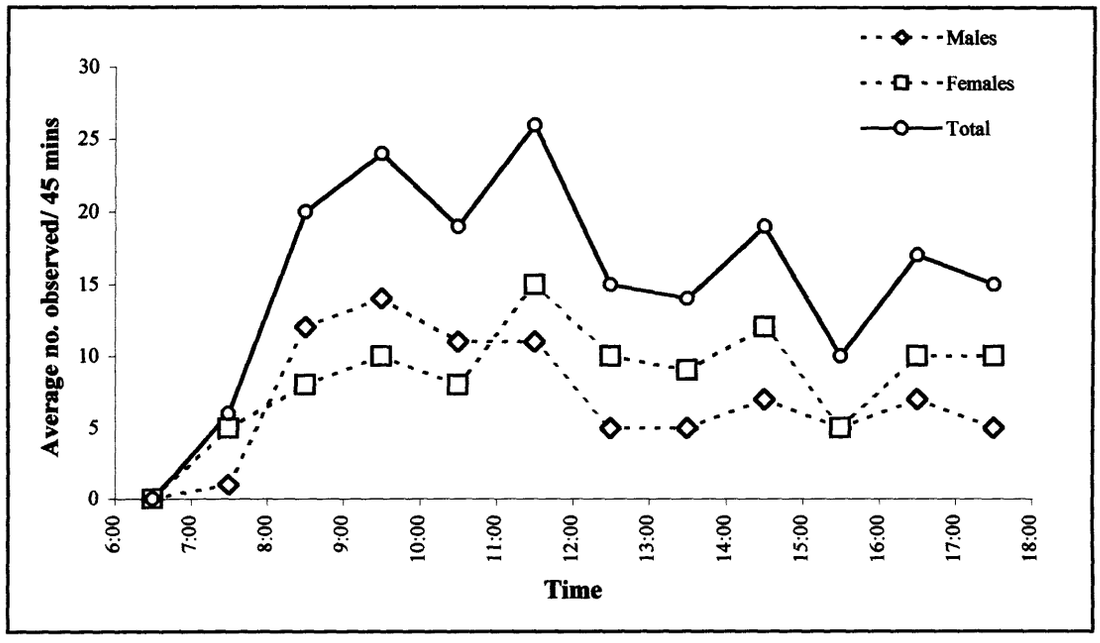

Figure 8-4. Diurnal activity of male and female *M. demolitor* in unsprayed refuge block at Witu on 4 April 97 (1 observer).

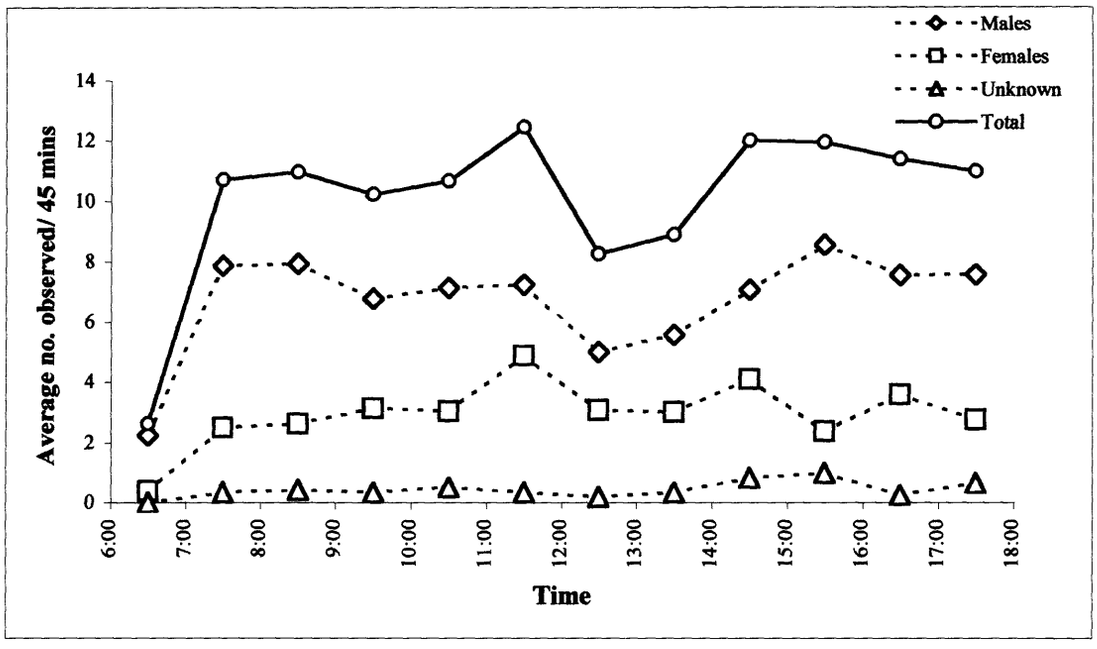

Figure 8-5. Diurnal activity of male and female *M. demolitor*, average of all studies.

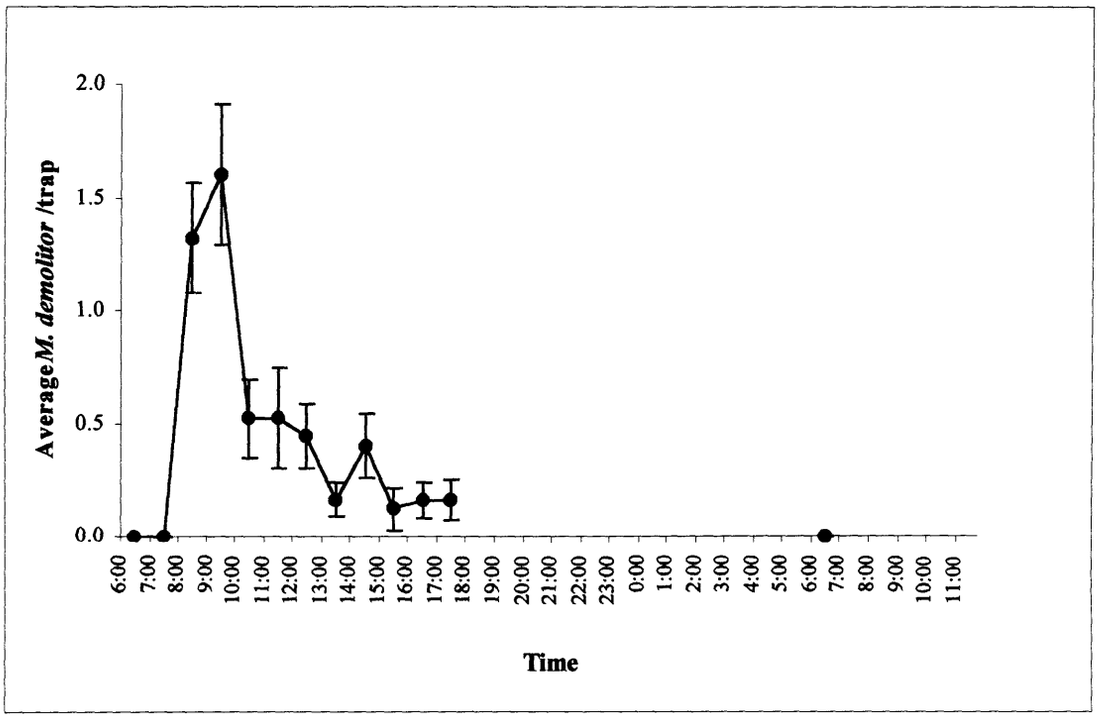

Figure 8-6. Average number of male *M. demolitor* caught per trap per hour from 0600 to 0600 the following day in an unsprayed irrigated block at Dalby (19 March 96). Sampling was carried out hourly from 0600 to 1800, and traps were sampled at 0600 the following day.

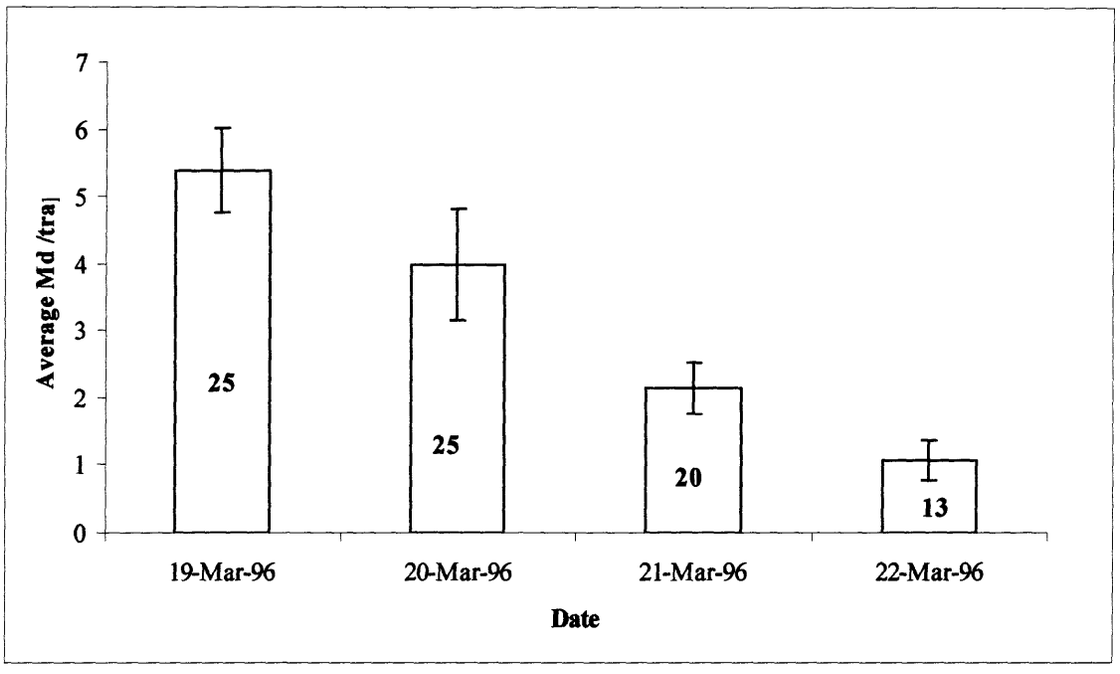

Figure 8-7. Average number of male *M. demolitor* caught per trap per day (19 to 21 March 96) in an unsprayed irrigated block at Dalby (Number of traps on each sampling occasion is displayed in each graph).

Discussion

Direct observation was a successful method for studying the diurnal behaviour of *M. demolitor*. This study showed that *M. demolitor* adults were active shortly after sunrise, between 0700 and 0800, and were then equally active throughout the day. This pattern of activity is similar to the pattern observed for *M. crociepes*, although *M. demolitor* did not display the peak in activity in the morning observed in *M. croceipes* and *M. croceipes* males were less active in the afternoon than in the morning (Powell and King, 1984). This was not observed in *M. demolitor*. It maybe that adult wasps shelter within the cotton plant canopy when not foraging. It must be investigated whether adults resting within the cotton canopy are sheltered from the effects of insecticide applications compared to active wasps. However, it is logical to suggest that applying knockdown contact insecticides when wasps are not active will reduce the mortality they cause.

This study showed that visual sampling in order to gain information on the densities of *M. demolitor* can be carried out at any time of the day without biasing the results. This is important if

M. demolitor numbers are to be considered when making spray decisions, because observations can be made with equal accuracy anytime throughout the day.

Male wasps were 2-3 times more common than female wasps. This may be due to different rates of immigration and emigration, or arrhenotokous parthenogenicity (virgin females produce male progeny, see p. 31). The differential sex ratio may also be due to differences in activity between the sexes. Male wasps may be more active than females, or females may be more actively foraging lower in the cotton canopy and are more easily missed by the observers. This differential sex ratio, either real or artificial, must be considered when consultants observe wasps in the field.

The virgin female trap catches in this study show a distinctive peak in the morning, declining till late afternoon. These data reflect the mid-morning peak in catches found in the response of *M. croceipes* males to sticky traps baited with virgin females, although *M. croceipes* males were not caught at all in the afternoon (Powell and King, 1984). These data show that if sticky traps baited with virgin females were to be used as a monitoring tool, sampling could be restricted to mid-morning when peak catches were recorded. It is unclear whether pheromone catches are a reflection of calling by females, answering by males, or of general male activity. It is unclear if the decline in virgin female trap catches over the four day trial period was due to females calling less due to age, or the females being stressed. The removal of male wasps through the "destructive" method of catching them may have contributed to the decline in male numbers. Unfortunately only limited data were collected in this study. This is an exciting area for future research.

Conclusions

This study shows that large numbers of adult *M. demolitor* were observed in the field, both male and female *M. demolitor* were inactive early in the morning and late afternoon and both male and female *M. demolitor* were equally active throughout the day. There was a distinct pattern in catches from sticky traps baited with virgin females, with males caught in sticky traps baited with virgin females mostly during mid morning, declining throughout the afternoon. No males were caught during the night.

This study suggests that farmers could minimise the disruption of insecticides by applying disruptive insecticides when adult *M. demolitor* are least active. Adult *M. demolitor* can be monitored at any time of the day without biasing results. If sticky traps baited with virgin females were used as a research or monitoring tool, trapping could be rationalised to mid-morning when peak catches were recorded.

Chapter 9

Preliminary study of the release-recapture of male *M. demolitor* adults

Abstract

The aim of this trial was to investigate the behaviour of male *M. demolitor* in response to sticky traps baited with virgin females. Males marked with fluorescent powder were released in the center of a grid of 48 traps, 20 metres apart. Data from this trial showed that fluorescent powder was a useful tool in this release-recapture study, catch efficiency of the sticky traps baited with virgin females was reduced after only 2 days in the field and that male *M. demolitor* were most likely to be caught in the closest sticky trap baited with a virgin female. Males did not generally move very far in response to these traps, but were certainly capable of long distance movement. This study showed that there was a large natural population of male *M. demolitor* (≈45/ha.) present in the biologically sprayed field. Implications for IPM in Australian cotton are discussed.

Introduction

The success of the sticky traps baited with virgin females (Chapter 6 p.211) at trapping male *M. demolitor* posed numerous questions, including how far males move in response to the calling females, how many males were responding to the traps, and from what distances were males coming. In addition to answering these questions, it was considered useful to investigate the feasibility of using fluorescent dust in mark-recapture trials with *M. demolitor*.

Mark-release-recapture studies using fluorescent dust have been carried out with success on populations of *Aedes* (*Stegomyia*) *simpsoni* (Theobald) (Diptera: Culicidae) in Uganda (Lutwama and Mukwaya, 1994). This study used six colours of fluorescent dust and a portable UV light source for marking insects on different days. The authors state that, although mortality due to the marking was not directly investigated, the fact that marked mosquitoes were recaptured over a number of days indicated that marking did not adversely affect the probability of recapture. Their

study successfully estimated the population size, survival rates and longevity of the adult mosquitoes in certain districts of Uganda.

The aim of this study was to examine the movement of male wasps in response to virgin females. These data are useful for interpreting the data from the sticky traps baited with virgin females as well as determining the distance males move in response to virgin females. This trial also provided useful data on populations of wild *M. demolitor* and their response to the sticky traps baited with virgin females.

Materials and methods

Sampling methods

Sampling was carried out at the Warra IPM site in the biological block (see Appendix 4) on 28 February and 2 March 1995. Sticky traps baited with virgin females similar to those used in Chapter 6 (p. 53) were used in this trial. *M. demolitor* females were stored normally until needed. Storage did not affect calling behaviour in females (see p. 32). Traps (48) were baited with a single female and set up in a 7 x 7 grid. Each trap was 20 m from its closest neighbour (the grid was total of 1.44 ha.) (Figure 9-1). The location of the trial grid in relation to the IPM trial site and the location of the normal traps used in routine monitoring (Chapter 6) is shown in Figure 9-2

Male *M. demolitor* from the laboratory culture (see Appendix 1), less than 48 hours old, were marked with fluorescent dust and released on two occasions at the center of the trial grid (Figure 9-1). Wasps were marked with fluorescent dust by sprinkling the dust over the wasps while held in a small plastic container. This method ensured adequate coverage of males, which remained marked for their lifetime. The first release was on 28 February 1995 and the second release was two days later on the 2 March 1995. On the first occasion, approximately 300 males were marked with yellow dust and on the second occasion, approximately 150 males were marked with orange dust. Traps were examined two days after the second release, on 4 March 1995, under UV light and the number of each colour of marked wasps as well as the number of unmarked or "wild" wasps were recorded. The number of marked males recaptured, both from day 0 and day 2, and the

number of "wild" wasps caught in each trap is shown in Table 9-1. The number of wasps caught along each axis of the grid is shown in Figure 9-2.

Male wasps were released at approximately 1100 on both release occasions from the center of the grid of traps. Wind speed was approximately 4-6 km/h from the NNW on both release occasions (Figure 9-1).

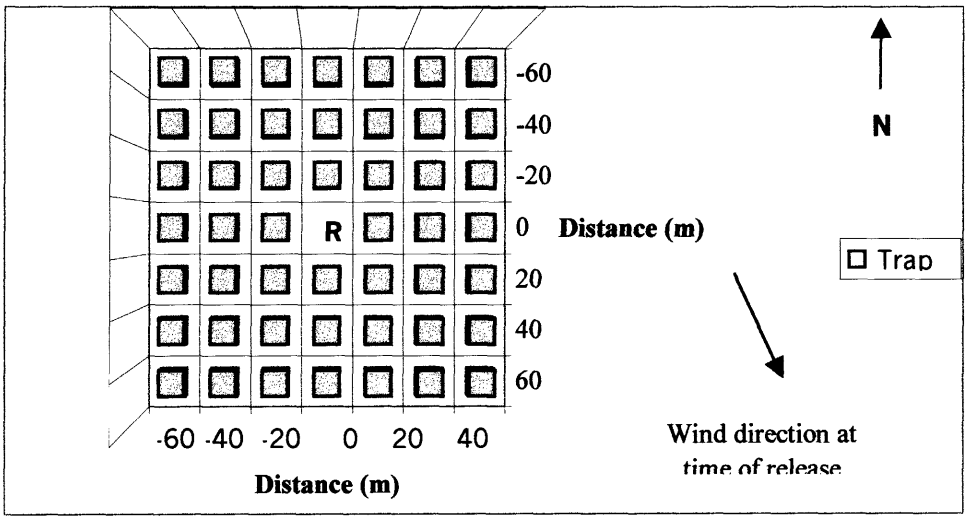

Figure 9-1. Arrangement of sticky traps baited with virgin females (view from above). R= release point.

Results and Discussion

On both release occasions, the traps closest to the release point recorded the most male wasps, especially the first trap down wind of the release point (Table 9-1) On the first release occasion the first trap downwind recorded 35 males which was over three times as many as the next highest catch. Of the eight closest traps to the release point, six recorded the highest or equal to the highest catches for the grid. On the second release occasion, the closest trap down wind also recorded the highest catch. However this catch was approximately 4 times less than on the first release occasion. On both release occasions the outer most row of traps recorded the lowest catches. Of the approximately 300 marked wasps released on the first occasion 103 were recaptured. On the second occasion, approximately 150 marked males were released and 27 were recaptured. While this trial was in progress, normal trapping with the sticky traps baited with virgin females was carried out at the time of the second release (see Chapter 6). 19 marked wasps were

caught in the grid of normal traps (Table 9-1). In these traps two marked wasps were trapped from the first release and six from the second release. Two wasps were recorded from traps over 300 m from the release point.

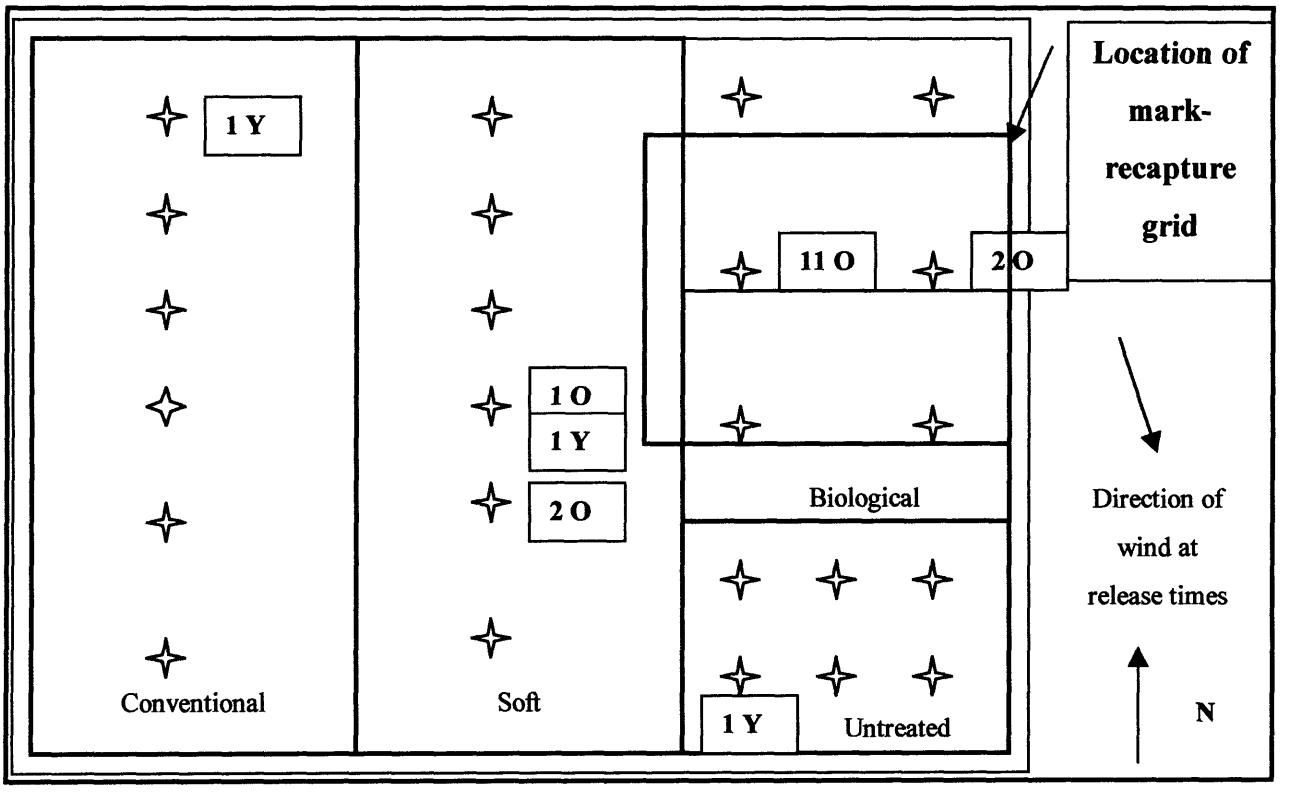

Figure 9-2. Arrangement of mark-recapture grid relative to the normal traps showing the number of wasps caught in these traps.

✦ = location of routine monitoring sticky female trap (Chapter 6), O= marked with orange dust, Y= marked with yellow dust.

During the trial period 90 wild male *M. demolitor* were trapped in the grid of traps, equivalent to 62.5 wasps per ha. (Table 9-1). Wild males were more evenly distributed across the grid than the released males. For the released wasps, the outside row recorded the least number of males. For the wild wasps the outside row recorded the highest number of catches, although the center rows recorded high catches as well (Table 9-1). These data show the variation in trap catches with 12 traps recording 0, 12 recording 1, 7 recording 2, 7 recording 3, 4 recording 4, 2 recording 5, 1 recording 7 and 1 recording 8. The traps recording a zero catch were more

frequently distributed toward the edge of the trial block, on the north and west sides (upwind) of the trial.

Table 9-1. The number of male *M. demolitor* adults caught in sticky traps baited with virgin females after release of males marked with florescent dust. Raw data.

Released day 0 (approximately 300 males released)								
Distance (m)	**-60**	**-40**	**-20**	**0**	**20**	**40**	**60**	**Total**
-60	0	0	0	0	1	0	1	**2**
-40	0	0	2	1	0	2	0	**5**
-20	0	9	0	5	4	1	0	**19**
0	0	0	7	**R**	35	0	0	**42**
20	1	2	2	11	4	0	1	**21**
40	0	0	1	2	0	4	0	**7**
60	1	1	1	0	1	2	1	**7**
Total	**2**	**12**	**13**	**19**	**45**	**9**	**3**	**103**
Released day 1 (approximately 150 male released)								
Distance (m)	**-60**	**-40**	**-20**	**0**	**20**	**40**	**60**	**Total**
-60	0	0	0	0	0	0	1	**1**
-40	0	1	1	0	1	1	0	**4**
-20	0	0	0	0	0	1	0	**1**
0	0	0	3	**R**	9	2	0	**14**
20	0	1	0	1	1	2	0	**5**
40	0	0	0	0	2	0	0	**2**
60	0	0	0	0	0	0	0	**0**
Total	**0**	**2**	**4**	**1**	**13**	**6**	**1**	**27**
Wild *M. demolitor*								
Distance (m)	**-60**	**-40**	**-20**	**0**	**20**	**40**	**60**	**Total**
-60	1	1	4	3	0	8	1	**18**
-40	7	3	2	0	0	1	1	**14**
-20	1	0	0	2	1	2	3	**9**
0	0	0	4	**R**	5	1	2	**12**
20	3	4	0	3	0	1	1	**12**
40	3	0	0	1	2	1	1	**8**
60	0	1	4	2	2	3	5	**17**
Total	15	9	14	11	10	17	14	**90**

Trapping efficiency was reduced between the two release dates. 30% of released males were recaptured after the first release, while only 20% were recaptured after the second release. The lower recapture for the second release date may have been due to less time being available for

recapture, the release of fewer wasps on the second date, or female calling declining with age. Table 9-1 shows that there was an edge effect in the catch numbers of the released males, especially on the first release occasion. This shows that marked males were generally caught toward the centre of the grid, closest to the release point, rather than evenly across the grid further away from the release point.

Although males were capable of considerable movement, they were generally trapped close to the release point. Males moving large distances were presumably searching for calling females. This study provides further support that the sticky trap data from Chapter 6 are an accurate reflection of the number of males present in each block.

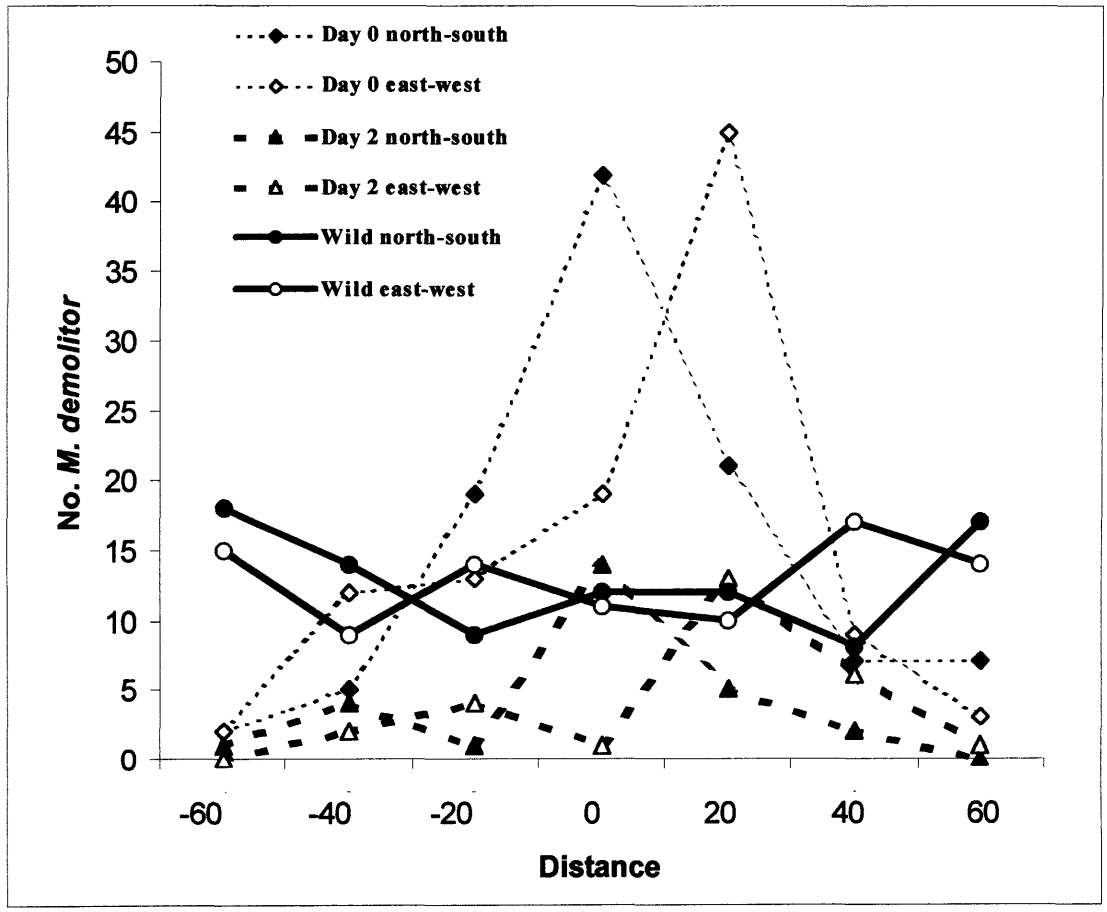

Figure 9-2. Total number for each axis of the grid of male *M. demolitor* caught in sticky traps baited with virgin females.

Wild males were equally trapped across the grid of traps. There was only a weak edge effect where more males were trapped in the outside row of traps (Figure 9-2). This indicates that a few indigenous males were drawn from the surrounding paddock, but this is not a major effect.

Figure 9-2 shows that males were not moving very far to become trapped and wind direction did not affect trap catches. This indicates that the wild population was present within the grid at the start of the trial and that there was limited immigration from outside the trial area. There was a large natural population of males present in the paddock at the commencement of the trial. This trial showed that there were 90 male wasps trapped from the 1.44 ha. of influence of the grid, equivalent to 62.5 wasps/ha. This estimate is lower that that found in Chapter 7 (ca. 300 male wasps per ha.) and probably reflects the lower density of wasps present at the time of the study.

This study demonstrated that fluorescent dust could be successfully used in release-recapture studies with adult *M. demolitor*. Fluorescent dust as a marker has many advantages. It is cheap, easy to use, different colours can be used on different release dates or locations, and large numbers of wasps can be marked quickly. Fluorescent powder has been previously used successfully for marking insects. Ramaswamy *et al.* (1985) found that *H. virescens* marked with florescent powder were not adversely affected. Laboratory observations indicated that most powder was groomed off by the wasps, except for a small patch on the rear of the thorax, which allowed easy recognition under the black light. This trial indicates that mate finding behaviour of male *M. demolitor* was unaffected by the fluorescent powder. However, any longer term effects on longevity and migration were not determined. The technique of using fluorescent dust was not used in the capture-recapture experiment (Chapter 7) because it was impracticable to use the black-light available in the field. However, with some modifications and refinement, a portable black-light could be used in the field.

Conclusions

Fluorescent powder was a useful tool in this release-recapture study. Male *M. demolitor* are most likely caught in the closest sticky trap baited with a virgin female. Male *M. demolitor* males do not move very far in response to these traps. At the time of this study there was a large natural population of male *M. demolitor* wasps (ca. 62.5 male *M. demolitor* adults/ha.) present in the biologically sprayed field.

Chapter 10

Base-line susceptibility and calibration of discriminating doses for *H. armigera* larvae to selected insecticides

Abstract

The aim of this study was to investigate the base-line susceptibility and discriminating doses for *H. armigera* larvae, to various insecticides, which were of suitable size for parasitisation by *M. demolitor* (0.005-0.148g). Methods for dosing *Helicoverpa* spp. larvae were examined and methods for topical and stomach application that conveniently produce reproducible data are described. Base-line dosage-mortality curves for second instar larvae were obtained for 7 stomach insecticides. These data show that the order of toxicity was chlorfluazuron (LC_{50}=0.009 μg ai/100mL insecticide/diet mixture) > lufenuron (LC_{50}=0.0016 μg ai/100mL insecticide/diet mixture) > spinosad (LC_{50}=0.0018 and 0.0023 μg ai/100mL insecticide/diet mixture) > methoxyfenozide (LC_{50}=0.0046 μg ai/100mL insecticide/diet mixture) > profenofos (LC_{50}=0.0345 μg ai/100mL insecticide/diet mixture) > *B. thuringiensis* (LC_{50}=0.1896 μg ai/100mL insecticide/diet mixture) > thiodicarb (LC_{50}=1.8414 μg ai/100mL insecticide/diet mixture). Larvae were treated topically with 4 insecticides testing contact activity. These data show that the ability of larvae to survive contact activity increased dramatically with age and size. This response in descending order, was chlorfluazuron > spinosad > methoxyfenozide > profenofos. Chlorfluazuron became insoluble in acetone before a response was observed in the largest larvae (0.104g). Profenofos remained relatively active against larger larvae with only a 10-fold increase in LD_{50} from the smallest to the largest larvae. These data reinforce recommendations that insecticide applications in the field should be targeted at early instar larvae. These data will be used for the study of the interactions between *M. demolitor*, host larvae and selected insecticides. The sub-lethal effects of 7 stomach insecticides were studied on *H. armigera* larvae. It was demonstrated that larvae treated with sub-lethal doses had negligible weight gain at concentrations which produced quite low mortality (20-30%). The implications for IPM are discussed.

Introduction

Extensive work has investigated the effects of insecticides on *H. armigera* and to a lesser extent, *H. punctigera*. Because of the long history of insecticide resistance in *H. armigera*, most work has been concerned with the monitoring of insecticide resistance in this species. Studies have

therefore followed the recommendations of resistance testing, based on the topical application of contact insecticides to third instar (35 ± 5 mg) larvae (Anon, 1970).

Wilson (1974) published bioassay data of the response of *H. armigera* and *H. punctigera* larvae from the Ord irrigation area compared to other areas where insecticide use had been limited. LD_{50} values for *H. armigera* treated with DDT varied from 0.88 to 80.71 µg/larva, toxaphene from 13.61 to 22.53 µg/larva, methyl parathion from 0.21 to 0.58 µg/larva, endosulfan from 3.17 to 3.24 µg/larva and monocrotophos between 0.71 to 0.88 µg/larva. These values varied because of the spray history of the area. LD_{50} values for *H. punctigera* were DDT 0.88 µg/larva, toxaphene 3.70 µg/larva, methyl parathion 0.12 µg/larva, endosulfan 0.89 µg/larva and monocrotophos 1.00 µg/larva. Goodyer *et al.* (1975) studied the toxicity of selected insecticides to *H. armigera* collected from the Namoi Valley, northern New South Wales. LD_{50} values from this site were DDT 36 µg/larva, endrin 6 µg/larva, endosulfan 5.6 µg/larva, methyl parathion 0.33 µg/larva and monocrotophos 1.03 µg/larva. The LD_{50} for *H. punctigera* collected at Bathurst tested with DDT was 0.15 µg/larva. These data were comparable to data published by Wilson (1974). Some differences compared to the earlier data are explained by strain differences. Kay (1977) published toxicity data for *H. armigera* and *H. punctigera* from material collected from northern New South Wales and southern to central Queensland. LD_{50} values for *H. punctigera* tested with DDT ranged from 1.1 to 1.4 µg/larva. LD_{50} for *H. armigera* tested with DDT ranged from 5.8 to >100 µg/larva, endosulfan 3.7 to 5.2 µg/larva and methyl parathion 0.05 to 0.15 µg/larva. These data indicate that there was a discernible increase in resistance levels to DDT and endosulfan, but there was no resistance to methyl parathion. Kay *et al* (1983) published base-line LD_{50} values for DDT, DDT and camphechlor mixture, endosulfan, methyl parathion, methomyl, fenvalerate, deltamethrin, profenofos and carbaryl tested on *H. armigera* collected from northern NSW and southern Queensland. These data were from larvae collected from 1975 to 1980, and are useful as base-line reference data for a specific age class against specific insecticides.

Spray applications should be directed against neonates. Therefore, it is more appropriate to perform bioassays with first instars. There are many advantages of testing first instar larvae compared to 30-40 mg larvae, including less laboratory rearing time per sample and the ease of producing neonates *eu masse*. Gunning (1993) developed a method of testing first instar

Helicoverpa spp. Values for LC_{50} for a susceptible strain of *H. armigera* were fenvalerate 5.8 mg/L, endosulfan 1 100 mg/L and methyl parathion 29.1 mg/L. LC_{50} values for *H. punctigera* were fenvalerate 2.4 mg/L, endosulfan 1 000 mg/L and methyl parathion 10.3 mg/L. These values were compared with values obtained using the standard test procedure of testing 30-40 mg larva topically. LD_{50} values for *H. armigera* and *H. punctigera* respectively were fenvalerate, 0.02 and 0.012 µg/larva; endosulfan, 1.1 and 0.6 µg/larva and methyl parathion 0.16 and 0.08 µg/larva. It was determined that these data could be compared due to the similarity in slopes of the probit lines. Results are useful as baseline data for first instar larvae.

The susceptibility of insects to most contact insecticides decreases with size (Busvine, 1971). This has been demonstrated for *H. zea* (Graves *et al.*, 1963; Gast, 1959; McPherson *et al.*, 1956). Although this is obvious, it is not clear why dose should be related to size. Changes in tolerance to insecticides at different stages have often been related to changes in the cuticle or to changes in size. For example, larvae may be more sensitive to contact poisons immediately after a moult (Robertson, 1942). Dubbeldam and McCaffery (1997) found that there was a transitional weight of 19 mg above which *H. virescens* larvae were significantly more susceptible to cypermethrin. The reason for this reduced tolerance may be due to a metabolic change, thinning of the cuticle or changes in growth patterns. The rate of increase of any discriminating dose may vary between species and between toxins. It is therefore critical to consider different sized larvae separately when testing contact poisons. Susceptibility to stomach poisons also decreases with size (Busvine, 1971), although this difference is often offset by the increased consumption of toxins by larger larvae. This has been demonstrated in *Helicoverpa* spp. with NPV (Teakle *et al.*, 1985a; Teakle *et al.*, 1985b; Teakle *et al.*, 1983) and *H. virescens* exposed to thiodicarb (Atwood *et al.*, 1997a). It is important to consider size effects when testing the effects of stomach toxicants, as with contact poisons.

As discussed in Chapter 4, *M. demolitor* prefers to attack second instar (5-6 mg) larvae, but will attack up to 3rd or 4th instar larvae (p.29). Outlined above is the extensive work investigating the effects of insecticides on *H. armigera* and to a lesser extent *H. punctigera*. Unfortunately these previous studies have focused on larvae not suitable for oviposition by *M. demolitor*. In order to

test insecticides on larvae which are suitable for *M. demolitor*, data must first be generated from suitably sized larvae.

Insecticides have been shown to retard growth and prolong the development of larvae when sub-lethal doses are administered. *H. virescens* larvae fed nicotine incorporated into standard diet had a prolonged development period and reduced weight compared to control larvae (Gunasena *et al.*, 1990). Weseloh and Andreadis (1982) showed that when treated with *B. thuringiensis*, gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae), development was delayed compared to untreated hosts. Retarded growth and prolonged development of larvae, from *B. thuringiensis* paralysing the gut and temporarily preventing feeding (Heimpel and Angus, 1959), was shown to promote parasitisation in the field because larvae remain at a suitable size for the host longer (Weseloh *et al.*, 1983). The braconid parasitoid *Apanteles melanoscelus* (Ratzeburg) (Hymenoptera: Braconidae) was shown to attack significantly more gypsy moth, *L. dispar*, larvae in field areas where *B. thuringiensis* has been applied, compared to control plots (Dunbar *et al.*, 1973, cited in Weseloh and Andreadis, 1982). The retarded growth of *B. thuringiensis* treated larvae has been demonstrated in the laboratory (Weseloh and Andreadis, 1982) where it was shown that treated hosts were parasitised significantly more than untreated larvae of the same age. This means that larvae receiving sub-lethal doses of insecticides may be important as hosts for parasitoids.

The aim of this study was to provide comparative data on the toxicity of 7 stomach and 4 contact insecticides to larvae of suitable size for oviposition by *M. demolitor* (second and third instar *H. armigera* larvae). These data will be used in subsequent studies of the interactions between larval *M. demolitor*, the host *Helicoverpa* spp. and some commonly used insecticides. These data also provide valuable base-line toxicity data of second to fourth instar *Helicoverpa* spp. to some commonly used cotton insecticides and some relatively newer insecticides in cotton production. The sub-lethal effects of the insecticides will also be examined and implications for IPM discussed.

Materials and methods

Insects

H. armigera larvae were reared using methods described in Appendix 1. Test larvae were separated from the main culture and reared on stainless steel trays three-quarter filled with standard diet. Steel grids were pushed into the diet to separate larvae and reduce cannibalism. Newly laid eggs were sterilized using a UV steriliser (see Appendix 3), then sprinkled onto the test trays. Larvae developed normally until required. This method usually produced many more larvae than were required, so only healthy larvae of the desired size were used. Tests were initiated when larvae were early second instar larvae, weighing 4-5 mg.

Bioassay

Bioassay methods were determined after extensive preliminary testing. Methods such as insecticide incorporation into artificial diet (Barbosa *et al.*, 1986; Thurston and Fox, 1972), the droplet feeding method (Teakle *et al.*, 1992; Hughes *et al.*, 1986), the leaf dipping feeding method (Furlong and Wright, 1993) and surface application on artificial diet (Mascarenhas *et al.*, 1997) were trialed for stomach poisons. Topical application and the Potter tower method (Potter *et al.*, 1947) were tested for contact poisons. On the basis of convenience, reproducability and accuracy, the diet incorporation method was used for stomach poisons and topical dosing was used for contact poisons.

Larvae were held individually in cells, thereby virtually eliminating cannibalism. Test trays usually stayed free of disease throughout the test period, with only occasional outbreaks of the fungus *Aspergillus* spp. Any trays infected with *Aspergillus* were discarded. Insects had to be handled only once in the stomach poison trial and twice in the topical dosing; reducing stress on test insects and losses due to escapes and mechanical damage.

Insecticides were selected on the basis of use, either present or likely future, for control of *Helicoverpa* spp. in Australian cotton. Representative insecticides were selected from all families

except the pyrethroids and organochlorine insecticide. These latter compounds were not tested because of known resistance in the *H. armigera* culture and likely complications this would create.

Stomach poisons

Stomach poisons tested were *B. thuringiensis* (powder), chlorfluazuron, lufenuron, methoxyfenozide, profenofos, spinosad (NAF-85), spinosad (XDE-105) and thiodicarb (Appendix 2).

Bioassay methods for testing the stomach poisons involved incorporation of insecticides directly into standard *Heliothis* diet (see Appendix 1). Larvae were fed *ad libitum* for 8 days. The amount of diet consumed was not recorded. A new stock solution of each insecticide was formulated for each trial and diluted in distilled water to form a series of stock solutions. Test concentrations were prepared by serial dilution of the stock solutions in distilled water. Concentrations were prepared within a predetermined range (determined through extensive preliminary range-finding), causing mortality between 1 and 99%. At least six concentrations were used to establish each dose-mortality response curve and at least 24 larvae were used to establish each point. Each dose-response test was replicated at least twice and pooled only if the fiducial limits overlapped. Test solutions were made up to a volume of 10 mL in distilled water and then thoroughly mixed with 90 mL of standard *Heliothis* diet making a total volume of 100 mL. This amount treated 24 larvae, so that each cell was filled with 4.2 mL of the insecticide/diet mixture. Controls were made up with 10 mL of distilled water only. Insecticide mixtures were added only after the diet had cooled to < 50°C to prevent breakdown of the chemicals. Early second instar larvae (4-5 mg) were placed individually in each cell after the diet had cooled and solidified on the insecticide-diet mixture. Generally, the insecticide-diet mixtures were made in the morning and larvae were 'plated-out' in the afternoon. Larvae were held under controlled temperature, humidity and photoperiod conditions before and after testing (25°C, 60-70% relative humidity and a 14:10 light: dark photoperiod).

Topical testing

Larvae were tested in 12-well Costar® tissue-culture trays (see Appendix 3). Each cell was about half filled with standard *Heliothis* diet (see Appendix 1). This was adequate for each larva to complete development. Each larva was placed individually in a cell, and trays were covered with a

porous plastic sheet (see Appendix 3) to prevent escape. Larvae were usually "plated-out" in the morning and tested in the afternoon. Larvae were held in controlled conditions before and after testing (Appendix 1). All glassware, syringes and pipettes were rinsed in copious amounts of laboratory grade acetone (see Appendix 3). Costar® trays were cleaned in detergent and sterilised in a weak (≈2%) bleach solution (see Appendix 3).

Insecticides tested were technical grade chlorfluazuron, methoxyfenozide, profenofos and spinosad (DE-105) (Appendix 2). Bioassay methods were modified from the Entomological Society of America standard test method for determining resistance in *Heliothis* spp. (Anon, 1970). Testing started with early second instar larvae (4-5 mg) and was repeated daily for 4 days. Larvae were weighed each day. On the final day, larvae corresponded to the maximum weight of a parasitised larva.

A new stock solution of each insecticide was formulated in analytical grade acetone (see Appendix 3) for each trial and then serially diluted to the desired concentration for each replication. Solutions were refrigerated over the trial period. Concentrations were within a predetermined range (determined through extensive preliminary range finding) to produce mortality of between 1 and 99%. At least four points were used to establish each dose-mortality response curve and at least 24 larvae were used to establish each point. Each dose-response test was replicated at least twice and pooled only if the fiducial limits overlapped. Reproducibility was considered an adequate test of accuracy. Test solutions were applied in order of ascending concentration, starting with the control. Each larva was dosed with 0.5 μL of acetone solution containing the test solution applied to the dorsal thorax using a Hamilton 25 μL microsyringe (see Appendix 3) fitted with a repeating dispenser (see Appendix 3). Control insects were treated with acetone alone. Larvae were handled as little as possible to reduce mortality through mechanical damage, and escapees.

Analysis

Mortality for both studies was assessed after 8 days. This interval was considered appropriate as it takes approximately seven days for *M. demolitor* to complete development within its host. This was considered important for subsequent studies as it was assumed that moribund

larvae would provide a developing internal parasitoid with resources to survive. Larvae were considered dead only if there were no life signs.

Data were initially examined using log/probit plots before being analysed by probit analysis (Finney, 1971) using Probit 5 for Windows (Gillespie, 1993). Adjustments for natural mortality were made using Abbott's formula (Abbott, 1925). Lethal doses are expressed in terms of μg of toxicant per larva for the topical study and in lethal concentration for the stomach poisons.

Sub-lethal effects

In order to quantify the sub-lethal effects an insecticide had on *H. armigera*, surviving larvae were weighed after treatment. Larvae were weighed initially in groups of 10 and then individually after the first day to the nearest 1 mg, using a Sartorius balance (see Appendix 3). Larval weights were plotted against percent mortality. 95% confidence intervals were generated for the percent mortality data using methods described by Devore and Peck (1993).

One additional study examined the sub-lethal effects of chlorfluazuron on pupation of *H. armigera* at very low doses. Dosing methods were the same as described above for stomach poisons. The concentrations of chlorfluazuron used in this study were below the concentrations which produced a mortality response within 8 days in the normal base-line study. 48 second instar larvae, for each concentration, were exposed to the insecticides as described above and the number of days taken from insecticide exposure to normal pupation was determined.

Results

Bioassay

Stomach insecticides

Bioassay data for the stomach poisons tested on *H. armigera* are presented in Table 10-1. Lethal concentrations are displayed as the LC_{50} :g active ingredient (ai) per 100 mL of insecticide/diet mixture. Each larva was exposed to 4.2 mL of this mixture. Unfortunately, the

amount of diet consumed by each larva was not recorded. Although recording diet consumption would have created many sampling problems, it certainly would have generated valuable data.

The IGR compounds, chlorfluazuron and lufenuron, were found to be the most effective at the lowest concentration. Spinosad, tested in two formulations, was next. There was no significant difference between spinosad formulations. Methoxyfenozide, a moult accelerating compound, was next in effectiveness, about a 6-fold increase in concentration from chlorfluazuron. There was roughly a 10-fold increase to the organophosphate, profenofos, about a further 7-fold increase to *B. thuringiensis* and lastly, a 10-fold increase to the least effective compound, the carbamate, thiodicarb. Responses to most compounds were homogeneous, except for one of the spinosad formulation and the *B. thuringiensis* formulation. This is an expected response from a laboratory strain which has been cultured for a fairly long period of time.

A steep slope of the dose-response curve indicates genetic homogeneity, which is expected from laboratory reared larvae. The slopes obtained in this study were quite varied, ranging from 1.3 for *B. thuringiensis* to 7.9 for profenofos. Since the strain tested had been cultured for a long period of time (Appendix 1), it is expected that the slopes would be steep.

Data from this trial were used to test the interaction between larval parasitoids, their host and insecticides (see Chapter 12). Although no formal study was undertaken, observations during this trial indicated that the amount of diet consumed decreased with increasing dose.

Table 10-1. Response of 4-5 mg *H. armigera* larvae to selected stomach poisons (LC :g ai/100 mL insecticide/diet mixture).

Insecticide	Number Tested	LC_{50}*	Fiducial limits	LC_{99}	Fiducial limits	Slope
chlorfluazuron	329	0.0009 **a**	0.0008-0.0009	0.0017	0.0014-0.0022	6.3
lufenuron	328	0.0016 **b**	0.0014-0.0019	0.0041	0.0032-0.0053	4.8
spinosad (XDE-105)	487	0.0018 **b**	0.0015-0.0021	0.0125	0.0078-0.0202	2.3
spinosad (NAF-85)	423	0.0023 **b**	0.0019-0.0027	0.0093	0.0070-0.0120	3.2
methoxyfenozide	418	0.0046 **c**	0.0040-0.0050	0.0155	0.0108-0.0222	3.6
profenofos	335	0.0345 **d**	0.0320-0.0371	0.0606	0.0537-0.0683	7.9
Bacillus thuringiensis	423	0.1896 **e**	0.1298-0.2769	5.3778	1.4893-19.4182	1.3
thiodicarb	475	1.8414 **f**	1.5894-2.1335	7.7996	5.4255-11.2126	3.1

* Values followed by the same letter do not differ significantly (tested by overlapping fiducial limits).

Contact insecticides

The dose-mortality response curves for the four contact insecticides tested topically on *H. armigera* larvae are presented in Table 10-2. Included in this table are the average weights of the test larvae and the number of larvae tested on each occasion. The size range of larvae corresponds to the size range suitable for oviposition by *M. demolitor*. 5-13 mg larvae correspond to second instar larvae, 25-51 mg larvae to third instar larvae and 104-148 mg larvae to fourth instar larvae. These data show that as larvae size increased the LD_{50} also increased, to the extent that in some cases the compounds became ineffective. These data show that profenofos was the only compound which remained relatively effective against large larvae (up to 135 mg), while spinosad and methoxyfenozide remained active against medium larvae (up to approximately 28 mg). Chlorfluazuron was only effective against small larvae (8 mg) and was ineffective against 20 mg and 40 mg larvae. Chlorfluazuron became insoluble in acetone before a response was observed in the largest larvae (104 mg). These results are not unexpected as profenofos is the only insecticide tested which has a predominately contact action; the other products are predominately stomach poisons but do have some contact action.

All products tested except profenofos produced response curves with low slopes. The slopes obtained with profenofos were quite steep, ranging from 3.6-8.

Sub-lethal effects after exposure to insecticides

Larval weights were recorded in order to quantify sub-lethal effects of insecticide on *H. armigera* larvae. Figure 10-1 to Figure 10-8 show the average weights of surviving larvae tested with stomach poisons at various sub-lethal concentrations of selected insecticides, plotted against the percent mortality. 95% confidence intervals were generated for the percent mortality data using methods described by Devore and Peck (1993). These data show that the weight of larvae was reduced significantly even at doses where mortality was very low, in the order of 20-30%. The effect of sub-lethal doses of *B. thuringiensis*, chlorfluazuron, lufenuron, and both formulations of spinosad on *H. armigera* larvae was a significant reduction in weight. This reduction was in the order of approximately 2/3, even at doses where mortality was less than 30%. The effects of sub-lethal doses of thiodicarb were not as dramatic as with the other products because reductions in larval weights occurred only when mortality was greater than 50%. All data show that all doses reduced the weight of larvae significantly. In all cases, weight gain by treated larvae was negligible when larval mortality was greater than 50%.

Figure 10-9 shows the number of days until pupation of second instar *H. armigera* larvae after dosing with chlorfluazuron. This figure shows that the concentration of chlorfluazuron which delayed and reduced pupation of *H. armigera* was well below the concentrations which caused knock-down mortality within 8 days (Figure 10-2). The implications of these data are discussed in the next section.

Table 10-2. Response of *H. armigera* treated topically with contact insecticides (µg ai/larva).

Larval weight (g)	No. tested	LD_{50}*	Fiducial limits	LD_{99}	Fiducial limits	Slope
profenofos						
0.005 ±0.001	337	0.0235 **a**	0.0176-0.0314	0.0819	0.0473-0.1418	3.6
0.013 ±0.001	216	0.0265 **a**	0.0239-0.0294	0.0511	0.0429-0.0608	6.8
0.025 ±0.001	144	0.0472 **b**	0.0360-0.0618	0.1261	0.0348-0.4564	4.5
0.041 ±0.003	120	0.0679 **b**	0.0490-0.0941	0.1743	0.0877-0.3466	4.7
0.135 ±0.006	96	0.1984 **c**	0.1096-0.3594	0.3458	0.0584-2.0460	8.0
spinosad						
0.005 ±0.001	408	0.0131 **a**	0.0103-0.0168	0.1162	0.0746-0.1810	0.8
0.008 ±0.001	186	0.0279 **b**	0.0200-0.0390	0.2005	0.0927-0.4337	1.5
0.028 ±0.001	238	0.0534 **b**	0.0387-0.0737	0.7352	0.3195-1.6916	0.7
0.051 ±0.002	216	0.1966 **c**	0.1515-0.2551	1.1787	0.7122-1.9507	0.4
0.125 ±0.007	118	0.5327 **d**	0.2721-1.0429	3.5302	0.0893-13.9538	0.4
methoxyfenozide						
0.005 ±0.001	359	0.0179 **a**	0.0135-0.0238	0.2207	0.0902-0.5397	1.8
0.013 ±0.001	174	0.0296 **ab**	0.0215-0.0408	0.2326	0.0995-0.5435	2.2
0.026 ±0.001	185	0.0532 **b**	0.0372-0.0762	0.4475	0.1485-1.3484	2.1
0.042 ±0.003	230	0.2873 **c**	0.1435-0.4138	13.8521	1.8081-106.1221	1.1
0.148 ±0.007	240	0.4785 **c**	0.2977-0.7690	18.1081	4.0177-81.6133	1.2
chlorfluazuron						
0.005 ±0.001	478	0.0273 **a**	0.0226-0.0328	0.1292	0.0961-0.1738	2.9
0.008 ±0.001	225	0.0515 **b**	0.0365-0.0727	0.9656	0.3135-2.9739	1.5
0.020 ±0.001	220	0.1205 **c**	0.0754-0.1925	4.0443	0.6032-27.1169	1.3
0.040 ±0.002	240	1.6608 **d**	1.012-2.7259	138.8892	16.7096-1154.4340	1.0
0.104 ±0.008	216	405172 **e**	**	**	**	0.2

* Values followed by the same letter do not differ significantly (tested by overlapping fiducial limits).

** Concentrations became insoluble in acetone before a response was observed.

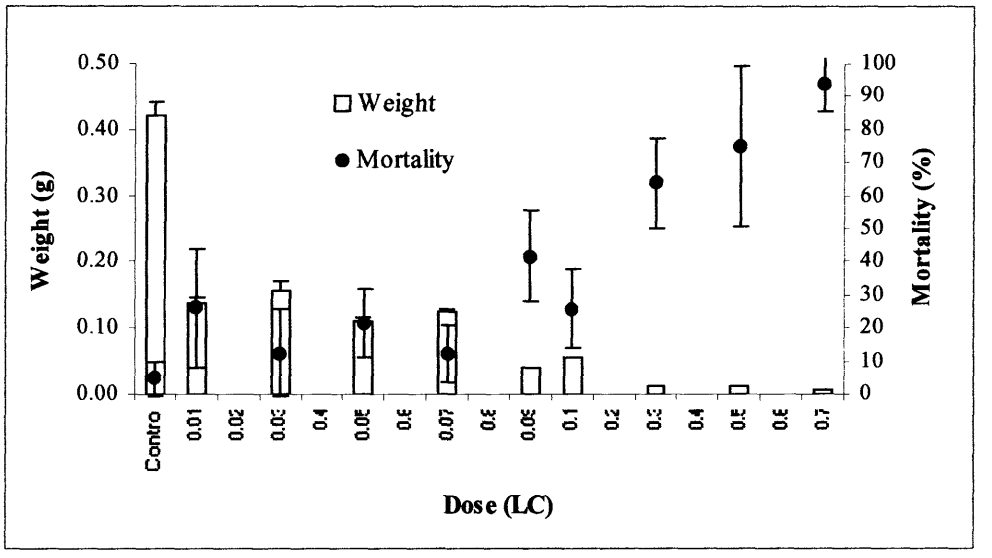

Figure 10-1. Weight and mortality of *H. armigera* larvae, after dosing with *B. thuringiensis*. Error bars for larval weight are standard errors of the means and error bars for percent mortality are 95% confidence limits.

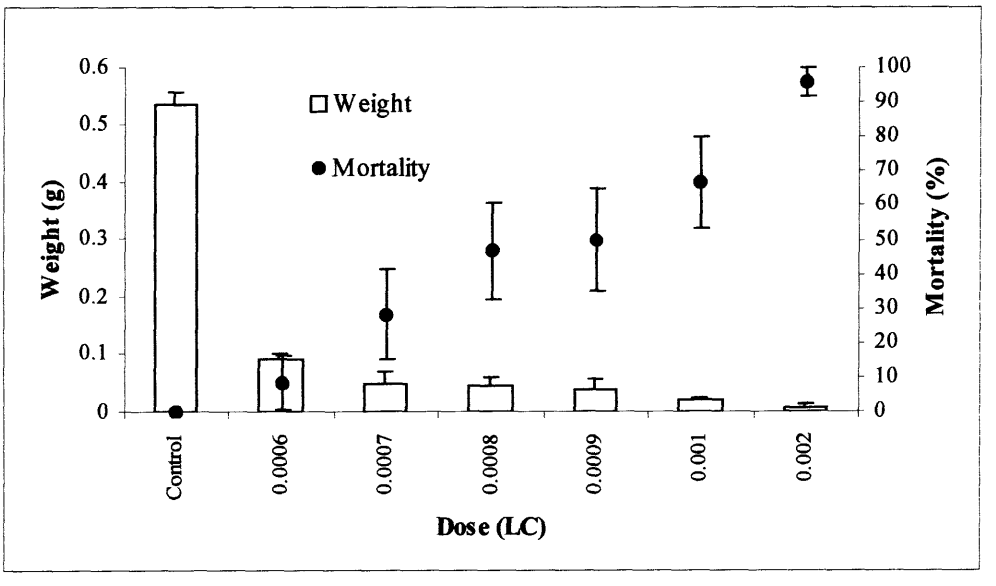

Figure 10-2. Weight and mortality of *H. armigera* larvae, after dosing with chlorfluazuron. Error bars for larval weight are standard errors of the means and error bars for percent mortality are 95% confidence limits.

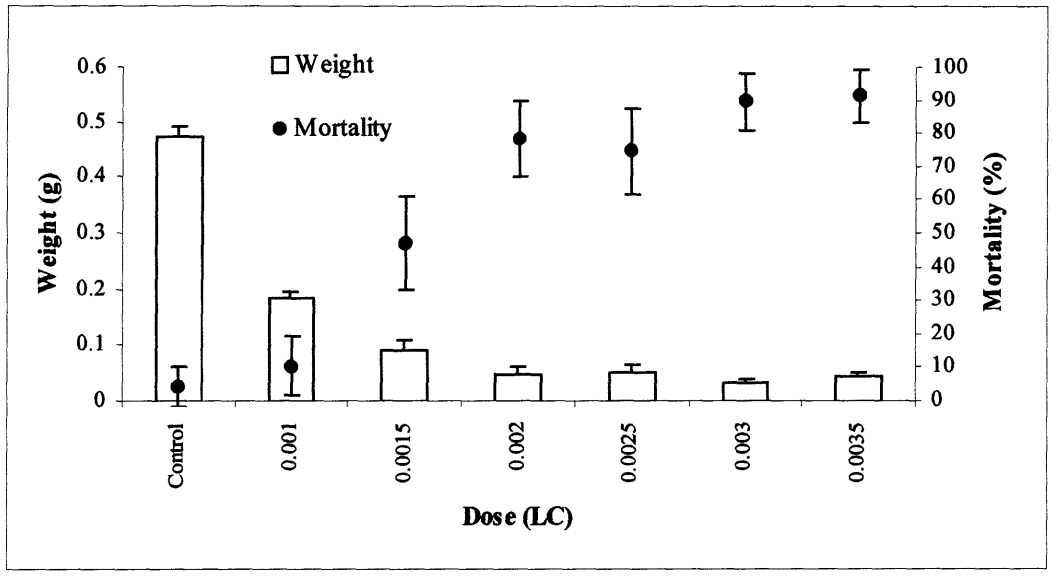

Figure 10-3. Weight and mortality of *H. armigera* larvae, after dosing with lufenuron. Error bars for larval weight are standard errors of the means and error bars for percent mortality are 95% confidence limits.

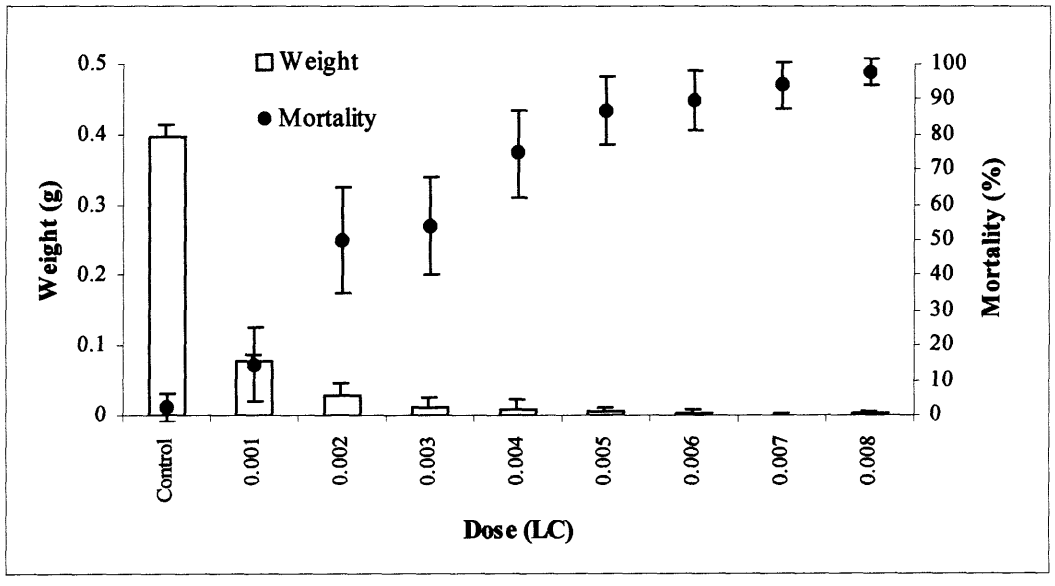

Figure 10-4. Weight and mortality of *H. armigera* larvae, after dosing with spinosad (NAF-85). Error bars for larval weight are standard errors of the means and error bars for percent mortality are 95% confidence limits.

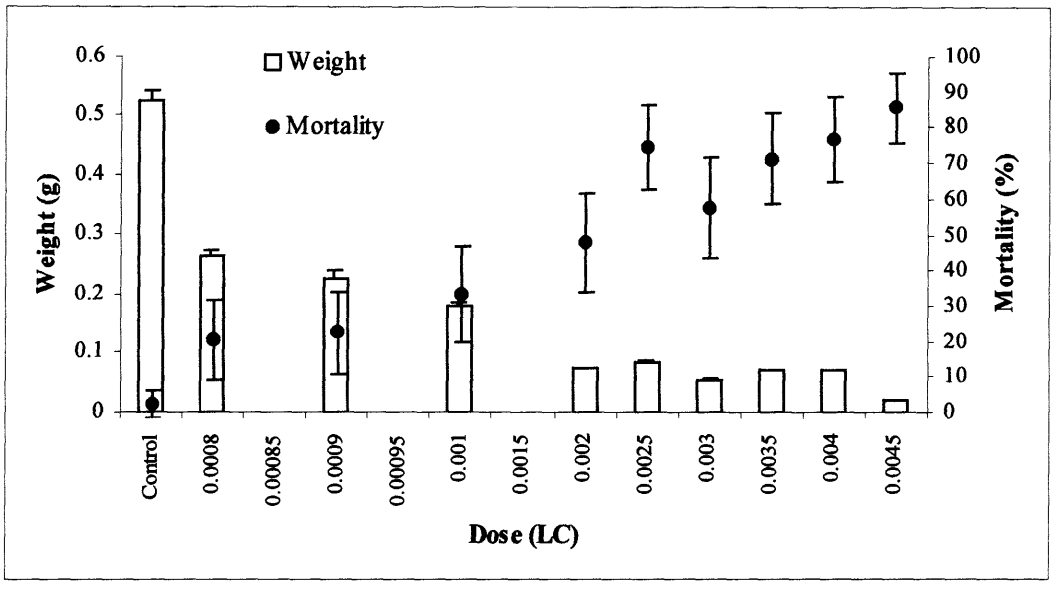

Figure 10-5. Weight and mortality of *H. armigera* larvae, after dosing with spinosad (XDE-105). Error bars for larval weight are standard errors of the means and error bars for percent mortality are 95% confidence limits.

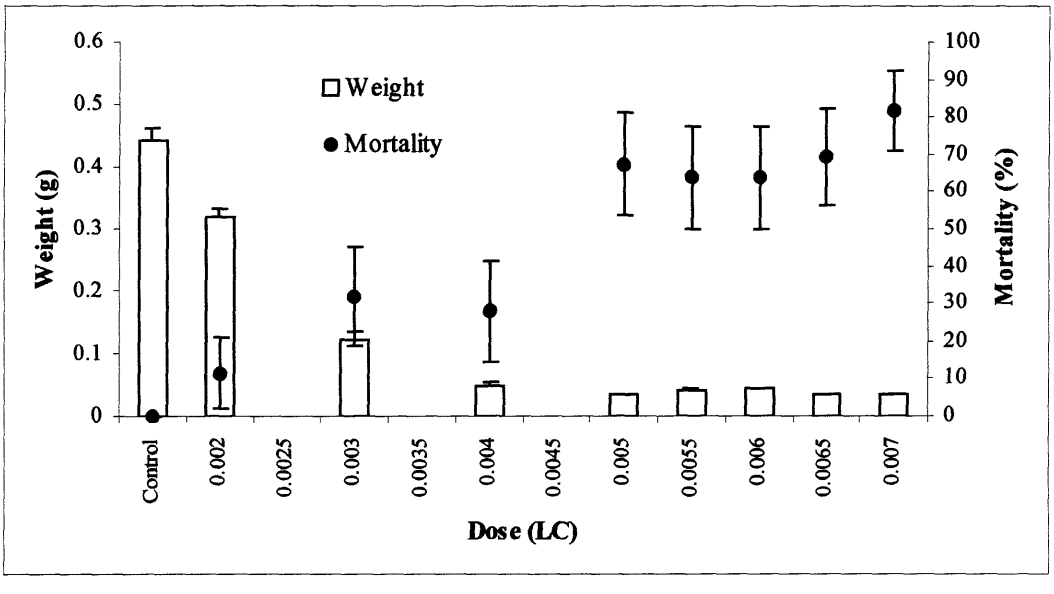

Figure 10-6. Weight and mortality of *H. armigera* larvae, after dosing with methoxyfenozide. Error bars for larval weight are standard errors of the means and error bars for percent mortality are 95% confidence limits.

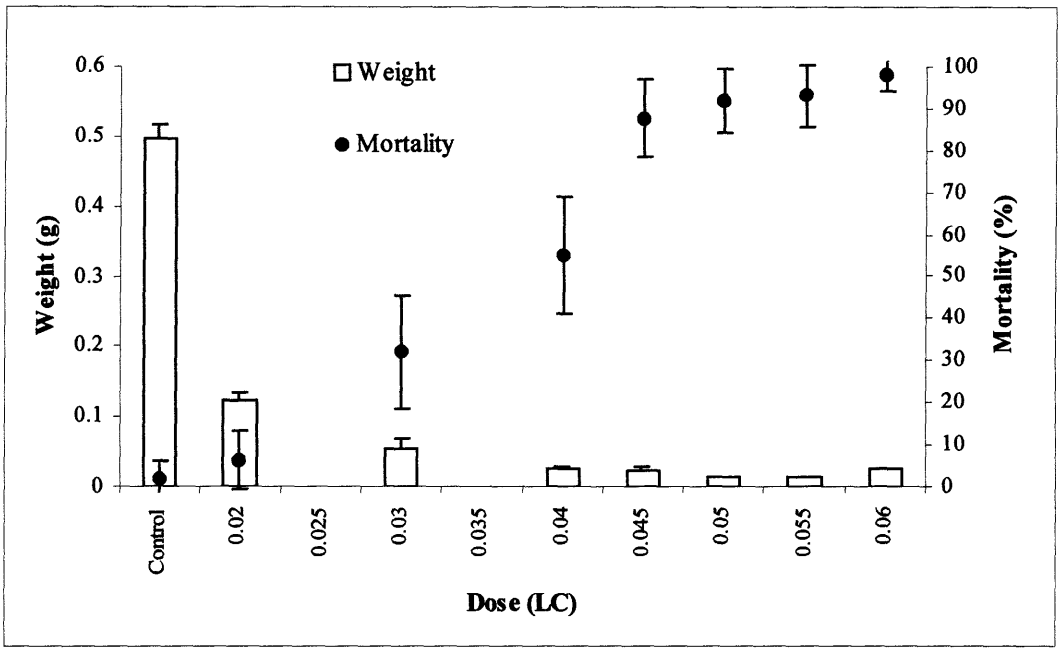

Figure 10-7. Weight and mortality of *H. armigera* larvae, after dosing with profenofos. Error bars for larval weight are standard errors of the means and error bars for percent mortality are 95% confidence limits.

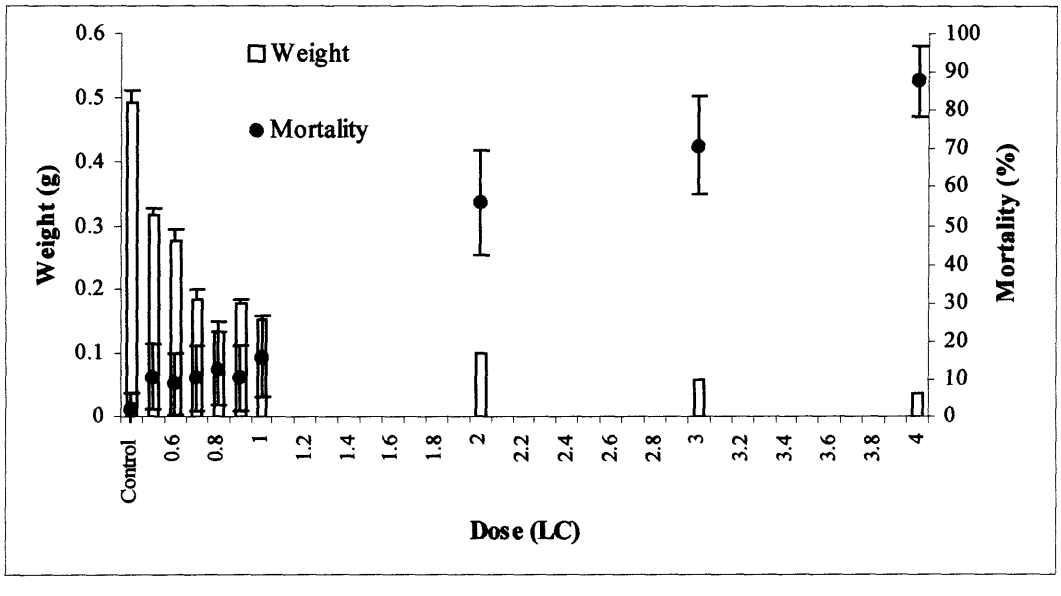

Figure 10-8. Weight and mortality of *H. armigera* larvae, after dosing with thiodicarb. Error bars for larval weight are standard errors of the means and error bars for percent mortality are 95% confidence limits.

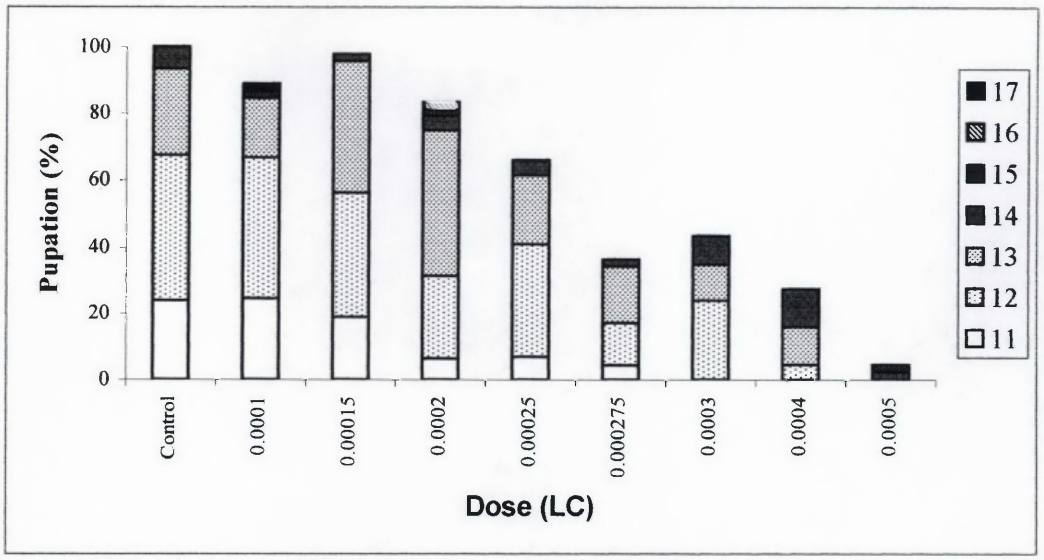

Figure 10-9. Days after dosing with sub-lethal concentrations of chlorfluazuron until pupation of second instar *H. armigera* larvae.

Discussion

Bioassay

The bioassay data from the 7 stomach insecticides tested (Table 10-1) show that the IGR compounds (see p. 14) chlorfluazuron and lufenuron as well as the MAC compound (see p. 14), methoxyfenozide, were the most effective against *H. armigera* at the lowest concentrations. The new spinosyn compounds were also very effective. There was no significant difference between the two formulations of spinosyns tested.

Bioassay data from the contact insecticides (Table 10-2) show that the sensitivity of larvae to contact insecticides decreased dramatically with age and size. This response was greatest for chlorfluazuron followed by spinosad, methoxyfenozide, and profenofos was the least affected. Chlorfluazuron became insoluble in acetone before a response was observed in the largest larvae (104 mg). Spinosad and methoxyfenozide had similar efficacy up until day 4 of testing. However, after day 4, there was a large increase in the dose of methoxyfenozide required to achieve the LD_{50}. This indicates that spinosad was slightly more effective against larger larvae. Profenofos remained relatively active against larger larvae with only a 10-fold increase from the smallest to the largest

larva. This is expected as profenofos is the only insecticide tested with a primary contact action. These data reinforce recommendations that insecticide applications in the field should be targeted at early instar larvae.

Large differences in discriminating doses (from 2 to 6-fold) have been observed from *H. armigera* and *H. punctigera* collected from different areas. These differences have been attributed to strain differences rather than differences from resistance.

Different species and closely related species respond differently to the same compound. Wilson (1974) found that tolerance to different insecticides, not due to insecticide resistance, varied widely between *H. armigera* and *H. punctigera*. Species specificity is caused by differences in the uptake of poison, penetration of the poison, degradation of the poison and the intrinsic toxicity after penetration. Conversely, Teakle *et al.* (1992) found that both *H. armigera* and *H. punctigera* were equally susceptible to *B. thuringiensis*, so studies need not be duplicated on both species. The discriminating dose response to specific insecticides will most probably change between closely related species, but it is not unreasonable to expect that trends will be similar. No data were obtained for *H. punctigera* in this study because of the lack of a suitable laboratory reared colony. Attempts were made to start a *H. punctigera* colony, however, this colony failed to produce sufficient larval numbers. It would have been beneficial to test *H. punctigera* larvae, especially with the pyrethroids and endosulfan. These were not tested because of known resistance in *H. armigera*. The data produced with *H. armigera* are adequate to test the interactions with *M. demolitor* and insecticides described in Chapter 12.

The method of feeding stomach poisons incorporated in artificial diet is rather crude. This method provides no data on the amount of poison eaten or absorbed, nor does it discriminate fully between contact and stomach action. Some stomach poisons may be repellent or cause vomiting, therefore reducing the uptake of the poison. The amount of poison absorbed will also depend on the time it is retained in the intestine. Toxicity of poisons may vary with temperature, water-solubility and in turn how the pH of the insect gut affects this solubility. Sometime poisons may pass through the target insect with very little absorption of the toxin by penetration through the gut. These factors are unavoidable and cannot be eliminated whichever method is used. After

testing methods such as feeding treated leaf punches, surface treating artificial diet, and the droplet feeding method, each method had advantages and disadvantages. As a large number of trials with different insecticides were planned, including extensive preliminary work associated with each trial, convenience and reproducibility were considered crucial. Therefore the diet incorporation method was chosen.

Accuracy in these bioassays was considered critical. Often, laboratory studies are more appropriate than field studies as variables are reduced. The only uncertainty in laboratory studies is the concentration of the solution. However, laboratory tests are time consuming and labour intensive. Stomach poisons tested by direct feeding methods where feeding is *ad libitum* are imprecise owing to the uncertainty of uptake. Accuracy may be improved by using artificial diet which eliminates any possible substrate effects. How the laboratory generated data described here relate to field data is unclear. It is difficult, and often unwise, to extrapolate data obtained from the laboratory to the field. There are many variables which must be considered and a generalised relationship between laboratory doses and field rates has not been described. Williams (1973) showed that as much as 80% of pesticide applied aerially in the field is not deposited on the crop. Williams (1973) described a method for extrapolating laboratory data into the field, for the western spruce budworm, *Choristoneura occidentalis* Freeman (Lepidoptera: Tortricidae). They suggested that laboratory lethal dose values could be multiplied by a factor of 3. Although laboratory studies are somewhat artificial and removed from the real world, they are critical as a first step in examining trends in susceptibility to insecticides, and necessary to show that certain insecticides are non-toxic.

Data produced in this study are useful as base-line dose responses to the tested insecticides. These data will be used in Chapter 12 for investigating the development and survival of *M. demolitor* larvae developing in host *H. armigera* larvae exposed to selected insecticides.

Sub-lethal effects

Under field conditions, larvae commonly receive sub-lethal doses of insecticides. The effects of these sub-lethal doses on pest larvae have been poorly studied. Ali and Watson (1982) observed sub-lethal effects on *H. virescens* larvae treated with *B. thuringiensis*. They demonstrated

reduced weight gain, which may relate in the field to reduced crop damage. Larvae dosed with sub-lethal concentrations in this study showed significantly reduced weight gain, compared to control larvae, at insecticide concentrations which caused less than 20-30% mortality. Target insects which receive a sub-lethal dose may die during pupation or emerge as sterile adults (M. Thirugnanam, unpublished data, 1995). Dosing with chlorfluazuron in this study caused delayed and reduced pupation of *H. armigera* at very low doses. Often, at very low concentrations of all products, larvae were observed to pupate successfully. However, adults would fail to emerge or were deformed if they did. This is important in the field, as future generations will be reduced. Other sub-lethal factors which may be important include effects on adult longevity, fecundity, egg viability or F_1 larval survival. These aspects were not examined in this study. It is clear that sub-lethal effects are often underestimated in the field, and if the larvae affected by them are providing a food source for predators, their presence may be desirable. Larvae receiving sub-lethal doses gain negligible weight, indicating they would cause little damage to the crop, and in the case of chlorfluazuron, pupation was delayed and reduced. It may therefore be advantageous for farmers to target larvae with a sub-lethal dose, thereby reducing harm to beneficials and spray costs.

The use of reduced insecticide application rates in the field is already occurring in cotton. Some studies have found that multiple applications of low rates of certain insecticides are better than a single higher rate. This has been shown for Tracer® for outbreak situations of beet armyworm (Hendrix, *et al.*, 1997).

Larvae receiving sub-lethal concentrations of insecticide may be a suitable source of food for predators or hosts for parasitoids such as *M. demolitor*. Successful parasitism of gypsy moth larvae which survived *B. thuringiensis* exposure was generally high, decreasing somewhat with later parasitism but not with increased *B. thuringiensis* concentration (Wesloh and Andreadis, 1982). Wesloh and Andreadis (1982) showed that larvae exposed to *B. thuringiensis* were attacked more readily than unexposed larvae. Substances which retard development of larvae but do not harm parasitoids will increase parasitism. Preliminary investigations in the laboratory were carried out to determine if larvae receiving sub-lethal doses were suitable as hosts for *M. demolitor*. A group of larvae surviving a variety of sub-lethal doses of chlorfluazuron were parasitised 8 days after exposure. Although most larvae failed to produced a parasitoid, a small

number produced a *M. demolitor* adult. This is encouraging and requires further study in the field to show that larvae treated with sub-lethal doses are a suitable source of food for predators and hosts for parasitoids.

This study has important implications for resistance in *H. armigera*. It is unclear how the use of sub-lethal concentrations in the field would affect the development of resistance in *H. armigera*. It was thought that *H. armigera* resistance may have developed due to the widespread use of low concentrations of pyrethroid against sorghum midge, or by insects recovering from non-lethal doses (Anon, 1983). This has been questioned (Forrester *et al.*, 1993). Whether the use of low or high doses promote the incidence of resistance is unclear (see Chapter 2).

Conclusions

This study generated baseline data for second instar *H. armigera*. The IGR compounds, chlorfluazuron and lufenuron as well as the MAC compound, methoxyfenozide, and spinosad were the most effective stomach compounds tested. These data are a useful reference tool and will be used in the study on the interactions between *M. demolitor* larvae, host and insecticides. These data are significant for IPM because the compounds found to have greatest activity on *H. armigera* are reported to be less detrimental to beneficials. The sub-lethal data generated in this trial show that although affected larvae do not die, their weights are significantly reduced, therefore resulting in reduced damage to the crop. Thus larvae affected by sub-lethal doses will cause negligible damage in the crop, and may be suitable sources of food for predators or hosts for parasitoids.

Chapter 11

Toxicity of insecticides to adult and pupal *M. demolitor*

Abstract

The aim of this study was to investigate the effects of 10 insecticides, either currently or potentially used for control of *Helicoverpa* spp. or *C. dilutus* (green mirid) in cotton, on adult and pupal *M. demolitor*. The IGR compound, chlorfluazuron and MAC compound, methoxyfenozide, were essentially harmless to adult *M. demolitor*. Toxicity in descending order of the tested products to adult *M. demolitor* was as follows: primicarb < endosulfan ≤ dimethoate ≤ cyhalothrin < profenofos < deltamethrin ≤ bifenthrin < spinosad. The pupal stage afforded *M. demolitor* some protection from the effects of insecticides. However, the pyrethroids were disruptive at 10 times the adult LD_{50}, and highly toxic at 100 times the adult LD_{50}. Primicarb and endosulfan caused a slight increase in mortality of pupal *M. demolitor* at 100 times the adult LD_{50}. The implications of these results for IPM in Australian cotton are discussed.

Introduction

Conservation of indigenous beneficials is considered crucial to IPM (see Chapter 3). This can be achieved by reducing the impact of pesticides on them, by using pesticides with a specialised mode of action, timing application to a stage when the beneficial is most tolerant, targeting pest species when they are most susceptible, or by using pesticides which are more harmful to the pest species relative to key beneficial species (see p. 25). By reducing the impact of pesticides on non-target insects, the action of indigenous beneficials will be increased and pesticide usage will be reduced.

Using pesticides with a specialised mode of action may conserve beneficials. The use of stomach poisons rather than contact poisons may reduce the impact on beneficials. Most parasitoids consume mostly nectar, which reduces potential insecticide consumption. For example, Bartlett (1966) studied two species of parasites, *Aphytis melinus* (Debach) (Hymenoptera: Aphelinidae) and *Metaphycus luteolus* (Timberlake) (Hymenoptera: Encyrtidae). He found that broad-spectrum organophosphates and carbamates were extremely toxic while the organochlorines

were almost non-toxic. Bartlett concluded that using stomach poisons could reduce the impact of insecticides on beneficials. Powell *et al.* (1986) studied the effects of the carbamate thiodicarb on *M. croceipes*. They found that they could not mix a concentrated enough dose, because the technical grade insecticide became insoluble in acetone, before any effect on the parasitoid was observed. This was due to the negligible contact action of thiodicarb. They recommended thiodicarb for use in any IPM program targeted at *Helicoverpa* spp.

Synchronising insecticide application with the most tolerant stage of the parasitoid's life cycle may conserve beneficials. Parasitoids are holometabolous. This means that the larval and adult stages are very different in body form and habit and are separated by a quiescent pupal stage. Bartlett (1964) suggests that the pre-pupal and pupal stages of most natural enemies were relatively immune to toxicants. Lingren *et al.* (1972) found that the pupal stage of *Campoletis perdistinctus* (Viereck) (Hymenoptera: Ichneumonidae) and *Apanteles* (= *Cotesia*) *marginiventris* (Cresson) (Hymenoptera: Braconidae) survived doses of one hundred times the LD_{50} determined for the adults. Ruberson *et al.* (1993) tested pupal *C. marginiventris* with field rates of selected insecticides. They found that malathion and high rates of profenofos and cyhalothrin caused significant mortality, while low rates of profenofos and cyhalothrin caused approximately 50% mortality. Thiodicarb and diflubenzuron caused slight mortality. Dumbre and Hower (1976) found that the alfalfa weevil parasitoid (*Microctonus aethipoidea* (Nees) (Hymenoptera: Braconidae)) was considerably more resistant to carbofuran, methoxychlor, methyl parathion and methidathion in the pupal stage than in the adult stage.

Targeting lower concentrations of insecticides at the most vulnerable stage of the pest can reduce disruption of beneficials. Like beneficials, pest insects have tolerant and susceptible stages in their life cycle. The juvenile stage may be the most susceptible. In addition, larger animals generally require greater doses for control (Busvine, 1971). As a result, if pest species, especially lepidopteran pests, are targeted early in the juvenile stage, lower doses are required for adequate control. Abu and Ellis (1977) used topical application to compare the toxicity of two carbamates (carbofuran and carbaryl), two organophosphates (malathion and phosmet) and an organochlorine (methoxchlor) to the adult alfalfa weevil, *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae) and its parasitoids, *Bathyplectes curculionis* (Thomson) (Hymenoptera: Ichneumonidae) and *M.*

aethipoidea. They found that all insecticides tested were more toxic to the parasitoid compared to the pest. However, the carbamates were relatively less toxic than the organophosphates or the organochlorine. They also found that if the pest was targeted as adults rather than larvae there was a selective advantage to the parasitoid.

Generally males are more susceptible to contact insecticides than females. This is thought to be because females are often larger than males, with larger doses required to invoke a response in larger insects (Busvine, 1971). Lingren *et al.* (1972) found that male *C. perdistinctus* were more susceptible than female parasitoids to certain insecticides. Studies have shown no significant difference in response to test insecticides between sexes of *M. croceipes* (Elzen *et al.*, 1989; Powell *et al.*, 1986). This was probably due to the lack of significant difference in the weight of sexes.

Many studies have recorded higher toxicity of insecticides to parasites relative to their host. Cate *et al.* (1972) found that the organophosphates, monocrotophos and disulfoton, and the carbamate, aldicarb, were more toxic to the parasitoid *C. perdistinctus* compared to its hosts, *H. virescens* and *Spodoptera frugipenda* (Smith) (Lepidoptera: Noctuidae). Rechov (1974) found that the parasitoid *Chenolus inanitus* (L.) (Hymenoptera: Braconidae) was far more susceptible to residues of the organophosphates, azinphos-methyl, parathion, chlorpyrifos and monocrotophos, the carbamate, methomyl, and the organochlorine, endosulfan, than the pest *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). Dumbre and Hower (1976) found that *M. aethiops* was more susceptible to carbofuran, methoxychlor, methyl parathion and methidathion, compared to adult weevils. For the most part, these studies recorded the toxicity of specific insecticides to specific parasitoids. Classifying insecticides into specific families and recording their effects on beneficials as groups is more relevant.

Recent studies have found that certain insecticides are relatively less harmful to parasitoids compared to the target species. Beneficials may be conserved through the use of these insecticides. Lingren *et al.* (1972) used topical dosing to determine the toxicity of insecticides to *C. perdistinctus* and *A. maginiventris,* important in the control of *H. zea* and *H. virescens.* They found that some organophosphates and organochlorines were not toxic to the parasitoid, although

others were very toxic. Plapp and Vinson (1977) studied *Campoletis sonorensis* (Carlson) (Hymenoptera; Ichneumonidae), a key parasitoid of *H. virescens*, by exposing parasitoids to insecticide residues on glass. They found that almost all the insecticides tested were more toxic to the parasitoids than to the pest. However, they concluded that synthetic pyrethroids and phosphorothiolates (s-alkyl organophosphates) were least harmful to parasitoids. Wilkinson *et al.* (1979) exposed *A. marginiventris* to insecticide treated filter paper. They found that synthetic pyrethroids were less toxic than the organophosphates to the parasitoid relative to the host. They stated that the pyrethroid, fenvalerate, could be used satisfactorily in an IPM system. Ruberson *et al.* (1993) found that *C. marginiventris* was highly susceptible to the pyrethroid, cyhalothrin, as well as the organophosphates, profenofos and malathion. However, the carbamate, thiodicarb, and diflubenzuron were essentially non-toxic. In general, the results of the above studies are mixed, although some conclusions can be drawn. They suggest that some insecticide groups, such as the synthetic pyrethroids (fenvalerate) and some specific insecticides from the organochlorines and organophosphates are relatively less harmful to beneficials than to the pest.

Strong circumstantial evidence suggests that *M. croceipes* is tolerant of certain insecticides, with high levels of parasitism recorded from heavily sprayed fields (King *et al.* unpublished data, cited in Powell and King, 1984). Extensive work has shown this species to be relatively non-susceptible to certain classes of insecticides used to control its hosts. Powell and Scott (1985) caged *M. croceipes* on field treated cotton plants. They found that residues of flucythrinate, thiodicarb and fenvalerate caused only 3.8%, 12.5% and 17.1% mortality respectively. These levels of mortality were considered acceptable. Powell *et al.* (1986) topically tested the toxicity of 14 insecticides to *M. croceipes*. They found that some insecticides, namely chlordimeform, diflubenzuron and thiodicarb were effectively harmless due to negligible contact action. Organophosphates, such as methyl parathion, chlorpyrifos, azinphos-methly, and malathion were all highly toxic. The organochlorine, toxaphene, was moderately toxic. Pyrethroids, such as fenvalerate and flucythrinate, and the carbamate, methomyl, were the least harmful insecticides tested. They concluded that an observed increase in field parasitism may have been due to a reduction in insecticide use and a change to pyrethroids rather than organochlorines and organophosphates. Elzen *et al.* (1987) tested *M. croceipes* topically with field rates of selected insecticides. Their results showed that *M. croceipes* was non-susceptible to the pyrethroid

fenvalerate. However, when the synergist chlordimeform was added, the knockdown effect of the pyrethroid was increased, which may increase indirect mortality. The carbamate, methomyl, was extremely toxic, contradicting earlier laboratory work of Powell *et al.* (1986). The organophosphate, acephate, was also extremely toxic. Elzen *et al.* (1987) concluded that thiodicarb and pyrethroid/chordimeform mixtures may conserve natural enemy populations. Elzen *et al.* (1989) exposed *M. croceipes* adults to residues of pyrethroid/formamidine mixtures and the carbamates, thiodicarb and methomyl. They found that only methomyl caused a significant increase in mortality compared to the other insecticides. These results agreed with their earlier study (Elzen *et al.*, 1987). Powell and Scott (1991) studied the effects of selected organophosphate, carbamate and pyrethroid insecticide residues on *M. croceipes*. Their results suggest that *M. croceipes* is relatively tolerant to all the tested insecticides except the organophosphates, profenofos and acephate, and the pyrethroid, bifenthrin. These results agree with past work.

In summary, *M. croceipes* is susceptible to organophosphates (except phosphates) and organochlorines (except toxaphene and DDT), tolerant of some carbamates (oxime compounds) and highly tolerant of most pyrethroids (except bifenthrin). Maneb (manganese ethylenebisdithiocarbamate, a fungicide) was harmless to *M. croceipes* adults (Felton and Dahlman, 1984). Bell *et al.* (1974) found that the bacterium *Serratia marcescens* Bizio was highly pathogenic to *M. croceipes* when ingested. The bacterium was practically harmless when topically applied.

M. demolitor has been studied less thoroughly than its American relative. Culin and Dubose (1987) tested the effects of insecticides on the larval stage of *M. demolitor* during development in the host *H. zea*. They found that insecticides such as chlordimeform, the organophosphate, methyl parathion, and the pyrethroid, fenvalerate, significantly reduced the percentage of parasitoid pupation. Other studies have found that parasitoids are more tolerant of insecticides while in the larval stage (Abu and Ellis, 1977; Dumbre and Howe; 1976; Croft and Brown, 1975; Duodu and Davis, 1974). This topic will be covered in more detail in Chapter 12. No work has been done on the tolerance of adults to insecticides. Circumstantial evidence, such as abundant *M. demolitor* in heavily sprayed cotton fields (D. Murray, *pers. com.* see Chapter 3 and Chapter 6), indicates that this species may be tolerant to some classes of insecticides.

This study reports the dose-response of adult and pupal *M. demolitor* to currently used or potential insecticides for control of *Helicoverpa* spp. and green mirids, *C. dilutus*, in cotton. Insecticides were selected from all families of insecticides and applied topically. The purpose of this study was to determine which insecticides are least toxic to *M. demolitor* and if *M. demolitor* is more tolerant during the pupal stage compared to the adult stage. These data will be compared to the effects on *Helicoverpa* spp. (Chapter 9). This information will be useful in recommending insecticide use in cotton IPM programs to ensure conservation of *M. demolitor*.

Materials and Methods

Insects

M. demolitor adults and pupae were used from laboratory reared insects using methods described in Appendix 1.

Bioassay

Insecticides were selected on the basis of use, either present or likely future, for control of *Helicoverpa* spp. and green mirids in cotton. Insecticides were from all families of insecticides. Test insecticides were technical grade chemicals, including alpha-cypermethrin, bifenthrin, chlorfluazuron, lambda-cyhalothrin, deltamethrin, dimethoate, endosulfan, methoxyfenozide, primicarb, profenofos and spinosad (Appendix 2).

Test cages for adults were made from plastic Solo® cups with the top and bottom cut out and replaced with cotton mesh to allow air circulation. A maximum of 10 wasps were held in each cage. Wasps were provided with honey which was smeared on the fabric and water which was supplied through a cotton dental wick. Honey and water were supplied daily. Wasps were held in controlled environmental conditions before and after testing (Appendix 1). Test cages for the pupae were small glass vials (10 cm long x 5 cm diameter) with pop-on lids. Lids had a small circle cut out (approximately 2 cm diameter) and replaced with fine wire mesh to allow air circulation. Ten pupae were held in each vial. All glassware, syringes and wasp cages were either disposed of

or washed with copious amounts of laboratory grade acetone to remove any insecticide residues after use. All reusable material was sterilised in a weak (≈2%) bleach solution.

Bioassay methods were modified from Powell *et al.* (1986). A new stock solution of each insecticide was formulated for each test on the basis of weight/volume of active ingredient in analytical grade acetone, then serially diluted to the desired concentration for each replication. Concentrations were prepared within a predetermined range, determined through extensive preliminary range finding. These ranges produced mortality of between 1 and 99%. Five to nine points were used to establish each dose-mortality response curve, and at least 25 wasps were used to establish each point. Each dose-response test was replicated at least twice and pooled only if the fiducial limits overlapped. Reproducibility of the tests was assumed to be an adequate test of accuracy.

Newly emerged wasps were sexed and males were transferred to test cages (maximum of 10 wasps per cage), and allowed to feed. Females were required for colony maintenance. Insects were used for tests when they were between 24-48 hours old. Wasps were anaesthetised with diethyl ether (see Appendix 3) for about 1 minute. Diethyl ether was used instead of CO_2 or cooling for convenience and because wasps took longer to recover. To avoid any adverse effects due to prolonged exposure to ether, wasps were dosed in small groups of 10 and treatment was carried out as rapidly as possible. Preliminary tests indicated that there were no apparent adverse effects or mortality attributed to the diethyl ether. Each wasp was dosed with 0.5 µL of test solution to the dorsal surface of the pronotum using a Hamilton 25 µL microsyringe fitted with a repeating dispenser. Acetone was applied first as the control, and doses were then applied in ascending order. After treatment, wasps were returned to controlled conditions and supplied with honey and water. Wasps were monitored for behavioral abnormalities and mortality was assessed 24 hours after treatment. Wasps were considered dead or moribund if they were incapable of coordinated movement, including normal walking.

Data were initially examined by hand using log/probit plots before being analysed by probit analysis (Finney, 1971) using Probit 5 for Windows (Gillespie, 1993). LD_{50}, LD_{99}, slope and 95% confidence intervals were determined. Differences between LD_{50}'s were indicated by non-

overlapping of confidence intervals. Adjustments for natural mortality were made using Abbott's formula (Abbott, 1925). Lethal doses for the topical study are expressed in terms of μg of ai per wasp.

Wasps weights were determined. Newly emerged wasps were sexed, anaesthetised and weighed to the nearest 1 mg (Sartorius Balance). Wasps were weighed in batches of between 7 and 14 depending on the number of wasps available at the time of weighing, and weights were averaged. A one-way analysis of variance was used to compare the weights of male and female wasps.

Pupae were tested using a similar method to adult dosing. Pupae were obtained attached to brown paper (Appendix 1) and divided into 4 equal groups, with at least 50 pupae in each group. Pupae which were in diapause were discarded (see p. 29). Pupae were treated less than 5 days after pupating. They were dosed with 0.5 μL of 1, 10, and 100 times the LD_{50} concentration of each insecticide, previously determined for the adults. Dosing was done using the Hamilton 25 μL micro-syringe as described for the adult wasps. Emergence of the wasps was scored a few days after emergence of the control group had ceased. Wasps were considered dead due to the insecticide if they failed to emerge normally from the pupal case. Occasionally wasps would die as they emerged, and these were considered as insecticide mortality, as insecticide residues on the pupal case or sub-lethal effects were the probable cause of death.

Results

Adult stage

Comparative toxicity, LD_{50} values along with the 95% confidence limits and slopes of the probit lines of the 11 insecticides applied topically to *M. demolitor* are presented in Table 11-1 in order of ascending toxicity. No dose-response curve was obtained for chlorfluazuron or methoxyfenozide as these insecticides became insoluble in acetone before a response was observed.

The two IGR compound were essentially non-toxic to *M. demolitor*. The carbamate, primicarb, was the least toxic of the insecticides tested. Generally the organochlorine, endosulfan, organophosphates, dimethoate and profenofos, and synthetic pyrethroid, cyhalothrin, were all very toxic to *M. demolitor*. These compounds were grouped together with a 22 fold increase in toxicity compared to the carbamate. There was about a 3 fold increase in toxicity from the highest to the lowest member of this group. The synthetic pyrethroids, bifenthrin, deltamethrin and alpha-cyhalothrin, were highly toxic with a 3 fold increase from the lowest member of the group. There was a significant difference between these three pyrethroids, with cyhalothrin significantly less toxic than deltamethrin and bifenthrin. Spinosad was extremely toxic to *M. demolitor*, and was by far the most toxic compound, with a 3.6 fold increase in toxicity compared to bifenthrin and a 750 fold increase compared to the carbamate.

There was considerable variation in the extremes of the dose-response curves. For this reason, the LD_{50}'s are of specific interest.

Wasp weights

Results indicate that there was no significant difference in weights of male and female wasps ($P = 0.386$). The mean weight of male wasps was 2.04 mg ± 0.05, (n=56) and female wasps was 2.12 mg ± 0.05 (n = 54).

Table 11-1. Response of male *M. demolitor* adults to topically applied insecticides (µg ai/wasp).

Insecticide	Number tested	LD_{50}*	Fiducial limits	LD_{99}	Fiducial limits	Slope
chlorfluazuron	**	**	**	**	**	**
methoxyfenozide	**	**	**	**	**	**
primicarb	320	0.6758 **a**	0.5481-0.8332	4.0504	2.4003-6.8350	2.5
endosulfan	120	0.0303 **b**	0.0200-0.0459	0.1180	0.0519-0.2685	3.3
dimethoate	200	0.0222 **b**	0.0185-0.0266	0.0594	0.0383-0.0922	4.5
lambda-cyhalothrin	120	0.0155 **bc**	0.0104-0.0233	0.1231	0.0274-0.5524	2.1
profenofos	326	0.0110 **c**	0.0094-0.0129	0.0285	0.0214-0.0380	4.7
deltamethrin	304	0.0035 **d**	0.0029-0.0042	0.0085	0.0048-0.0152	5.0
bifenthrin	225	0.0033 **d**	0.0025-0.0043	0.0098	0.0046-0.0209	4.1
spinosad	312	0.0009 **e**	0.0008-0.0010	0.0021	0.0016-0.0028	5.3

* LD values followed by different letters are significantly different (tested by overlapping fiducial limits).

** Doses became insoluble in acetone before there was any observed effect on adult *M. demolitor*.

Pupal stage

Results from the testing of *M. demolitor* while in the pupal stage are presented in Table 11-2. These data show that all compounds tested caused no mortality at the LD_{50} for the adult. The pyrethroids, cyhalothrin and deltamethrin, caused 95% and 100% mortality of pupae at 10 times the adult LD_{50} and all pyrethroids, (cyhalothrin, bifenthrin and deltamethrin) caused 95%, 100% and 100% morality of pupae at 100 times the adult LD_{50}. The carbamate, primicarb, and the organochlorine, endosulfan, caused a slight increase in mortality at the 100 times the adult LD_{50}. Endosulfan may have caused this increase when adults emerged as it is a very persistent compound. The IGR, chlorfluazuron, MAC, methoxyfenozide, and Naturalyte, spinosad, caused no increase in pupal mortality at all concentrations.

Table 11-2. Mortality of *M. demolitor* pupae (%) dosed with acetone, one, 10, and 100 times the LD_{50} determined for *M. demolitor* adults (see Table 11-1).

Insecticide	Control	LD_{50}	10 x LD_{50}	100 x LD_{50}
chlorfluazuron	*	*	*	*
methoxyfenozide	*	*	*	*
primicarb	5	6	6	20
endosulfan	16.7	14	10	32
profenofos	3.8	6.6	6.3	2.1
dimethoate	8.2	6	8	2
lambda-cyhalothrin	10.4	8.3	95	95
bifenthrin	6.1	2.1	8.2	100
deltamethrin	7.5	12.5	100	100
spinosad	4	0	0	4

Discussion

M. demolitor adults and pupae were not susceptible to the IGR compound, chlorfluazuron or the MAC compound, methoxyfenozide, as was expected. These compounds have a specialised mode of action, a very specific range of activity and little contact activity. Powell *et al.* (1986) found that diflubenzuron, an IGR compound, was inactive against *M. croceipes*. These compounds were among the most potent chemicals tested against *H. armigera* (Chapter 10) making them ideally suited for use in any IPM program.

The carbamate insecticide, pirimicarb, was relatively non-toxic to *M. demolitor*. Pirimicarb was the least toxic of the conventional insecticides tested. The LD_{50} was comparable to the figure obtained for *M. croceipes* tested with another carbamate, carbaryl (Powell *et. al.*, 1986), but was 53 fold greater than for *C. perdistinctus* tested with the same insecticide (Lingren *et al.*, 1972). Tests of the toxicity of field rates of carbamates on *M. croceipes* have found thiodicarb and oxamyl to be non-toxic, while methomyl was extremely toxic (Powell and Scott, 1991; Elzen *et al.*, 1989; Elzen *et al.*, 1987). Elzen *et al.* (1989) suggests that if *M. croceipes* escaped direct treatment with the carbamate, methomyl, in the field, they would probably survive. The low slope of the dose-response curve for primicarb indicates there was a large variation in the response to this compound. No comparison of dose-response can be made against *Helicoverpa* spp., as pirimicarb

has negligible activity against *Helicoverpa* spp. and is used for control of the cotton aphid, *Aphis gossypii*. An important carbamate compound, thiodicarb, is targeted at *Helicoverpa* spp. control in cotton. Thiodicarb was not tested against adult *M. demolitor* as it has little contact action (Powell *et al.*, 1986). The LD_{50} for thiodicarb for *H. armigera* (Chapter 10) was not significantly different to that obtained for pirimicarb for *M. demolitor*, indicating similar tolerances to some carbamate compounds.

The organochlorine, endosulfan, was toxic to *M. demolitor* adults. The LD_{50} for *M. demolitor* was about 10 fold greater than the result for another organochlorine, toxaphene, tested on *M. croceipes* (Powell *et. al.*, 1986). and *C. perdistinctus* (Lingren *et al,* 1972). Powell *et. al.* (1986) found only a 1.6 fold increase in toxicity between the carbamate and organochlorines they tested. Lingren *et al.* (1972), did not state the weight of *C. perdistinctus*, so it is unclear if this is a factor. Plapp and Bull (1978) tested the toxicity of endosulfan residues against *C. sonorensis*. The LD_{50} of 0.67 μg/vial was intermediate in toxicity compared to the other insecticides tested. England *et al.* (1997) tested endosulfan residues against *C. marginiventris*. Initially, endosulfan was very toxic but the long term effects were slight, with 100% surviving after exposure one day after treatment. Endosulfan resistance in *H. armigera* was known for the laboratory colony, so comparison between parasitoid and pest is not possible.

The organophosphates, dimethoate and profenofos, were highly toxic to *M. demolitor*. The high toxicity of organophosphates agrees with work with *M. croceipes* (Powell and Scott, 1991; Elzen *et al.*, 1987; Powell *et al.*, 1986), *C. sonorensis* and *C. marginiventris* (Ruberson *et al.*, 1993; Wilkinson *et al.*, 1979; Plapp and Vinson, 1977). Similar results were found for *C. perdistinctus*, although a few compounds were found to be relatively less harmful (Cate *et al.*, 1972; Lingren *et al.*, 1972). The doses obtained for *M. demolitor* generally agree with the published range of doses for *M. croceipes*, although they are at the toxic end of the range. The high toxicity of the organophosphates may be due to the rate of detoxification being insufficient to counteract the rapid rate of absorption. This was found to be the case for malathion tested on *M. croceipes*. Malathion was extensively metabolised to primary non-toxic metabolites. However, a small amount of the highly toxic malaoxon was produced, and proved fatal (Bull *et al.*, 1989).

All pyrethroids tested were very toxic to *M. demolitor*. Cyhalothrin was the least toxic and deltamethrin and bifenthrin the most toxic. Bifenthrin has been found to be highly toxic to *M. croceipes* (Powell and Scott, 1991). Previous studies have shown that mixed isomer pyrethroids, such as permethrin and cypermethin, were relatively safe to parasitoids compared to the phytophagous pest. However, pure isomer pyrethroids, such as lambda-cyhalothrin (Karate®), were very toxic. Ruberson *et al.* (1993) showed that adult *C. marginiventris* were highly susceptible to field rates of the pyrethroid, cyhalothrin. Work with *M. croceipes* (Elzen *et al.*, 1989; Elzen *et al.*, 1987; Powell *et. al.*, 1986) and other hymenopteran parasitoid species, such as *C. sonorensis* and *C. marginiventris* (Wilkinson *et al.*, 1979; Plapp and Vinson, 1977) have shown high levels of tolerance to certain synthetic pyrethroids. Fenvalerate was commonly found to be non-toxic to parasitoid species (Powell and Scott, 1991; Elzen *et al.*, 1989; Elzen *et al.*, 1987; Powell *et al.*, 1986). The relatively high levels of tolerance of *M. croceipes* to pyrethroids and DDT are probably attributable to factors associated with the interactions of these chemicals with target site receptors (Bull *et. al.*, 1989). Bull *et al.* (1987) indicated that methyl parathion and the cyclodiene compounds, dieldrin and aldrin, were clearly the most toxic; fenvalerate was least toxic. The *cis* and *trans* isomers of permethrin exhibited low levels of toxicity. The *cis* isomer was about 2.5 times more toxic than *trans*-permethrin. None of the pyrethroids used in the trial were mixed isomer pyrethroids, which is unfortunate because these are supposedly less toxic to parasitoids. High densities of *M. demolitor* have been observed in fields heavily sprayed with pyrethroids (D. Murray, *pers. com.*). This may be due to the use of mixed isomer pyrethroids. However, other factors such as the repellent properties of pyrethroids (Elliott, *et al.*, 1973) may be important.

Spinosad proved to be extremely toxic to *M. demolitor*. It is a new insecticide aimed at the control of Lepidopteran pests, and was by far the most potent compound tested. This insecticide was approximately 750 fold more toxic than the carbamate and approximately 10 to 30 fold more toxic than the other insecticides tested. Field trials have established that spinosad is non-disruptive to certain predator groups (Hendrix *et al.*, 1997; Murray and Lloyd, 1997; Peterson *et al.*, 1996) but may be disruptive to Hymenoptera (Murray, 1996). Hendrix *et al.* (1997) found that if wasps avoided direct initial contact with the insecticide, once dry, residues were harmless. This means that application timing is crucial for conservation of *M. demolitor* if spinosad is to be used. This must be tested for *M. demolitor* in the field.

This study (Table 11-2) shows that pupal *M. demolitor* were protected from insecticides at doses which caused significant mortality in the adult stage. However, the pyrethroids, cyhalothrin and deltamethrin, caused 95% and 100% pupal mortality at 10 times the adult LD_{50} and all pyrethroids, cyhalothrin, bifenthrin and deltamethrin, caused 95%, 100% and 100% mortality respectively of pupae at 100 times the adult LD_{50}. The carbamate, pirimicarb, and organochlorine, endosulfan, caused a slight increase in mortality at the 100 times the adult LD_{50}. Endosulfan may cause this increase when adults emerged as it is a relatively persistent compound. The IGR, chlorfluazuron, MAC, methoxyfenozide, and Naturalyte®, spinosad, caused no increase in pupal mortality at all concentrations. These data concur with those of Ruberson *et al.* (1993), who found that field rates of profenofos and cyhalothrin caused significant mortality, while low rates of profenofos and cyhalothrin caused approximately 50% mortality of pupal *C. marginiventris*. These results also agree with the study of Lingren *et al.* (1972) where *C. perdistinctus* and *A. marginiventris* pupae were essentially non-susceptible to most insecticides, although they did observe isolated mortality which appeared to be due to insecticide residues on the pupal case causing mortality upon adult emergence. Whether this protection is through the pupal case preventing penetration of the insecticides is not clear. It could also be due to developing pupae being less susceptible to insecticides, by mechanisms such as reduced metabolism of the developing pupae or better degradation of insecticides. These results show that if insecticides, with the exception of the pyrethroids, are applied while the parasitoid is in the pupal stage, then their impact is essentially negligible.

Due to a shortage of insects (most females were needed for colony maintenance), no insecticides were tested on females. This is most unfortunate because it is the female wasps that exert the control over *Helicoverpa* spp., not the males. It is assumed, because of the similar size of male and female *M. demolitor*, that the response to insecticides will be similar. Elzen *et al.* (1989) working with *M. croceipes* found no significant difference in response between males and females.

Some results of this study agree with the results of previous studies with hymenopteran parasitoids, such as *M. croceipes* and *C. peridistinctus*. Some values in the *M. croceipes* study are significantly greater and some values in the *C. peridistinctus* study are somewhat lower than the

values determined in this study. This poses the question, can concrete conclusions about the responses of groups of insects to different families of insecticides be made reliably? Different species of natural enemies respond differently to the same compound, even for closely related species such as *C. plutella* and *C. marginiventris* (Pietrantonio and Benedict, 1997). The results of Lingren *et al.* (1972) working with *C. perdistinctus* show that there can be great variation of responses to insecticides within the same families of insecticides. They obtained ranges of LD_{50} for the organophosphates tested of between 0.0004-2.0593 µg/insect (>5000 fold), and for the organochlorines of between 0.0009-0.2800 µg/insect (>300 fold). Powell *et al.* (1986) obtained ranges in their LD_{50}'s for the organophosphates of between 0.0132-0.3087 µg/insect (23 fold) and for the synthetic pyrethroids tested of between 0.2031-0.9399 µg/insect (4.6 fold). This means that it is very difficult to draw conclusions of the effects on beneficials due to the action of families of insecticides.

Some prior studies found that parasitoids are more susceptible to some insecticides than others. Comparisons of responses to these insecticides between the pest and the parasitoid, with very few exceptions, show that the parasitoid is many times more susceptible than the pest (Plapp and Bull, 1985; Plapp and Vinson, 1977). Bull *et al.* (1989) compared the LD_{50}'s for *M. croceipes* and *H. virescens* in terms of µg/gram of insect weight. They found that the ratios were fairly similar or in fact favoured the parasite. This means that although one insecticide may be relatively less toxic to the parasitoid than another, any dose that provides adequate control of the target pest species will kill the adult parasitoid. Care must be taken when comparing insecticides. In the study of Plapp and Vinson (1977), they compared 25-50 mg *H. virescens* larvae to the parasitoid, although much smaller larvae would be targeted for control. A more feasible approach may be to target the pest when the parasitoid is in a less susceptible phase in its life cycle. The relative toxicity of products is probably irrelevant, as the dose targeted at any pest insect will be very much higher than any predetermined laboratory LD_{50}, and is likely to be higher than beneficials could survive.

The results of this trial do not consider any sub-lethal effects on the wasps, such as reduced host searching, mobility, fecundity, longevity, and mate finding behavior. Effects on development and rates of predation and parasitism have also been reported (Croft, 1977 cited in Croft and Whalon, 1982). The sub-lethal effects of pyrethroids on beneficials are virtually unknown, although

repellency has been recorded. Elzen *et al.* (1989) found that when *M. croceipes* were exposed to plants treated with pyrethroids, their ability to locate hosts and even fly was reduced. They also found that carbamates and pyrethroids significantly affected the parasitoid's foraging behavior. Testing the efficacy of insecticides against parasitoids is not the best way of testing the impact of insecticides on beneficials, because beneficial impact can be reduced without an increase in beneficial mortality (Franz *et al.*, 1980). A more appropriate method would be to test the number of hosts parasitised per female between unsprayed and sprayed treatments. This is very difficult and probably inappropriate as a first step in investigating the effects of insecticides on beneficial populations. Toxicology tests are preferred because of their greater accuracy and reproducibility (Franz *et al.*, 1980).

How these data generated in this study relate to what is happening in the field is unclear. Williams (1973), working with the western spruce worm suggests that field doses of 3 times the laboratory discriminating doses will be adequate. However, it is doubtful such a simple extrapolation is valid. For instance, Powell and Scott (1991) caged *M. croceipes* on newly sprayed cotton plants treated with synthetic pyrethroids, carbamates and organophosphates in the field. They found that apart from bifenthrin, acephate and profenofos, >70% of wasps survived. However, work by Rechav (1974) with the braconid *C. inanitus*, a parasitoid of *S. littoralis*, showed that residues from all insecticides tested, including organophosphates, an organochlorine and a carbamate were toxic to the parasitoid for longer than they effectively controlled the pest. Rechav (1974) concluded that this was a major problem in the development of an IPM program in cotton. It is of course possible to show that certain insecticides are non-toxic. Results indicating that a certain substance is harmless under stringent lab conditions will most probably be confirmed in the field, but not necessarily *vice versa*.

Any effects on susceptibility of test insects because of inbreeding in the laboratory culture are unknown. It is assumed that such effects were negligible. An attempt was made to collect *M. demolitor* from an area which had received low selection pressure from insecticides, (central Queensland), in order to obtain baseline data. Unfortunately low incidence of parasitism in collected larvae made establishment of a susceptible culture impossible. Inbreeding depression (Lincoln *et al.*, 1982) is the phenomena whereby discriminating doses are lowered as a result of

inbreeding. This was observed in *S. exigua* after six generations (Wolfenbarger *et al.*, 1997). Most published studies of the effects of insecticides on beneficials have used insects which had been cultured for long periods of time. It must be acknowledged that the effects of using cultured test material may have affected the results. It is very difficult to establish baseline data from field collected material, because of the large number of insects required to carry out this work. As it was, numbers of cultured insects were never adequate to supply demands.

Laboratory studies such as those presented here are valuable for describing the response of *M. demolitor* to various groups of insecticides. The fact that such relatively small differences in LD_{50} were significantly different indicates that the assay procedure is precise. However, field studies are needed to validate these data. Future research should target field collected *M. demolitor* for insecticide tolerances to insecticides, using the work described here as baseline data. Future work could also evaluate the effects of insecticide residue on *M. demolitor* under field conditions.

The effects of using diethyl ether as an anesthetic are unclear. An anaesthetic such as ether shuts the sodium channels that the neurotransmitter acetylcholine uses to excite nerve cells, and at the same time opens chloride channels that the neurotransmitter GABA uses to inhibit other nerve cells (Knight, 1999). How this affects the action of insecticides on nerve channels, especially the antichlolinesterase compounds, is unclear. Ether was used in this study due to ease of handling and convenience. Although no obvious mortality effects were observed, interactions between insecticides and ether should be examined further.

The variation in response of *M. demolitor* to insecticides may help in developing IPM programs which include biological and chemical control of the insect pests of cotton. The selection of insecticides should be determined on the basis of their effects on *M. demolitor* and other beneficial insects in all aspects of their life cycle as well as the effects on the target pest insect. Although some insecticides may be relatively less toxic to *M. demolitor* than others, it is important to consider that, with the exception of the IGR compounds, they are all toxic to some degree and are therefore expected to disrupt *M. demolitor* populations. It is therefore important that disruptive sprays are avoided or are applied when adult wasps are expected to avoid initial contact with the insecticide. Elzen *et al.* (1989) suggested a similar situation for *M. croceipes*, that if wasps escaped

direct treatment with insecticides such as methomyl in the field, they would probably survive. The use of insecticides has two impacts on natural enemies. Insecticides not only reduce natural enemy numbers directly but also reduce their food source. The reduction in their food resources may prevent successful colonization of the field (England *et al.*, 1997). Bull *et al.* (1989) suggested that for *M. croceipes*, the observed tolerance to certain insecticides may be enhanced through a selection program. If a high level of insecticide tolerance could be induced in a laboratory population, and if economical methods for mass production were available, *M. croceipes* could have an excellent potential for use in an augmentative biological control program. This potential is similar for *M. demolitor*.

Conclusions

This study shows that the toxicity of tested compounds, in ascending order were chlorfluazuron = methoxyfenozide < primicarb < endosulfan ≤ dimethoate ≤ cyhalothrin < profenofos < deltamethrin ≤ bifenthrin < spinosad. This study showed that the pupal stage of *M. demolitor* was protected from the effects of insecticides, except the pyrethroids.

A long term goal is to rank the selectivity of registered insecticides against the more important beneficial insects so as to provide recommendations for IPM programs. In this study, only the IGR compounds and the carbamate, pirimicarb, were of lower toxicity to the parasitoid than the pest. None of the other insecticides tested in the present study were acceptable for use in an IPM program. However, targeting insecticide applications when *M. demolitor* is in the pupal stage will reduce the impact of most disruptive insecticides, and field studies will probably modify these results slightly, because of differences between stomach and contact action and in the persistence of residues.