Chapter 12

Development and survival of larval *M. demolitor* in host *H. armigera* larvae exposed to selected insecticides

Abstract

The aim of this study was to investigate the impact on the development and survival of larval *M. demolitor* when parasitised host *H. armigera* larvae were exposed to 7 stomach and 4 contact insecticides. Tests were carried out using predetermined LD_{99} for unparasitised *H. armigera* larvae for each insecticide (Chapter 9). Parasitised larvae were exposed to the discriminating dose 0 to 7 days following oviposition. It was determined that the direct impact on developing larval *M. demolitor* after exposure of the host to stomach and contact insecticides was negligible. However, all insecticides tested had a dramatic indirect impact on the larval parasitoid through mortality of the host. This study shows that parasitisation by *M. demolitor* reduced the susceptibility of the host to stomach poisons and to lesser degree to contact poisons late in the parasitoids development. This was thought to be due to reduced feeding in the case of stomach poisons, although other factors such as reduced metabolism may be important. The implications of this study for IPM in Australian cotton are discussed.

Introduction

Previous studies have demonstrated that some adult hymenopteran parasitoids are tolerant of some insecticides during the adult and pupal stages. This has been demonstrated for *M. demolitor* and *M. croceipes* (Elzen *et. al.*, 1987; Powell *et. al.*, 1986; see Chapter 11). However, the impact of insecticides on larval parasitoids developing within host larvae has not been studied in great detail. Some studies have indicated that the larval stage of parasitoids may be protected from the effects of insecticides. Effects of insecticides on developing parasitoids may include an increase in parasitoid survival, a decrease in parasitoid survival, an increase in parasitoid development time and disruption of parasitoid development. Often, parasitoid development is affected by host mortality, although the larval parasitoid is unaffected. Insecticides have also been shown to promote the action of parasitoids through prolonging the development time of the host larvae, so that larvae remain suitable for parasitism for longer.

Exposure of parasitised host larvae to insecticides has been shown to disrupt parasitoid larval development. This can be through a disruption in pupation, cocoon formation, egression or death of the larval parasitoid. Atwood *et al.* (1997b) studied the effects of *B. thuringiensis* and thiodicarb on the development of *M. croceipes* larvae within host *H. virescens* larvae, using a method of diet incorporation. They found that *M. croceipes* emergence from the host was inversely related to insecticide concentration. Overall, their tests indicate that thiodicarb had a greater impact than *B. thuringiensis*. Atwood *et al.* (1997a; 1995) found that pupation of *Cotesia marginiventris* Cresson (Hymenoptera: Braconidae) was significantly reduced when host *H. virescens* larvae were exposed to *B. thuringiensis* or thiodicarb. However, they found that *C. marginiventris* emergence was increased when exposure of the parasitised host to insecticide was delayed 48 hours after parasitism. Felton and Dahlman (1984) examined the effects of the fungicide maneb (manganese ethylenebisdithiocarbamate) to larval *M. croceipes* developing in *H. virescens*. They found that maneb was toxic to larval *M. croceipes* when parasitised hosts were fed relatively low concentrations of the fungicide. Parasitoid emergence was stopped and cocoon formation was inhibited. Horton *et al.* (1986) studied the effect of the fungicides benomyl and thiabendazole on *M. croceipes* in *H. zea* larvae which were fed either artificial diet with the fungicides incorporated within or applied to soybean foliage. Both fungicides significantly reduced successful parasitoid survival, measured by percent emergence of larval parasitoids. McNeil (1975) suggested that juvenile hormone insecticides were highly detrimental to *Aphidius nigripes* (Hymenoptera: Braconidae), a parasitoid of the potato aphid, *Macrosiphum euphorbiae* (Thomas) (Homoptera: Aphididae). He found that all parasitoids died as pupae even at very low concentrations, which were much lower than the effective doses for the pest. Furlong and Wright (1993) studied the effects of the chitin biosynthesis inhibitor (teflubenzuron) on *Cotesia plutellae* Hellén and *Diadegma semiclausum* Kurdjumov, parasitoids of the diamondback moth, *Plutella xylostella* L. They found that the insecticide had no apparent effect on the number of cocoons formed by either species, but induced mortality in the transition from pupa to adult, when chitin biosynthesis is particularly active.

In addition to parasitoid mortality, pesticides have been shown to prolong the larval stage of the parasitoid when host larvae are treated. Vinson (1974) found that parasitised *H. virescens* treated with juvenile hormone significantly prolonged development times of *C. nigriceps*. He also

showed that parasite numbers were reduced and the sex ratio was affected. Ahmad and Forgash (1976) found that topically treating parasitised gypsy moth larvae with carbaryl increased the development times of larval *A. melanoscelus*. Whether this was due to carbaryl affecting the parasitoids' development, or whether carbaryl selectively killed those larvae with faster developing parasitoids is unknown. They also suggested that male parasitoids were more sensitive to carbaryl. Gunasena *et al.* (1990) studied the effects of nicotine on the growth, development and survival of larval *Campoletis sonorensis* (Cameron) (Hymenoptera: Ichneumonidae) developing in *H. virescens*. They found that low concentrations of nicotine prolonged larval development and disrupted larval egression. They also found that parasitoid survival was reduced due to mortality of the host. Whether the prolonged parasitoid larval development in the host resulted from indirect effect of nicotine stress on the host or as a direct effect of nicotine on the parasitoid in the host haemolymph is unknown (Self *et al.*, 1964). Although it was shown that nicotine may enter parasitoid tissue (Barbosa *et al.*, 1986), nicotine consumption by *Manduca sexta* (L.) has also been shown to adversely affect development of the gregarious parasitoid, *Cotesia congregata* (Say) (Barbosa *et al.*, 1986; Thorpe and Barbosa, 1986; Thurston and Fox, 1972). All of these studies have shown that pesticides can be disruptive to parasitoids developing within their host.

Studies have shown that the mode of application of insecticides to the host larva can affect the susceptibility of larval parasitoids. Smilowitz *et al.* (1976) studied the effects of a juvenile hormone mimic (Altozar®) on the survival of larval *Hyposoter exiguae* (Viereck) (Hymenoptera: Ichneumonidae), a parasitoid of the cabbage looper, *T. ni*. They found that when larvae were fed the insecticide there was no effect on the developing parasitoid. However, when applied topically parasite mortality ranged between 5-95%. Juvenile hormone mimics generally affect insects prior to the pupal moult. It was suggested that as the host stops feeding prior to the parasitoid's last moult, the dose received by the parasitoid was insufficient to affect development. Temerak (1980) studied the effects of *B. thuringiensis* on *Bracon brevicornis* Wesmael (Hymenoptera: Braconidae) developing in the pink borer larvae, *Sesamia cretica* Lederer (Lepidoptera: Noctuidae) by injecting *B. thuringiensis* into the body cavity of larvae. He found that parasitoid survival, measured by cocoon formation, was reduced. Temerak (1980) stated that hosts were rendered nutritionally unsuitable for the parasitoids due to *B. thuringiensis*. The method of injecting *B. thuringiensis* is artificial as this is not the normal mode of infection. Thompson *et al.* (1977) showed that immature

Glypta fumiferenae (Viereck) and *Apanteles fumiferenae* Viereck, parasitoids of the western spruce budworm *Choristoneura occidentalis* Freeman (Lepidoptera: Tortricidae), acquired lethal doses from the host larvae in which the toxin had been activated. Niwa *et al.* (1987) showed that parasitoids may become infected with *B. thuringiensis* spores and crystals during development within infected hosts. These parasitoids apparently matured normally, but were unable to egress from the host. Whether this was due to *B. thuringiensis* infection, stress to the host or the early death of the host due to *B. thuringiensis* is unclear. These examples show that the method of exposure to pesticides influences the susceptibility of the larval parasitoid to pesticides.

Previous studies have demonstrated that often parasitoids are not directly affected by the pesticide, but are affected indirectly through host mortality. Parasitoids may increase susceptibility, decrease susceptibility or have no effect on the susceptibility of their hosts to insecticides. If host susceptibility to insecticides is increased due to the action of the parasitoid, then it is clear that parasitoids directly influence their own survival against toxicants. However, if the insecticides do not affect the developing parasitoid, and host development is slowed and prolonged, then pesticides may actually promote parasitoids by allowing larval parasitoids to complete development before the host dies. The timing of exposure to pesticides of the host after parasitism has also been demonstrated to be important in determining host susceptibility.

Culin and Debose (1987) studied the effect of chlordimeform, methyl parathion and fenvalerate on larval *M. demolitor* in *H. zea* hosts. They found that the number of parasitoids completing development was reduced because of host mortality. Parasitism by *M. demolitor* did not increase the susceptibility of the host to these insecticides. Abu and Ellis (1977) studied the survival of alfalfa weevil, *Hypera postica* parasitised with *Bathyplectes curculionis* (Thomson) (Hymenoptera: Ichneumonidae) and *Microctonus aethiopoides* Loan (Hymenoptera; Braconidae) treated with carbamates, carbofuran and carbaryl, organophosphates, malathion and phosmet, and the organochlorine, methoxychlor. They found that there was no incidence of parasitoid mortality in hosts surviving insecticide treatment, indicating the parasitoids were protected from the effects of the insecticides. However, they found that parasitised weevils were more susceptible to certain insecticides although this difference was not statistically significant.

Atwood *et al.* (1997b) demonstrated that *H. virescens* parasitised by *M. croceipes* were more susceptible to *B. thuringiensis* and thiodicarb compared to unparasitised larvae. Atwood *et al.* (1995) demonstrated the same trend with the parasitoid *Cotesia marginiventris* when *H. virescens* larvae were exposed to *B. thuringiensis*. Gunasena *et al.* (1990) found that *H. virescens* larvae parasitised by *Campoletis sonorensis* (F.) (Hymenoptera: Ichneumonidae) were 3.4 times more susceptible to nicotine than non-parasitised larvae. Ahmad and Forgash (1976) found that gypsy moth larvae parasitised by *A. melanoscelus* were about 3.3 times more susceptible to carbaryl than unparasitised larvae. They suggested that oviposition-injury stress was responsible for the greater sensitivity. Dumbre and Hower (1976) showed that *Bathyplectes curculionis* (Thomson) (Hymenoptera: Ichneumonidae) egg development did not influence development of third instar larvae of the alfalfa weevil (*Hypera postica*) (Gyllenhal). However, parasitoid larval development increased host susceptibility to carbofuran, carbaryl, malathion, phosmet and methoxychlor by almost one-half. Yanes and Boethel (1983) showed that parasitised loopers were more susceptible to the leaves of a resistant soybean variety compared to non-resistant varieties. This indicated that stress due to the developing parasitoid may be an important factor. Explanations for greater susceptibility of parasitised larvae include lower activity of several detoxification enzymes (Fix and Plapp, 1987), biochemical changes as a consequence of parasitism and teratocyte development, oviposition stress or weakening of the host due to increased stress, and reduced feeding due to parasitism.

Studies have shown that parasitisation can decrease the susceptibility of host larvae to pathogens, such as NPV. It has been demonstrated that infection with NPV is suppressed in *H. armigera* after parasitisation by *M. demolitor*, if two days development time is allowed before exposure to NPV (Yanes and Boethel, 1983; Teakle *et al.*, 1983; Teakle *et al.*, 1985b). This also occurs in *T. ni* parasitised by *Hyposoter exiguae* (Viereck) (Hymenoptera: Ichneumonidae) (Beegle and Oatman, 1975; 1974). These results are supported by circumstantial evidence from the field, with an aerial application of NPV to sorghum causing little disruption to parasitism by *M. demolitor* in *H. armigera* (Teakle *et al.*, 1983). The reason for suppression of NPV infection is suggested to be through reduced feeding (Chapter 5) or internal damage to the host. This effect was also demonstrated by Smilowitz *et al.* (1976), who studied the effects of a juvenile hormone mimic on the development of *Hyposoter exiguae* (Viereck) (Hymenoptera: Ichneumonidae) in the

host, the cabbage looper, *T. ni.* They found that parasitoid survival was increased when the insecticide was administered through diet rather than topically. Parasitisation reduced feeding in the host, consequently, the dose of insecticide was reduced and parasitoid survival was increased.

Some studies have demonstrated that parasitisation has no effect on the susceptibility of the host larvae to toxins. For example, Wilkinson and Ignoffo (1973) showed that juvenile hormone mimics had no effect on the parasitoid *Apanteles rubecula* Marshall, following treatment of the host *P. rapae.*

Insecticides may actually increase the rate of parasitism of host larvae in the field. Some insecticides, when administered in a sub-lethal dose, are thought to retard the development of the larval host. As development is slowed, the larvae remain suitable as hosts for their respective parasitoids for longer. This has been demonstrated for the gypsy moth (*L. dispar*) and for the western spruce budworm (*Choristoneura occidentalis* Freeman (Lepidoptera: Tortricidae)) treated with *B. thuringiensis.* Wesloh and Andreadis (1982), Wollam and Yendol (1976) and Ahmad *et al.* (1978) studied the effects of *B. thuringiensis* on *Apanteles* (=*Cotesia*) *melanoscelus* (Ratzeburg) within the gypsy moth. It was found that *B. thuringiensis* did not influence parasitoid survival nor was *B. thuringiensis* influenced by the parasitoid. *B. thuringiensis* treated hosts were more readily parasitised, because of their delayed development. Fusco (1980), Ticehurst *et al.* (1982) and Weseloh and Andreadis (1982) indicated a synergism between *B. thuringiensis* and parasitoids such as *C. melanoscelus* against the gypsy moth. Sub-lethal doses of *B. thuringiensis* delayed development in the gypsy moth. This increased the time during which larvae were susceptible to parasitoid attack. However, infected gypsy moth larvae were smaller and produced fewer parasitoids (Wallner *et al.*, 1983). Photonegativity and non-feeding behavior of parasitised western spruce budworm enabled more parasitised larvae to survive in *B. thuringiensis* treated areas (Hamel, 1977). There was no significant difference in total parasitism between treatment and control, however, the total composition of the overall parasitoid complex differed appreciably. This could result in benefits as larvae are targeted earlier. Hamel (1977) found that *Apanteles fumiferenae* Vier. (Hymenoptera: Braconidae) and *Glypta fumiferenae* (Vier) (Hymenoptera: Braconidae), two species of parasitoid which attack first instar larvae, were increased due to *B.*

thuringiensis treatment, while three parasitoids of later instar larvae and pupae were suppressed. This indicated a lag in host development.

It is clear from the above review that larval parasitoids may be affected by the exposure of hosts to toxins. Whether this is due to the direct effects of the toxin on the developing parasitoid or stresses on the host due to toxins is unclear in many cases. Parasitoids may be protected by their hosts from direct contact with pesticides, although it has been shown that toxic substances can be acquired by developing parasitoids. Often, the effects on parasitoids are quite subtle and the main effect is due to host mortality, depriving parasitoids of the proper requisites to complete their life cycle. Because *M. demolitor* attack *Helicoverpa* spp. early in development, some larvae targeted with insecticide sprays will also be parasitised by *M. demolitor*. The object of this chapter was to determine the effects of selected insecticides on the development of larvae *M. demolitor* in *H. armigera*.

Materials and methods

Insects

Insects were reared using methods described in Appendix 1. The procedure for preparing larvae for testing is described in Chapter 10 (p. 115).

Test larvae were parasitised by exposing approximately 1500 second instar larvae to a mixture of male and female *M. demolitor*. Parasitoids were allowed to sting *ad libitum* for about 4 hours. Preliminary testing and past experience (D. Murray, *pers. com.*), showed that this method resulted in less that 1% of unparasitised larvae. In this time adult *M. demolitor* were supplied with honey and water, while *H. armigera* larvae were supplied with a small amount of standard *Heliothis* diet. On completion of stinging, larvae were "plated-out" on trays containing diet, and wasps were returned to the culture. Both parasitised and unparasitised larvae were transferred to Costar® trays, and dosed with insecticide when they reached the required age.

Bioassay

Insecticides were selected on the basis of either present or future use for control of *Helicoverpa* spp. in cotton. Insecticides were from selected families of insecticides. Due to known insecticide resistance in the laboratory colony of *H. armigera*, the pyrethroids and endosulfan were not tested. In each case, larvae were treated with the LD_{99}, as determined in Chapter 10.

Topical testing

Testing was carried out using the methods described in Chapter 10 (p. 116). Insecticides were technical grade chemicals, including chlorfluazuron, methoxyfenozide, profenofos and spinosad (NAF-85) (see Appendix 2). Testing started with early second instar larvae (5 to 6 mg) and continued daily until parasitoid egression (7 days after oviposition). Larvae were approximately 38 mg at the end of testing (see Table12.2).

Stomach poisons

Testing was carried out using the methods described in Chapter 9 (p. 116). Emulsifiable concentrate formulations were used unless otherwise noted, including *B. thuringiensis* (powder), chlorfluazuron, lufenuron, methoxyfenozide, profenofos, spinosad (NAF-85), and thiodicarb (see Appendix 2). Larvae were tested from day 0 through to day 7 following oviposition. Larvae were placed individually in each cell on the insecticide/diet mixture after the diet had cooled and solidified. Larvae were fed the insecticide/diet mixture continuously until mortality and egression was assessed 8 days after treatment. Testing was done only in the morning. Larvae were held under controlled conditions (Appendix 1) before and after testing.

Analysis

Mortality for both studies was assessed 8 days after treatment with insecticides. This interval was considered appropriate as it takes seven days for *M. demolitor* to complete development within its host. Parasitoid emergence was scored eight days after the initial test and each day after that. Survival of *M. demolitor* was defined as larval egression from the host and not successful parasitoid pupation because *Heliothis* diet was an unsuitable substrate for pupation.

Larvae were weighed before and after treatment. Larvae were weighed initially in groups of 10 on the first day, then individually to the nearest 1 mg (Sartorius Balance).

The methods described above were used as they were the most convenient, and most reproducible. Cannibalism was virtually eliminated. Test trays were generally free of disease throughout the test period, with only occasional outbreaks of *Aspergillus* spp. Insects were handled only once in the stomach poison trial and twice in the topical dosing, reducing losses from escapes and mechanical damage.

Results

Table 12.1 shows the percent mortality of unparasitised and the percent egression of *M. demolitor* larvae from parasitised *H. armigera* larvae after treatment of larvae with the LD_{99} of selected stomach insecticides, 0-7 days after parasitisation. These data show that *M. demolitor* survival, expressed as percent egression from the host, was reduced. They indicate that if the host survived long enough for the parasitoid to complete larval development, then the parasitoid emerged from the host normally. However, parasitoid larval survival was reduced due to host mortality in all cases. Chlorfluazuron reduced parasitoid survival through host mortality for 5 days, lufenuron for 4 days, spinosad for 6 days, profenofos for 5 days, methoxyfenozide for 5 days, thiodicarb for 6 days, and *B. thuringiensis* for 5 days.

Table 12.2 shows the percent mortality of unparasitised and the percent egression of *M. demolitor* larvae from parasitised *H. armigera* larvae after treatment of larvae with the LD_{99} for similar size class of selected contact insecticides, 0-7 days after parasitisation. These data show that for chlorfluazuron, as host mortality was reduced, *M. demolitor* larval egression increased. This increase occurred 1 day after parasitisation. The data for methoxyfenozide show a similar trend, however, the last data entry for day 4 shows 100% mortality for unparasitised larvae. This entry is perhaps an error caused by a mistake in the dosing level. The data for profenofos shows that parasitoid survival was reduced over the entire period of parasitoid larval development, with parasitoid egression only rising to 33%. The data for spinosad indicates that parasitoid larval

survival was inhibited throughout the period of the parasitoid's larval development. These data will be discussed later.

All data reported are from single experimental runs. Time constraints prevented re-testing all products.

Discussion

This study shows that treating *H. armigera* larvae parasitised by *M. demolitor* with insecticides had a negligible direct impact on the developing larval parasitoids. However, insecticides had a dramatic indirect impact on parasitoids through mortality of the host. This can be seen in the data as there were no cases with any product at any time, where unparasitised host mortality was low and parasitoid egression was also low as would be expected if parasitoid larvae were affected by the insecticides. This has been found in previous studies. Croft and Brown (1975) and Abu and Ellis (1977) found that larval parasitoids early in development usually died after their hosts were killed because they were deprived the proper requisites to complete their life cycle, not due to the direct effects of the pesticide. In the present study there was no evidence of parasitoid mortality within hosts surviving insecticide treatment.

This study showed that parasitisation reduced the susceptibility of the host to stomach insecticides and to a lesser degree to contact poisons. Larval parasitoids certainly did not increase the susceptibility of the host to insecticides.

Insecticides with a slow rate of kill may promote parasitoids by allowing the larval parasitoid enough development time to complete development before the host is killed. This can be seen with the data obtained from testing methoxyfenozide.

Table 12-1. Egression of *M. demolitor* (%) from parasitised *H. armigera* larvae and host mortality (%) from unparasitised *H. armigera* larvae (number treated) treated with the LC_{99} (see Chapter 10, Table 10-1) of selected stomach insecticides.

		Treatment with insecticide 0-7 days after parasitisation								
	LC_{99}	Control	0	1	2	3	4	5	6	7
chlorfluazuron										
Parasitised	0.0017	**100** (24)	**0** (24)	**0** (24)	**0** (24)	**0** (24)	**0** (24)	**0** (24)	**100** (23)	**100** (24)
Unparasitised		*	*	*	*	*	*	*	*	*
lufenuron										
Parasitised	0.0041	**95.8** (24)	**0** (24)	**0** (24)	**4.2** (24)	**62.5** (24)	**65.2** (23)	**95.8** (24)	**100** (18)	**95.7** (23)
Unparasitised		**0** (24)	**100** (24)	**100** (24)	**91.7** (24)	**95.8** (24)	**79.2** (24)	**66.7** (24)	**54.5** (22)	*
spinosad (NAF-85)										
Parasitised	0.0093	**100** (24)	**0** (24)	**0** (24)	**0** (24)	**4.2** (24)	**0** (24)	**0** (24)	**0** (24)	**72.0** (24)
Unparasitised		**4.8** (21)	**95.8** (24)	**95.7** (23)	**91.7** (24)	**75.0** (24)	**83.3** (24)	**95.8** (24)	**95.8** (24)	**97.7** (23)
methoxyfenozide										
Parasitised	0.0155	**100** (24)	**0** (24)	**0** (24)	**0** (24)	**4.2** (24)	**21.7** (24)	**50.0** (24)	**83.0** (24)	**75.0** (24)
Unparasitised		**0** (24)	**100** (24)	**87.5** (24)	**95.8** (24)	**95.8** (24)	**100** (24)	**91.7** (24)	**68.2** (22)	**50.0** (24)
profenofos										
Parasitised	0.0606	**100** (24)	**0** (24)	**0** (24)	**0** (24)	**12.5** (24)	**66.6** (24)	**50.0** (24)	**95.8** (24)	**100** (24)
Unparasitised		**0** (24)	**100** (24)	**100** (24)	**95.7** (23)	**75.0** (24)	**60.9** (23)	**50.0** (24)	**20.8** (24)	**0** (24)
B. thuringiensis										
Parasitised	5.3778	**88.9** (18)	**0** (23)	**0** (22)	**0** (22)	**0** (22)	**0** (20)	**30.0** (20)	**90.9** (22)	**96.9** (32)
Unparasitised		**4.3** (23)	**100** (22)	**100** (22)	**100** (23)	**100** (24)	**100** (24)	**100** (24)	**100** (24)	**100** (9)
thiodicarb										
Parasitised	7.7996	**100** (24)	**0** (24)	**0** (24)	**0** (24)	**0** (24)	**0** (24)	**0** (24)	**0** (24)	**70.8** (24)
Unparasitised		**0** (24)	**100** (24)	**100** (24)	**100** (24)	**100** (24)	**100** (24)	**100** (24)	**100** (24)	**0** (24)

* Data missing

Table 12-2. Egression of *M. demolitor* from parasitised *H. armigera* larvae or host mortality (%) from unparasitised *H. armigera* larvae treated with the LD_{99} (see Chapter 10, Table 10-2) of selected contact insecticides.

	Treatment with insecticide 0-7 days after parasitisation								
	control	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
profenofos									
Parasitised									
No. tested	22	24	24	24	24	24	24	24	24
Weight (g)	0.005	0.005	0.006	0.006	0.012	0.020	0.027	0.031	0.039
Dose (µg/larva)	na	0.08	0.05	0.05	0.05	0.13	0.13	0.17	0.17
Egression (%)	81.8	0	4.2	8.3	0	0	4.2	25.0	33.3
Unparasitised									
No. tested	23	24	24	24	24	*	*	*	*
Weight	0.005	0.005	0.008	0.025	0.041	*	*	*	*
Dose (µg/larva)	na	0.08	0.05	0.13	0.17	*	*	*	*
Mortality	13.0	83.3	100	100	87.5	*	*	*	*
spinosad (NAF-85)									
Parasitised									
No. tested	23	23	20	24	24	24	24	24	24
Weight (g)	0.005	0.005	0.007	0.011	0.017	0.030	0.033	0.030	0.039
Dose (µg/larva)	na	0.12	0.20	0.20	0.20	0.74	0.74	0.74	0.74
Egression (%)	89.0	0	0	0	0	8.3	0	4.2	0
Unparasitised									
No. tested	24	23	24	24	*	*	*	*	*
Weight	0.005	0.005	0.008	0.028	*	*	*	*	*
Dose (µg/larva)	na	0.232	0.401	1.47	*	*	*	*	*
Mortality	0	87.0	100	95.8	*	*	*	*	*
methoxyfenozide									
Parasitised									
No. tested	11	19	22	24	24	23	24	22	23
Weight (g)	0.005	0.005	0.006	0.011	0.019	0.021	0.038	0.040	0.035
Dose (µg/larva)	na	0.22	0.23	0.23	0.44	13.85	13.85	13.85	13.85
Egression (%)	90.9	0	9.1	25.0	29.2	87.0	91.7	95.5	91.3
Unparasitised									
No. tested	24	24	24	24	24	24	*	*	*
Weight	0.005	0.005	0.009	0.026	0.032	0.128	*	*	*
Dose (µg/larva)	na	0.22	0.23	0.44	13.85	18.10	*	*	*
Mortality	8.3	75.0	95.8	91.7	66.7	100	*	*	*
chlorfluazuron									
Parasitised									
No. tested	19	24	22	17	12	19	24	22	24
Weight (g)	0.005	0.005	0.008	0.009	0.020	0.026	0.032	0.038	*
Dose (µg/larva)	na	0.13	0.96	0.96	4.04	4.04	4.04	4.04	*
Egression (%)	94.7	0	20.8	88.2	91.7	100	100	100	100
Unparasitised									
No. tested	23	24	24	24	24	24	24	24	24
Weight	0.005	0.005	0.008	0.024	0.074	0.140	0.205	0.363	*
Dose (µg/larva)	na	0.13	0.96	4.04	138.89	138.89	138.89	138.89	*
Mortality	0	95.8	54.2	20.8	0	8.3	4.2	0	0

* Not tested.

The LC_{99} and LD_{99} were chosen for each compound as it was felt that if parasitoids survived after testing with this extremely high dose, they would certainly survive when lower doses were used. This is possibly a mistake in the methodology of this study. There is considerable variation associated in the extremes of the probit dose-response line (Copenhaver and Mielke, 1977) and more useful data may have been generated with testing with the LC_{50} and LD_{50}.

Insecticide resistance in a host insect may confer some protection to the larval parasitoid. This is only the case if the primary resistance mechanism is lower rate of accumulation, reduced penetration, increased degradation or excretion, as opposed to an alteration in the target site. Furlong and Write (1993) suggested that diamondback moth larvae resistant to acylurea conferred some protection to developing parasitoid species. It was assumed that if host larvae survived long enough for the parasitoid to complete development, then resistant *H. armigera* treated with pyrethroids which are parasitised by *M. demolitor* would presumably produce a parasitoid.

The tests with stomach insecticides in this study were carried out with continuous insecticide exposure. This is a very artificial scenario, compared to what would normally occur in the field. It has been shown that lepidopteran larvae may recover from initial doses of insecticides, as demonstrated for *B. thuringiensis* (Fast and Regeniere, 1984; Ali and Watson, 1982).

Dissection of host larvae would have probably provided more reliable data. However it was considered that the most useful data were generated by letting parasitoids egress normally. Parasitoid egression was used to determine parasitoid survival rather than adult emergence or parasitoid cocoon formation as *Heliothis* diet is often an unsuitable substrate for parasitoid pupation.

This study showed that selective pesticides, such as *B. thuringiensis*, which are reportedly harmless to beneficial insects, may have a significant effect on beneficials. Microbial pathogens infecting the host larva may also have deleterious effects on the parasitoid through infection of the parasitoid (Bell *et al.*, 1974), reduced adult number and vitality (Bell *et al.*, 1974) and decline in parasitoid size and longevity (Laigo and Tamashiro, 1967). Field tests on the emergence of *M. croceipes* after treatment with *B. thuringiensis* showed that there was negligible impact on the

parasitoid (Atwood, 1997b). Bell *et al.* (1974) found that the rate of emergence of *M. croceipes* was depressed 25.5% when the bacterial pathogen *Serratia marcescens* (Bizio) infected *H. zea*. Parasitoids may become infected while developing within the host (King and Bell, 1978). *M. croceipes* required 1.1 days longer to complete development in *H. zea* than was required by the fungus *Nomuraea rileyi* to kill the host larvae and sporulate. Parasitised larvae were predisposed to infection. Fungus inhibited development of *M. croceipes* if host larvae were infected with *N. rileyi* prior to or within 1 day after parasitisation. Parasitisation was reduced after treatment with fungus, probably due to decreased susceptibility of host larvae for parasitoid development because of increased host age. Reduced parasitisation 1 day after treatment was probably due to antagonism between pathogen and parasitoid.

The study described in this chapter showed the importance of testing the effects of pesticides over the complete life cycle of beneficials. It demonstrated the importance of knowing the life history and behavior of the parasitoid, so that application of disruptive insecticides can be timed to coincide with the most tolerant stage of the parasitoid. The impact of pesticides on beneficials may be reduced, and if care is taken, many insecticides may be utilised in an integrated program.

Use of certain pesticides at dosages lowered so as to kill less than 100% of the pests may promote parasitoids. This has been previously demonstrated for *B. thuringiensis* treatment of the gypsy moth and western spruce budworm. Results from this study showed that if the host survived long enough for the parasitoid to complete development then the larval parasitoid was unaffected by the pesticide. Applications of sub-lethal doses of insecticides may promote parasitoids. This aspect warrants further evaluation in the field.

Transgenic cotton (Ingard®) may affect parasitoids. Yanes and Boethel (1983) examined the effects of plant resistance to the soybean looper on the development of larval *M. demolitor*. They found that host plant resistance resulted in a significant difference in the number of parasitoids completing development. Parasitised larvae fed susceptible leaves yielded a higher number of completed parasitism than those fed resistant leaves. This shows that the lethal and sub-lethal affects of *B. thuringiensis* toxins are important.

Conclusions

This study showed that *M. demolitor* larvae developing within parasitised host larvae were unaffected by insecticide applications. However, larval parasitoids were indirectly affected by insecticides through host mortality. Larval parasitoids did not increase the susceptibility of their host to the insecticides, in fact parasitisation reduced the susceptibility of the host to stomach insecticides and to a lesser degree for contact insecticides.

This study shows that any *M. demolitor* present in the larval stage at the time of a disruptive insecticide application will be unaffected directly by the insecticide, however, mortality may arise due to the mortality of the host. The use of insecticides with slow rates of kill may promote parasitism by allowing parasitoid larvae enough time to complete development.

Conclusions

This thesis contributes to IPM in Australia by presenting data on aspects of the ecology and effects of insecticides on *M. demolitor*, in order to promote conservation and increase the role of *M. demolitor* in IPM. *M. demolitor* is a critical component for the success of IPM in Australian cotton, attacking *Helicoverpa* spp. early in it's larval development but after significant mortality at the egg stage has occurred. This provides a distinct advantage over egg parasitoids, such as Trichogramma spp. These are often overwhelmed by massive egg numbers and although a high percentage of eggs are parasitised, many eggs are left to develop into damaging larvae.

M. demolitor was shown to be the dominant larval parasitoid in southeast Queensland. *M. demolitor* is present in the cotton crop at the critical stage of the crop's development. High rates of parasitism of *Helicoverpa* spp. larvae by *M. demolitor* were recorded in the field (between 50% and 90%). The levels of parasitism recorded in this thesis were comparable to previous studies (Murray *et al.*, 1996; Shepard *et al.*, 1983a; Forrester, 1981; Titmarsh, 1981) and actually may indicate higher than previously recorded parasitism. This thesis enhances our understanding of parasitoids in IPM in Australian cotton. Previously the role of parasitoids in controlling *Helicoverpa* spp. had been underestimated (Hearn and Fitt, 1992).

These data presented in this thesis showed that *Helicoverpa* spp. larvae parasitised by *M. demolitor* cause insignificant damage to the cotton crop (approximately 5% of an unparasitised larvae) and parasitised larvae should be tolerated and not included in larvae checks to determine spray decisions. Although the results presented agree with previous studies on *M. demolitor* (Murray and Rynne, 1992), it must be confirmed that the observed reduction in feeding translates to reduced feeding damage in the crop. The technique for distinguishing *Helicoverpa* spp. larvae parasitised by *M. demolitor* requires a small amount of training. However, once mastered it is quick, easy and reliable. The hind portion of the larvae is squeezed until it "pops". The developing larval parasitoid larvae can be readily identified as a small white larvae, approximately 2-5 mm, which will wriggle slightly for a few minutes before it dies (Unpublished observations).

Methods of monitoring *M. demolitor* in the field were studied. It was determined that an estimate of percent parasitism, sticky traps baited with virgin females, and direct observations were useful tools for research or for ongoing monitoring of *M. demolitor*. Suction sampling, yellow coloured water traps and traps baited with *H. armigera* larvae proved unsuccessful at monitoring *M. demolitor*. Successful monitoring tools were used to study some aspects of the ecology of *M. demolitor* in this thesis. Direct observations by walking up cotton rows and spotting adult wasps proved the most useful method for monitoring *M. demolitor* in the field. This technique is quick and easy and could easily be adopted by crop scouts for determining the presence/absence and even an estimation of the number of adult wasps present in the cotton field. If data on wasp sex ratios is required, wasps can easily be caught and sexed in a standard sweep net and the sex determined by examining the colouration of the wasp's abdomen (see p. 31 and Figure 4-2). The other two successful monitoring methods, percent parasitism and sticky traps baited with virgin female wasps, could be used in intensive studies of *M. demolitor*. These methods proved much more labour intensive than direct observations and are better suited to scientific studies rather than daily monitoring by crop scouts. They have potential for collecting valuable reliable data on presence/absence, wasp activity, wasp populations, effects on the pest and female calling in the field.

Adult *M. demolitor* were determined to be equally active throughout the day, but were inactive early in the morning and late evening. This study showed that monitoring of adult *M. demolitor* by crop scouts can be carried out at any time of the day without biasing results. This is important because the reliability of checks made in the afternoon is the same as checks made in the morning. The effects of some disruptive insecticide sprays can be reduced by timing applications for periods of adult inactivity. This reduces direct exposure to the insecticide and once dry, residues of some insecticides are harmless to *M. demolitor* (Hendrix *et al.,* 1997). This approach has been taken by manufactures of insecticides who recommend that applications not be made when honey bees are actively foraging. This study showed that *M. demolitor* adults were inactive in the morning and late evening and therefore, if insecticides such as spinosad were applied at these times, there affect on adult *M. demolitor* may be reduced. These data agree with studies previously published overseas (Hooper *et al.*, 1991; Powell and King, 1984) and improve our understanding of the Australian species.

Large numbers of adult *M. demolitor* were observed in the field. A preliminary study estimated that there were approximately 300 male wasps per ha. This study showed that a relatively small number of female wasps were causing a high rate of parasitism (approximately 60%) of pest larvae and indicates that inundative release of *M. demolitor* may be economically feasible. Obviously a detailed economic assessment of the feasibility of inundative releases of *M. demolitor* must be undertaken before this approach can be recommended. However, this study showed than there may be a role for government departments, such as the Department of Primary Industries or CSIRO to make releases of adult *M. demolitor* in key cropping areas. The cost of rearing host insects makes a program like this very expensive. However, if an artificial diet could be devised for larval *M. demolitor*, a program like this may become a reality. Attempts at making a artificial diet for larval *M. crociepes* have been carried out in the USA with limited success (King *et al.*, 1985a), however, there may be potential for some progress on *M. demolitor* here in Australia.

A release-recapture study showed that fluorescent powder could be used to mark wasps. This study showed that the catch efficiency of the sticky traps baited with virgin females is reduced after only two days in the field and that male *M. demolitor* were not moving very far in response to these traps. While this study showed that fluorescent dust can be used successfully to mark adult *M. demolitor* in intensive scientific studies, it also showed that 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. This indicates that the natural population of *M. demolitor* occurring in the field is most likely large enough to warrant conservation.

Baseline data for the toxicity of selected insecticides to second instar *H. armigera* were obtained. The IGR compounds, chlorfluazuron and lufeneron as well as the MAC compound, methoxyfenozide, and spinosad were the most effective stomach compounds tested. The sub-lethal data generated in this trial show that although affected larvae do not die, their weights are significantly reduced. This indicates reduced damage to the crop, and larvae affected by sub-lethal doses will cause negligible damage to the crop while being suitable sources of food for predators or hosts for parasitoids. These data were used in a study on the interactions between *M. demolitor* larvae, hosts and insecticides, which further increase our understanding of baseline toxicity of some

new compounds to *Helicoverpa* spp. This knowledge is crucial as new compounds become registered for use on this pest in cotton.

This study examined the effects of commonly used insecticides for control of *Helicoverpa* spp. in cotton on *M. demolitor* throughout its life cycle, in an attempt to recommend insecticides or management techniques which could be used to conserve natural populations of *M. demolitor*. It was found that *M. demolitor* was relatively more tolerant of certain insecticides during its larval and pupal stages. The tonicities of tested compounds to adult *M. demolitor*, in ascending order were chlorfluazuron = methoxyfenozide < primicarb < endosulfan ≤ dimethoate ≤ cyhalothrin < profenofos < deltamethrin ≤ bifenthrin (alpha-cypermethrin) < spinosad. Using the data generated on the baseline toxicity to *Helicoverpa* spp., it was determined that only the IGR compounds and the carbamate, pirimicarb, were of lower toxicity to the parasitoid than to the pest. Comparison of responses to insecticides between pest and parasitoids in the literature has shown, with very few exceptions, that parasitoids are more susceptible than the pest (Plapp and Bull, 1985; Plapp and Vinson, 1977). 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 or larval stage will reduce the impact of most disruptive insecticides. This study showed that *M. demolitor* larvae developing within parasitised host larvae were not directly affected by insecticide applications. However, larval parasitoids were indirectly affected by insecticides through host mortality. Larval parasitoids did not increase the susceptibility of their host to the insecticides. Parasitisation reduced the susceptibility of the host to stomach insecticides and to a lesser degree for contact insecticides. The use of insecticides with slow rates of kill may promote parasitism by allowing parasitoid larvae enough time to complete development.

By monitoring the activity of adult wasps through direct observations, determining the level of parasitisation of *Helicoverpa* spp. larvae in the crop by squashing larvae and by observing the presence of *M. demolitor* pupae (see p. 29) in the field at the time of an insecticide application recommendation, crop scouts can use the toxicity data presented in this thesis to modify insecticide spray recommendations, or time applications to conserve naturally occurring populations of *M. demolitor* in the field. This can be achieved by selecting softer insecticides when adult wasps are present or by adjusting the timing of sprays to coincide with less susceptible stages of *M. demolitor*

or periods of adult inactivity. Data presented in this thesis showed that pupal *M. demolitor* and late instar larval *M. demolitor* were less susceptible to insecticides compared to adult or early larval instars.

Methods for conserving indigenous *M. demolitor* are outlined in this thesis. Methods which warrant further investigation include breeding and release of insecticide resistant parasitoids, avoidance of harmful cultural techniques, maintenance of diversity of alternative hosts, nursery crops for *M. demolitor*, and the provision of artificial food supplements. Other aspects which are of interest for further study are interactions with ascovirus, and studying the substances injected into the host by *M. demolitor* to disrupt development, which may be useful in developing specific insecticides.

The work presented in this thesis has contributed to IPM in Australian cotton by presenting data on the ecology and effects of insecticides on *M. demolitor*. Through selection of softer insecticides and by implementing management techniques recommended in this thesis, the already significant impact *M. demolitor* has in IPM in Australian cotton through *Helicoverpa* spp. mortality, can be increased.