Introduction

"We stand now where two roads diverge.....The road we have long been traveling is deceptively easy, smooth super-highway on which we progress with great speed, but at its end lies disaster. The other fork of the road- the one 'less traveled by'- offers our last, our only chance to reach a destination that assures the preservation of our earth" Rachel Carson, *Silent Spring*, 1962.

The shape of agriculture has changed remarkably in the last 20 years. Farmers are expected to produce food and fiber at sufficient quality and ever increasing quantity to feed the world's increasing population. Losses to pests (insects, weeds and pathogens) are estimated at 35-48% of production (Pimentel, 1976). Farmers have many new tools in their fight against these pests. Genetically modified plants have arrived; plants that express the genes that produce the toxins of bacteria and plants that are resistant to selected herbicides. Before these rather recent developments, pesticides were hailed as the great breakthrough in agriculture and their use became widespread.

Insecticides are simply chemical compounds used to control insect pests; there is a vast array of chemicals currently registered for this purpose. It is a simple indisputable fact that insecticides work. According to McGahan *et al.* (1991) a $27.2 million investment in chemical control of *Helicoverpa* spp. in Australia can increase production by $210.4 million. This results in a cost/benefit ratio of about 1:7.6. Unfortunately, the tremendous success of the early insecticides sent agriculture down a path which has created a dependency on their use. Insecticide use carries a price; this price is rarely considered when economic figures like those above are determined.

The price of insecticide use can be measured in the number of insecticide resistant pests, pest resurgence, the destruction of pollinating species, and the creation of secondary pests, damage to natural ecosystems and waterways and the associated dangers to human health, through insecticide residues in by-products or accidental exposure to insecticides. Warnings of the side-effects of insecticide use were brought to the attention of the public in 1962, in Rachel Carson's

famous book, *Silent Spring*. Carson (1962) claimed that pesticides were being used carelessly and their use had become infectious, workers often relying on pesticides when other methods of control had existed before their development. Although pesticide misuse has certainly declined since the publication of *Silent Spring*, there is great room for further improvement.

Cotton is grown in Australia with almost total reliance on insecticides for control of pest species. This results in a disproportionate use of pesticides when compared to other crops (Fitt, 1989). Cotton production requires large inputs, with insect control considered necessary to ensure maximum yield and quality. To understand the complex problems of cotton production and why there is a need to use such large quantities of pesticides to produce cotton economically, I must first examine how cotton is produced in Australia. Chapter 1 briefly examines the cotton production system in Australia. Chapter 2 examines the biology of the key pests of cotton, *Helicoverpa* spp. and Chapter 3 reviews research into integrated pest management of *Helicoverpa* spp. With this knowledge, I examine ways of reducing the dependence on insecticides and focus on a key beneficial insect of Australian cotton ecosystems, namely *Microplitis demolitor* Wilkinson (Hymenoptera: Braconidae). I will examine the biology of *M. demolitor* (Chapter 4), the effects on the feeding behavior of the pest (Chapter 5), incidence of adult wasps in Australian cotton (Chapter 6), estimations of natural populations (Chapter 7), diurnal behaviour (Chapter 8) and a release/recapture study of adult *M. demolitor* (Chapter 9). The effects of insecticides on the host, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (Chapter 10) and the different life stages of *M. demolitor* (Chapter 11 and Chapter 12) will then be investigated. From this discussion it will become clear that by increasing the role of naturally occurring beneficial insects, such as *M. demolitor*, the dependence on insecticides will be reduced.

Although great reductions in pesticide dependence have been made, many of the old practices have continued. The aim of the modern cotton industry in Australia is to be sustainable; this depends of course on the definition of "sustainable". If sustainable is defined as 30 years, then most modern agricultural systems are doing just fine. However, if sustainable is defined as "forever" then few would pass. This does not have to be so. With a reduction in the dependence on pesticides and a shift to true integrated pest management (IPM), the sustainability of cotton production in Australia would be ensured. Without this reduction of insecticides, it is doubtful that

the cotton industry will cease to exist overnight. However, the use of many insecticides will become restricted and the costs of producing cotton will eventually become uneconomic.

This thesis is a contribution to a combined effort in research in an attempt to reduce the dependence on insecticides by increasing the role of *M. demolitor* in the cotton ecosystem. It proposes methods of producing cotton in a sustainable manner in the short and distant future.

Chapter 1

Cotton Production in Australia

Introduction

Cotton is the world's most important fibre crop. In Australia, modern cotton production commenced in the early 1960s and has grown to become the fourth largest rural export for Australia, with Australia exporting nearly $1 billion of cotton each year (Slack-Smith *et al.*, 1997). This chapter will briefly investigate cotton production in Australia.

What is cotton?

Cultivated cotton belongs to the family Malvacae and the genus *Gossypium*. In Australia, cotton is produced from varieties of *G. hirsutum* and *G. barbadense*. Varieties differ in disease and pest resistance, adaptation to mechanical harvesting, and length of growing season. Temperature broadly determines the regions where cotton may be grown (Hearn and Fitt, 1992).

Why is cotton cultivated?

Cotton is grown for fibre and seed. Sometimes trash consisting of old cotton plants is fed to livestock, although this practice is discouraged due to high levels of insecticide residues in the trash. Fibre quality is determined largely by variety. Varieties producing long finer fibre return better premiums, but may require more exacting climatic and agronomic conditions (Hearn and Fitt, 1992). Seed harvested from cotton is used for oil, and human or livestock consumption.

Where is cotton grown in Australia?

About 70% of the cotton produced in Australia is grown in northern and western NSW (299 300 ha.), and most of the rest in central and southern Queensland (138 800 ha.) (ABARE,

1998). The majority is irrigated with raingrown (dryland) cotton accounting for approximately 20% of the area and about 10% of production (Slack-Smith *et al.*, 1997). Cotton production has recently recommenced in the Ord valley, Western Australia, many years after it ceased due to insecticide resistance (Wilson, 1974).

How is cotton grown in Australia?

Cotton is a risky crop to grow. It requires large inputs (fertilizers, insecticides and herbicides), initial monetary capital and management expertise. Water is the most important input often determining yield.

A wide variety of pathogens attack cotton. However, diseases generally do not cause extensive loss of production (Ebbels, 1980), with the recent exception of fusarium wilt, which threatens continued cotton production in some cotton growing areas. Resistant varieties and cultural control play crucial roles in disease management. There is a distinct difference between pest and disease management in cotton. While pests are controlled predominantly by chemical use, diseases are managed mainly by non-chemical means.

The physiology and high value of cotton contribute to making the cotton crop especially susceptible to insect damage. Factors listed in Hearn and Fitt (1992) include:

- cotton has a high economic value. This means that the level of economic damage is reached with relatively few insects;
- high inputs are required to grow cotton in an intensive system, so there are high incentives to implement control measures to protect any investment;
- damaged cotton fruit (squares or bolls) are readily shed; and
- cotton has a prolonged fruiting period, which makes it susceptible to insect damage for an extended period and allows some pests to develop through several generations.

After water availability, insects are the second most important factor determining the success of a cotton crop. Hargreaves (1948, cited in Reynolds *et al.*, 1982) listed 1326 species of insects found on cotton worldwide. Room (1979b) recorded 500 species of insect and spiders in

Australian cotton fields. However, only 3 species were recorded as major pests, 16 minor pests and 31 as parasites and predators. In the cotton ecosystem there are numerous parasites and predators of the herbivorous species, often described as "beneficials". Room (1979b) estimated that about half of the species collected in cotton were predators to some extent, with only a small number having a significant impact on the pest species.

The key pests of cotton are *H. armigera* and *Helicoverpa punctigera* Wallengren. They routinely cause economic damage in Australian cotton. Biological and ecological characteristics of these species contribute to making them devastating pests and makes their control difficult. These characteristics include:

- they cause damage as larvae rather than adults, thereby requiring early detection and control;
- larvae feed preferentially on the fruiting structures, rather than on leaves;
- larvae become entrenched in protected sites (flowers and squares) on the cotton bush, which makes control with insecticides difficult;
- adults are highly mobile and may invade from other crops or even from different localities;
- larvae are highly polyphagous, with many alternate wild and crop hosts;
- adults have high fecundity;
- they have short life cycles and reproduce very rapidly; and
- *H. armigera* tends to develop insecticide resistance rapidly.

Conclusions

Growing cotton in Australia poses many challenges. The most challenging aspect is insect control, which at present is achieved with almost total reliance on insecticides. This reliance is unsustainable and an IPM system must be implemented. In order to implement an IPM system, the key pests (*H. armigera* and *H. punctigera*) must be studied in detail and any weaknesses exploited. This is the topic of the next chapter.

Chapter 2

Helicoverpa: A review

Introduction

The genus *Helicoverpa* includes some of the most important insect pests throughout the tropics, subtropics and to a lesser extent temperate regions (Jackson *et al.*, 1989). There are five species of *Helicoverpa* in Australia (Matthews, 1991; 1999), however, only *H. armigera* and *H. punctigera* are major pests of Australian agriculture (White *et al.*, 1995; Zalucki *et al.*, 1986; Common, 1953), including cotton (Zalucki *et al.*, 1986; Wardhaugh *et al*, 1980).

Why are *Helicoverpa* spp. such devastating pests?

Characteristics which make *Helicoverpa* spp. devastating pests include difficulty of identification between *H. armigera* and *H. punctigera*, wide distribution, high polyphagy, high fecundity, facultative diapause, high mobility, voracious feeding and the development of insecticide resistance (Fitt, 1989; Jackson *et al.*, 1989).

Identification

All life stages of *H. armigera* and *H. punctigera* can be distinguished by morphological characteristics, although identification is often unreliable (Daly and Gregg, 1985). Discrimination between *H. armigera* and *H. punctigera* at an early stage is crucial for efficient pest management, in order to avoid targeting *H. armigera* with insecticides to which it is resistant (Gunning and Easton, 1994b).

Distribution

H. armigera occurs throughout the agricultural cropping areas of eastern and northern Australia (Zalucki *et al.*, 1986) and the Ord irrigation area (Wilson, 1974). *H. punctigera* is

endemic and ubiquitous to Australia, and is considered the dominant species in inland and southern Australia (Wright, 1970; Common, 1953). Australia wide distribution maps for both species were published in the Commonwealth Institute of Entomology (1969; 1968), Common (1953) and Zalucki *et al.* (1986), and for Queensland in Kirkpatrick (1961). Distribution maps may reflect only the effort in searching rather than their actual distribution. Recently, *H. armigera* has been recorded throughout central Australia (Fitt *et al.*, 1995), though this species is rare in these areas (Gregg *et al.*, 1995).

Crops attacked

H. armigera and *H. punctigera* are highly polyphagous and have been recorded from a wide range of hosts in many families (Zalucki *et al.*, 1986; Wardhaugh *et al.*, 1980; Kirkpatrick, 1961; Common, 1953). *H. punctigera* attack only dicotyledonous plants, while *H. armigera* attacks both dicotyledonous and monocotyledonous plants. *H. punctigera* has a wider total host plant range than *H. armigera*, although *H. armigera* has a wider range of cultivated crop hosts (White *et al.*, 1995; Zalucki *et al.*, 1994; 1986). Both species attack a wide range of agricultural crops (Fitt, 1989; Zalucki *et al.*, 1986; Wardhaugh *et al.*, 1980; Wright and Nikitin, 1964; Common, 1953; Miller, 1945; Evans, 1943). Cotton is not a preferred host of either species and in many areas is attacked only after preferred hosts have senesced (Fitt, 1989).

On the Darling Downs, southern Queensland, the sowing and harvesting cycles of the common crops provide *Helicoverpa* spp. with a sequence of crops which can be attacked, as they become attractive during flowering (Titmarsh, 1992). Larval numbers peak on reproductive chickpeas and vegetative maize (October), reproductive maize and sunflower (mid to late December), cotton, sorghum, soybean and late sunflower (mid January to early February) and then cotton, mungbean, pigeonpea and soybeans (March) (Titmarsh, 1992). As the season progresses the number of *Helicoverpa* spp. increase and, with the destruction of insecticide susceptible individuals, the frequency of resistant individuals in the population increases.

Feeding habits

Helicoverpa spp. are voracious feeders. As larvae grow the damage they cause increases rapidly. Noctuid larvae consume 50% of the total food consumption during the last instar (Wilson

and Gutierrez, 1980). *Helicoverpa* spp. tend to feed preferentially on the flowering and fruiting parts of the plant. This exacerbates economic damage (Cullen, 1969; Hardwick, 1965; Kirkpatrick, 1961) and results in larvae becoming entrenched in protected feeding sites, making them difficult to target with insecticides. Preferential feeding on flowers and squares also disrupts normal flowering, causing the crop to flower for longer and therefore remain attractive for longer (Parsons, 1940).

Fecundity and development

Both species of *Helicoverpa* have high fecundity and a relatively short generation time. Fecundity is influenced by temperature, humidity and nutrition. (Adjei-Maafo and Wilson, 1983; Nadgauda and Pitre, 1983). There are no estimates of realised fecundity in the field. However, laboratory studies have estimated that females lay approximately 1000-1500 eggs throughout their adult life (Fitt 1989; Cullen, 1969). Eggs take approximately 4 days to hatch in both species (Kirkpatrick 1962). After egg hatch, larvae develop through five to seven instars (with six being usual) before pupating in the soil (Twine, 1978b). Pupal *H. armigera* develop in approximately 10 days (Twine, 1978a), *H. punctigera* pupae develop in approximately 13 days. After emergence, adult *H. armigera* live for about 10 days if adequate food is available (Broadly and Butler, 1983).

Diapause

Both species of *Helicoverpa* exhibit facultative diapause, although *H. armigera* are far more likely to diapause successfully. Diapause is important in maintaining local populations during periods when conditions are unfavourable or plant hosts are unavailable. Diapausing pupae are tolerant of adverse cold and dry conditions (Edger *et al.*, 1983; Roome, 1979a) and low humidity (Ditman *et al.*, 1940). Diapausing *H. armigera* pupae are responsible for crop infestations in the next season (Wilson, 1983) and is very important in the selection of insecticide resistance in this species. Substantial parasitism of overwintering *H. armigera* pupae by Ichneumonidae and Tachinidae have been recorded (Wilson, 1983). Cultivation also causes heavy mortality and is widely publicised under the banner of "pupae busting" (Shaw, 1999).

Mobility

Both species of *Helicoverpa* may undertake local and interregional movements (Fitt, 1989), although it is unclear whether only *H. punctigera* undertake regular seasonal long-range migration (Wilson, 1983; Anon, 1980; Wardhaugh *et al.*, 1980), or whether both species regularly migrate (P. Gregg, *pers. com.*). Long range migration enables moths to escape unfavorable conditions and reinvade new habitats, including inter-regional crop rotations. *H. punctigera* populations increase in inland Australia as a result of favourable autumn and winter rains. Moths generally migrate as habitat deterioration occurs (Gregg *et al.*, 1995; 1993; Farrow and McDonald, 1987) to cropping areas during spring on northerly airflows ahead of cold fronts (Gregg *et al.*, 1993; Dale *et al.*, 1992; Farrow and McDonald, 1987). *H. armigera* may migrate in response to unfavourable conditions, such as inadequate nectar supplies (Roome, 1975b; Cayrol *et al.*, 1974), but may also persist in large numbers in cropping areas during late summer, autumn and winter as diapausing pupae (Morton *et al.*, 1981). The migratory habit of *H. punctigera* tends to inundate cropping areas with moths which have not been exposed to insecticides, thereby diluting the effects of selection pressure for insecticide resistance. As a result, insecticide resistance in this species is negligible (Fitt, 1989).

Resistance in *Helicoverpa* spp.

Insecticide resistance in *H. armigera* is widespread and displayed against many products. Resistance to DDT (Goodyer and Greenup, 1980; Goodyer *et al.*, 1975; Wilson, 1974; Twine and Kay, 1973), endosulfan (Gunning and Easton, 1994a; Kay *et al.*, 1983), carbamates (Gunning *et al*, 1992), organophosphates (Gunning *et al*, 1998) and pyrethroids (Gunning *et al.*, 1984; Anon, 1983) has been reported in Australia. Insecticide resistance in *H. armigera* stopped the growing of cotton in the Ord valley (Michael and Woods, 1980; Wilson, 1974).

Insecticide resistance in field populations of *H. punctigera* was not reported until 1997 (Gunning and Easton, 1994b). Gunning *et al.* (1997) reported isolated insecticide resistance to the pyrethroid, fenvalerate, and to endosulfan and some carbamates in *H. punctigera*, but this resistance does not appear to have been sustained. Pyrethroid resistance had previously been reported after laboratory selection (Forrester *et al.*, 1993), indicating that *H. punctigera* certainly has the potential to develop resistance, but rarely does so in the field due to its ecology.

Resistance management strategy

In response to the discovery of *H. armigera* resistance to pyrethroids in 1982-83, the Australian cotton industry introduced an insecticide resistance management strategy. This strategy was designed to combat the increasing pyrethroid resistance levels by the rotation of unrelated chemical groups on a per generation basis. It recommended the restriction of pyrethroids to three sprays per season targeted at only one generation of *H. armigera* per year (Forrester and Bird, 1996; Daly, 1988; Sawicki and Denholm, 1987; Daly and McKenzie, 1986). This strategy resulted in the maintenance of moderate resistance levels and the continued effectiveness of many pyrethroids until the 1990's (Daly *et al.*, 1988; Daly, 1988). Recently, resistance to many insecticides, especially pyrethroids, has reached critical levels and their use in modern cotton production is under serious threat. As a result, the use of insecticides must be reduced to slow the ever increasing levels of resistance.

Economic impact of *Helicoverpa* spp.

Studies have estimated the economic impact of *Helicoverpa* spp. in Queensland and Australia. The annual cost of *Helicoverpa* spp. control and damage in Queensland crops was estimated at $16 million ($8-28 million) in 1980 (Alcock and Twine, 1981). Wilson (1982) estimated the Australia wide control to be $25 million in 1980 (Wilson, 1982). In 1988/89 this value had risen to $73.3 million in Australia, including $27.8 million for insecticide control and $45.5 million due to loses in production (McGahan *et al.*, 1991). In 1997 the estimated damage caused by *Helicoverpa* spp. to all Australian crops was $934 million per annum (range $426-$1,139 million per annum) (Adamson *et al.*, 1997). Estimates of losses in cotton alone are $385 million per annum (range $199- $460 million per annum), with the cost of managing *Helicoverpa* spp. in cotton at $87 million per annum

Helicoverpa spp. cause economic loss through loss in production, cost of insecticides and their application, insect monitoring and restrictions of crop choice. Losses are particularly significant in high value crops, including cotton, tobacco, sweet corn and many horticultural crops (Dale *et al.*, 1992; Fitt, 1989). Adamson *et al.* (1997) estimated the cost of controlling *Helicoverpa* spp. in cotton was 75% of the total management costs of *Helicoverpa* spp. control in

all crops, including grain legumes, field and horticulture crops combined. Often losses occur due to reduction in quality, rather than loss in production (Murray, 1995a), which is often not considered in estimations of the economic impact of *Helicoverpa* spp.

Future

Research on techniques of controlling *Helicoverpa* spp. through cultural and biological means has the potential to reduce the damage caused by *Helicoverpa* spp. and reduce the amount spent on control. McGahan *et al.* (1991) estimated that a 1% improvement in control effectiveness of *Helicoverpa* spp. in Queensland's crops would result in a $0.45 million per annum increase in their value. This value only considers improved production. If insecticide use was reduced as a result, savings would exceed this value. This clearly underlines the economic importance of implementing an IPM strategy in Australian Cotton.

Conclusions

From a understanding of the biology and ecology of *Helicoverpa* spp. it is possible to focus on methods of controlling this pest. From this knowledge, an integrated system of control can be implemented, rather than relying solely on insecticides. The integrated management of *Helicoverpa* spp. in Australian cotton is the topic of the next chapter.

Chapter 3

Integrated Pest Management of *Helicoverpa* spp. in Australian cotton

"Killing off many insects of minor importance helps the plants grow faster, so that the crop is usually matured earlier and the farmer has a better chance to get it properly harvested" USDA Yearbook 1952.

Introduction

Integrated pest management (IPM) is the practice where pest species are managed using the most appropriate control method. IPM aims to deal with the pest complex as part of the whole production system, rather than as a collection of separate individual problems. Pest management options available in an IPM scheme include chemical control (with rotation of chemical groups), biological control (including pathogens and natural enemies), cultural control, behavioural control (semiochemicals), use of economic thresholds, crop management and host plant resistance. Chemicals may be a important part of the IPM package, but they are certainly not the sole tool. An IPM strategy aims to make maximum use of natural mortality and selective or "softer" control practices, minimise selection for insecticide resistance, use insecticides only when necessary and use selective rather than broad-spectrum insecticides. The complete elimination of the pest is neither feasible nor desirable; some pests in the crop must be tolerated. An IPM strategy should always be flexible to incorporate new developments (Roome, 1979a).

The following discussion of IPM concentrates on management of *Helicoverpa* spp. as the key pest of Australian cotton. Some examples from other countries, crops and pests are included where relevant, especially for *H. zea* and *Heliothis virescens* (Fabricius) from the USA. However, the focus will predominantly be on *H. armigera* and *H. punctigera* as pests in cotton in Australia.

Insecticides in IPM

In IPM, each pesticide application is judged on the basis of the potential positive outcomes compared against all the negative outcomes, not solely against lost profit. Applications of insecticides represent purposeful environmental contamination and can be justified only when the benefit/risk ratio is clearly in favour of insecticide use (Metcalf, 1982).

Banning insecticides or the enforcement of strict guidelines for their use is not the answer. An environmental tax may be useful only if any penalties are balanced with bonuses for good practice. Education on best management practices and the elimination of unnecessary insecticide use would be a leap in the right direction. This is being addressed by the Australian cotton industry through the introduction of the Best Management Practices program (Cotton Research and Development Corporation. Narrabri, NSW). This program encourages growers to take responsibility for the environment through training manuals and seminars on reducing the impact on the environment.

Chemical control of *Helicoverpa* spp.

Control of *Helicoverpa* spp. in cotton is achieved almost solely through insecticide application (Twine, 1989). The high economic value of cotton and the biology and ecology of the crop (Chapter 1) and pests (Chapter 2), results in this high insecticide use.

Side-effects of insecticide use targeted at *Helicoverpa* spp.

Insecticides targeted at *Helicoverpa* spp. have been demonstrated to cause unwanted side effects. Resurgence of *Helicoverpa* spp. populations, the creation of secondary pests (Roome, 1979a; Boyer and Bell, 1961; Ripper, 1956; Newson and Smith, 1949) and the disruption of non-target arthropods are well documented (Lytton-Hitchins *et al.*, 1996; Parvin *et al.*, 1988, cited in King and Coleman, 1989; Waller *et al.*, 1988; Bishop and Blood, 1980; DeBach, 1974; Carson, 1962; Ripper, 1956; Gaines, 1954; Huffaker and Kennet, 1953). More direct effects on humans (Clarke and Churches, 1992), live-stock (Edge, 1996; Roome, 1979a) and the environment (Edge, 1996; Barret *et al.*, 1991) have recently been highlighted.

Despite obvious adverse effects from insecticide over-use, an attitude of over-kill persists within the Australian cotton industry. This is primarily due to the economics of insecticide making other pest options economically unpalatable. It is only practical problems, such as the ineffectiveness of key insecticides, that are forcing change. Perhaps the long term ecological effects of insecticide over use should be considered.

Cultural control

Cultural control can be defined as the manipulation of the environment to reduce rates of pest increase and damage (Pedigo, 1989). Cultural control generally aims to exploit any "weak-links" in the life cycle of the pest. Cultural techniques for *Helicoverpa* spp. management include destruction of pupa through soil cultivation (Marshall *et al.*, 1996; Hopkins *et al.*, 1972), trap crops (Anon, 1997), nursery crops (Mensah and Harris, 1996; Walker *et al.*, 1996), uniform early planting dates, and the use of rapidly fruiting cultivars (Mensah and Harris, 1994).

Crop management

Management of the cotton crop can have significant implications for the impact of *Helicoverpa* spp. Factors such as fertilizer use, irrigation, sowing dates and crop rotation can influence the damage caused by *Helicoverpa* spp. (Hearn and Fitt, 1992). Plant nutrition and chemical defences are known to influence prey and host dynamics (Scriber and Slasky 1981). Crop rotation can seriously impact on pest numbers in subsequent seasons, as some crops can breed huge numbers of moths. However, crop rotations with attractive crops can have a positive effect by attracting female *Helicoverpa* spp. females away from the cotton crop.

Other methods of cultural control

Novel methods of control have been investigated. These include sex pheromones (Betts and Gregg, 1993), repellents, antifeeding compounds and chemosterilants (Hopper, 1986). The potential for control of *Helicoverpa* spp. through cultural methods has not been exploited fully and work in this area is continuing.

Host plant resistance

The development of plants which are tolerant to insect attack has the potential to reduce insecticide reliance (Hearn and Fitt, 1992). Cotton varieties vary in attractiveness to ovipositing adults (antixenosis) and suitability for growth and development of *Helicoverpa* spp. larvae (antibiosis). Host plant resistance characteristics can be categorised as phenological (earliness, i.e. fast maturing, to avoid pests), morphological (increased yield, to compensate for damage), glabrousness and nectariless (to reduce attractiveness to *Helicoverpa* spp.), okra leaf and frego bract (to improve insecticide coverage), or allelochemical (gossypol and tannins, to reduce larval survival and development and repel *Helicoverpa* spp.) (Thomson, 1987; Niles, 1980). Many resistance characteristics may increase plant tolerance to pests by only small amounts, but these characteristics contribute to the overall IPM approach to controlling *Helicoverpa* spp.

Genetically modified or Ingard® cotton

Developments in genetic transformation have seen the introduction of genes expressing the *B. thuringiensis* (Bt) toxin into high yielding plants. Bt plants (Ingard®) were introduced for commercial production in 1996 (Long *et al.*, 1997). Bt toxins have a narrow spectrum of activity, are harmless to non-target insects, are biodegradable in the environment and target pests at their most susceptible stage. The first release of Bt plants was based on the Cry 1A(c) toxin (Long *et al.*, 1997). Alternative Bt toxins, virus genes and feeding inhibitors are being considered for future transgenic lines (Oakeshott and Gregg, 1995). Use of the Ingard® gene has been responsible for a reduction in the number of insecticide sprays by about 52-68% (Long *et al.*, 1997; Pyke, 1997).

The threat of resistance to Bt cotton is serious, if this technology is not managed efficiently (Fitt, 2000). In an attempt to prevent resistance occurring, a proactive insecticide resistant management plan was implemented (Fitt, 1997; 1995).

Genetically modified (GM) crops have a great role to play in IPM. If they become the dominant variety grown (>50%), it is hoped that the lessons of the past are not forgotten and that GM crops are used in an integrated approach rather than as the sole management tool. It must be ensured that we do not step off the pesticide treadmill only to land onto the GM crops treadmill.

Compensation

The ability of the cotton plant to compensate for damage caused by insects has been long recognised. Early-season leaf damage rarely affects yield, unless seedlings are killed (Bottrell and Adkisson, 1977; Dunnam *et al.*, 1943). Growth is indeterminate (Wilson *et al.*, 1972) and cotton plants only mature about one-third of the fruit they produce. Compensation for loss of early fruit occurs at yields less than 5 to 7.5 bales/ha. Dryland crops will rarely exceed this so compensation is very likely. Tugwell and Waddle (1974) stated that 80-95% of fruit (primarily squares) shed by a typical plant is due to climatic conditions and the rest to insects. Compensation must be considered in IPM of cotton. It is one aspect that is largely ignored by farmers.

Natural mortality

Natural mortality and cannibalism of *Helicoverpa* spp. in the field can often be significant. Natural mortality commonly occurs during the egg and first instar stages, due to wind and rain dislodging eggs, extreme climatic conditions and egg infertility (Dillon *et al.*, 1994; Titmarsh, 1992). Cannibalism in *H. armigera* may also be an important factor and is readily observed in the laboratory (Twine, 1971). However, the importance of this factor in the field is unclear, although one study found cannibalism to be insignificant in controlling *H. zea* in sorghum (Kinzer and Henderson, 1968). Natural mortality should be considered to avoid unnecessary insecticide use by overestimating the potential damage to the crop from egg counts. As environmental factors are the primary cause of natural mortality, management is difficult. Plant breeding which increases exposure of *Helicoverpa* spp. eggs laid in the canopy or plants which move more in response to the wind, might contribute to natural mortality.

Biological control of *Helicoverpa* spp.

Biological control of *Helicoverpa* spp. though the action of beneficials (predators and parasitoids) and pathogens is a crucial component of any IPM package (King and Coleman, 1989). Research has shown that natural enemies are capable of contributing substantially to pest mortality. However, their full potential is usually never realised due to extensive insecticide use (Smith *et al.*, 1976), the effects of which can last for up to three or four years (Debach and Rosen, 1991;

Debach, 1974). As a result, the actions of natural enemies are often inadequate to suppress pest numbers to a satisfactory level to prevent economic injury (Ives *et al.*, 1984). Pathogens are generally free from the disrupting effects of insecticides. The effectiveness of pathogens as biopesticides is presently being re-evaluated and shows great promise.

Pathogens of *Helicoverpa* spp.

Two pathogens have been exploited as commercial control agents of *Helicoverpa* spp. These are *B. thuringiensis* and the *Helicoverpa* nucleopolyhedrovirus (NPV). Pathogens have certain advantages over chemical insecticides. They are specific to the pest, have no resistance problems, no toxic residues and are relatively cheap to produce, develop and register (Lisansky, 1984). They are a simple technology, which can be produced at a local level, are compatible with other chemicals and existing application technology and can be self-perpetuating. Disadvantages compared to chemical insecticides include environmental instability, slow speed of action, short persistence, and the need for good application coverage since they usually need to be ingested. They often have a narrow host range. This is considered a disadvantage by chemical companies, as products have a reduced market and often minor pests normally controlled by *Helicoverpa* spp. control measures require specific treatment (Roome, 1975a). The major disadvantage of pathogens is that it usually takes a few days for effective *Helicoverpa* spp. control and feeding, although reduced, continues until shortly before death (Eid *et al.*, 1985; Teakle *et al.*, 1985a; 1985b; Stacey *et al.*, 1977).

Compared to synthetic insecticides, pathogens do not compete in efficacy. With slower rates of kill, narrow target range and questionable effectiveness against high *Helicoverpa* spp. numbers, pathogens appear to be a poor substitute. However, they are an important component of IPM, especially early season, and when used in conjunction with high beneficial numbers, pathogens can achieve satisfactory levels of control.

Natural enemies of *Helicoverpa* spp.

Some control of *Helicoverpa* spp. by beneficial insects has long been recognised (van den Bosch *et al.*, 1969; Lingren *et al.*, 1968a; Ridgway, *et al.*, 1967; Ewing and Ivy, 1943; Fletcher and Thomas, 1943), although the actual effectiveness of beneficial insects in controlling *Helicoverpa*

spp. is still unclear (Stanley, 1997; Stanley and Gregg, 1994). Natural enemies of *Helicoverpa* spp. have been recorded from many groups (see Zalucki *et al.*, 1986; Knutson, 1985; or Roome, 1979b for a detailed lists of beneficials). Roome (1979b) published a list of 16 species of parasitoids and 24 species of predators (of about 500 species of arthropods collected) which attack *Helicoverpa* spp. in the Namoi Valley. The actions of natural enemies may be promoted through classical introduction, augmentation or by conservation of natural indigenous beneficials.

Classical biological control

Classical biological control involves the introduction of exotic species of natural enemies of *Helicoverpa* spp. Several species have been released into Western Australia to control *Helicoverpa* spp. These include *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) from the USA (Woods, 1981); *Cotesia kazak* Telenga (Hymenoptera: Braconidae); *Apanteles marginiventris* (Cresson); *Hyposoter didymator* Thunberg (Hymenoptera: Ichneumonidae); and *Campoletis chlorideae* (Uchida) (Hymenoptera: Ichneumonidae) (Michael *et al.*, 1984). Attempts to introduce *C. kazak* and *H. didymator* into southern and central Queensland were made during 1991-2 (Murray and Rynne, 1992). However, establishment of these species has not been confirmed (D. Murray, *pers. com.*). Attempts at classical control have met with little success in other parts of the world because natural enemies have not been able to establish or exert acceptable control over highly fecund pests (Johnson *et al.*, 1986; Cate, 1985). Classical biological control should target introduced pests which lack their own natural enemy complex. This certainly does not apply to *H. punctigera* which is clearly endemic and probably does not apply to *H. armigera* which is likely to be so.

Augmentation

Augmentation, or inundation of predators and parasitoids, is achieved through field releases of laboratory reared insects. Augmentation of natural enemies attempts to artificially increase the beneficial population to a level at which they provide effective control. The difficulty of mass-producing predators and parasitoids at a cost competitive with other control strategies and in sufficient numbers for their timely release is the major limiting factor in augmentation. Factors such as host density, beneficial density, beneficial fecundity, insecticide hazards and host location mechanisms must be considered for a successful inundative biological control program. Some

predators and parasitoids have been trialed in augmentation programs targeted at *Helicoverpa* spp. in cotton, with varying success (Ridgway and Jones, 1969; 1968).

The predator *Chrysopa carnea* Stephens (Neuroptera: Chrysopidae) has been used for augmented control of *H.* zea and *H. virescens* in the U.S.A. (Ridgway and Jones, 1969; 1968). Control by *C. carnea* was estimated at up to 95% and yield was increased 3-fold (Ridgway and Jones, 1969). These data clearly vindicate the potential of inundative releases in IPM systems in cotton.

Egg parasitoids have been used extensively in augmentative control, also with varying success (King *et al.*, 1985a; 1985b; 1985c; Ridgway and Morrison, 1985; Li, 1984; Ridgway *et al.*, 1977). Stinner *et al.* (1974) recorded a parasitism rate of 66-80% in *H. zea* and *H. virescens* by *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) even after interference by insecticide drift after releases of between 77,500-397,500 adult parasitoids per acre. Twine and Lloyd (1982) recorded a parasitism rate of *Helicoverpa* spp. eggs by *Trichogramma* spp. of 49.4% (range 11.7% to 100%). This level of control was considered inadequate. Murray *et al.* (1994) recorded a 40% increase in egg parasitism after releases of *T.* nr. *brassicae* or *T. funiculatum* (total of 73% parasitism in the release area). Egg parasitoids attack *Helicoverpa* spp. early in its development, and this has the advantage that little damage has occurred to the crop. However at this early stage, little natural mortality has occurred, and egg parasitoids often suffer from overwhelming pest numbers. Often, the numbers of *Helicoverpa* spp. eggs are just so great that even an 80-90% reduction in egg numbers is insufficient to prevent economic damage.

Larval parasitoids have the advantage over egg parasitoids because of the great natural mortality which has already occurred in the egg and early instars. This means that any reduction in pest numbers will reduce damage significantly, and a lower percent reduction in pest numbers will often result in an acceptable level of control. The larval parasitoid *Campoletis sonorensis* (Cameron) (= *C. perdistinctus* (Viereck)) (Hymenoptera: Ichneumonidae), has shown potential in augmentative releases in large field cage trials in the USA. These trials have shown adequate parasitism of *H. virescens* of 85-95% for ca. 2 months (Lingren, 1977). Lewis and Gross (1989) used small scale plots to estimate the number of wasps required to achieve desired levels of

parasitism after augmentative releases. They estimated that between 900-1,500 female *Microplitis crociepes* (Cresson) (Hymenoptera: Braconidae) per ha. were required to achieve 80% parasitism on soybeans. The same study and the study of Lewis *et al.* (1972) estimated that 900-1500 female *Cardiochiles nigriceps* (Viereck) (Hymenoptera: Braconidae) per ha. were required for adequate parasitisation of *H. virescens*. Tillman (1995) compared the rates of parasitism by *C. nigriceps* and *M. crociepes* after field releases in small-scale plots. She found that *C. nigriceps* parasitised more pests (30%) than *M. crociepes* (7%) and suggest that *C. nigriceps* was better suited to inundative releases than *M. crociepes*. Murray *et al.* (1994) reported only a 10% increase in parasitism by the native braconid *M. demolitor* after augmentative releases in southeast Queensland. However, insufficient release rates may have been responsible for this poor control. Lack of host location mechanisms in laboratory reared insects is an important factor and may have contributed to the small increase in parasitisation seen in the above study (see p. 32).

Inundative releases of beneficials are not yet economically feasible in cotton in Australia or USA (King *et al.*, 1985a). Considerable research has been devoted to developing techniques for mass-rearing beneficial species for augmentative releases. Cost effective methods for rearing, storing and releasing viable natural enemies must be developed. As far back as 1980, *M. demolitor* was recognised as having potential in an inundative release program in Australia (Anon, 1981). There may be potential for a government/industry funded inundative release program of *M. demolitor* in cotton in Australia. A 1% improvement of control of *Helicoverpa* spp. in Queensland would result in a $450 000 increase per annum in production (McGahan *et al.*, 1991). It will be shown in Chapter 6 that parasitism of *Helicoverpa* spp. may reach approximately 80% in naturally occurring populations. This means that only a small area receiving 80% parasitism is needed to result in an average of 1% for the entire area. *M. demolitor* has the added advantage that it is self-perpetuating, so that any early season releases will have season long benefits. However, until augmentation becomes a reality, conservation of naturally occurring beneficials is essential.

Naturally occurring beneficials

Naturally occurring populations of beneficials are the dominant form of biological control in most cropping systems and must be utilised to their full capacity if IPM is to become a reality (Luck *et al.*, 1988). Naturally occurring predators and parasitoids were estimated to be responsible

for between 50-90% of mortality of the egg and first two larval stages of *Heliothis* spp., in cotton in the USA. Ridgway and Lingren (1972) suggested that 75% of *Heliothis* spp. eggs and larvae are killed by predators. Two approaches can be used in the conservation of indigenous populations of beneficials. Firstly, sampling of beneficial populations is carried out and pest management decisions are modified accordingly. Alternatively, selective insecticides are used and it is assumed that beneficial populations are present and exerting some control over *Helicoverpa* spp. (Ives, 1981). Farmers mostly use the second approach, because the impact of specific species is not known. The amount of time needed to sample predators is considered unrealistic and field-sampling techniques have not been fully assessed (Stanley, 1997). In Australia, there is no specific use made of biocontrol agents in commercial cotton production, although in some instances, knowledge of their activity is used in pest management systems (Twine, 1989).

Predators

The extent to which naturally occurring predators control *Helicoverpa* spp. is unclear. A diverse array of predacious beneficials have been recorded from the cotton crop (Room, 1979b). However, many are general predators and prey on other insects, especially aphids and jassids (Stanley, 1997). Predators of *Helicoverpa* spp. are probably confined to about 10 to 15 families. Specific studies have demonstrated that predators play a significant role in *Helicoverpa* spp. control. Lingren *et al.* (1968b) used large field cages to demonstrate that indigenous populations of larval *C. carnea* and adult *Geocoris punctipes* (Say) (Hemiptera: Lygaeidae) reduced egg and larval populations of *H. zea* on cotton. Van den Bosch *et al.* (1969) used field cages to show that indigenous populations of *G. pallens*, *Nabis americoferus* Carayon (Hemiptera: Nabidae), and *C. carnea* significantly reduced populations of bollworm larvae in cotton in California.

Proving which predators are effective against *Helicoverpa* spp. is difficult. Stanley (1997) found that predators generally offered only small and unpredictable contributions to *Helicoverpa* spp. mortality. Cage studies on *Dicranolaius bellulus* (Guérin-Méneville) (Coleoptera: Melyridae), and *Mallada signata* (Schneider) (Neuroptera: Chrysopidae) showed that at realistic *Helicoverpa* spp. densities (<20 eggs/m) predation was insignificant (≈0.2 eggs/beetle/day). Stanley (1997) suggested that predation rates may be low and variable because of the presence and abundance of alternative prey, variable environmental conditions, differences in development stages of predators

or expression of plant defences. Knowledge of the effectiveness of predators, or predation rates is more important than the actual number present. Predation rates undoubtedly depend on factors such as age (and therefore size) of prey, environmental conditions, search ability and availability of alternative prey. Predators may migrate out of the cotton crop if insufficient prey numbers are present. Although it is unclear whether predators contribute significantly to *Helicoverpa* spp. mortality, predators are known to be extremely important in controlling secondary pests, such as aphids and mites, so the conservation of predators is crucial for successful IPM.

Parasitoids

Indigenous parasitoids have been demonstrated to be crucial to many successful IPM programs. Parasitoids differ from parasites in that they kill their host during normal development. Parasites do not intentionally kill their host. Parasitoids are known to attack all life-stages of *Helicoverpa* spp. and are very much more host specific than predators, therefore their presence is much more easily related to pest control in the field.

As already discussed, egg parasitoids often face overwhelming pest numbers. Murray *et al.* (1996) has recorded natural egg parasitism of between 50-70% by *Trichogramma* spp. Twine (1973) recorded only 7.92% parasitism of *Helicoverpa* spp. eggs on the Darling Downs, mostly by *Telenomus* sp. nr *triptus* (Nixon) (Hymenoptera: Scelionidae). These levels of parasitism in isolation would be inadequate under high pest pressure to reduce *Helicoverpa* spp. numbers below threshold.

Parasitism of the pupal stage of *Helicoverpa* spp. has the disadvantage that damage has already been done in the current generation, but it reduces the number of pests in the next season. This is especially important in insecticide resistance management. Wilson (1983) estimated parasitism of diapausing pupae in all crops in the Namoi valley to be 18.3% (range between 9.8 to 34.7%). The dominant parasitoids were *Heteropelma scaposum* (Morely) (Hymenoptera: Ichneumonidae) and *Carcelia noctuae* Curran (Diptera: Tachinidae). Kay (1981) also found *H. scaposum* and *Carcelia* sp. to be the dominant parasitoids of *Helicoverpa* spp. in southeastern Queensland

Parasitism of larval *Helicoverpa* spp. is very important because extensive natural mortality of egg and neonate larvae has already occurred, and negligible damage has been done to the crop. Species of parasitoids in the USA include the braconids *M. crociepes*, *C. nigriceps*, which only parasitises *H. virescens*; and *Campoletis* sp. and *Cotesia* (=*Apanteles*) *marginiventris* (Cresson) (Pair *et al.*, 1982; Burleigh and Farmer, 1978; Smith *et al.*, 1976; Young and Price, 1975; Graham *et al.*, 1972; Shepard and Sterling, 1972; Bottrell *et al.*, 1968; Lewis and Brazzel, 1968; Chamberlin and Tenhet, 1926). King *et al.* (1985d) demonstrated that *M. crociepes* was the dominant parasitoid (>90% parasitism) of *H. zea* and *H. virescens* in the USA.

Estimates of percent parasitism by *M. croceipes* in the USA range from 4-23% in the 1960s and 1970s (King *et al.*, 1985d), 15-18% in 1964-1966 (Lewis and Brazzel, 1968), 20% (0-47%) in 1975 (Burleigh, 1975), 30.9% in 1981, 50% in 1982 (King *et al.*, 1985d) and 57% in 1982 (Powell and King, 1984). Some of the fields studied had received extensive insecticide applications. Parasitism by *M. croceipes* has been recorded as high as 70% (range 0-70%) (Burleigh and Farmer, 1978; Smith *et al.*, 1976; Bottrell *et al.*, 1968). The steady increase in parasitism, especially by *M. crociepes*, is attributed to reduced insecticide use and the use of selective insecticides, including some pyrethroids (see page 174), chlordimeform, methomyl and microbial insecticides rather than organochlorine and organophosphate insecticides. Generally, higher parasitism rates in the USA occurred early and late season, and were associated with lower rates of insecticide use. Higher rates of parasitism have been recorded in fields with no insecticide use (68.3%) compared to those where insecticides were used (44.3%) (King *et al.*, 1985b; Lewis and Brazzel, 1968). Seasonal patterns in parasitism have been identified. Parasitism increased during early and mid-season but declined as the season progressed (King *et al.*, 1985d; Burleigh, 1975; Lewis and Brazzel, 1968). There was no significant difference in the rates of parasitism by *M. crociepes* of *H. zea* or *H. virescens* (King *et al.*, 1985d; Powell and King, 1984).

M. demolitor

The dominant parasitoid of *Helicoverpa* spp. in many cropping areas of Australia, including southeast Queensland, is a species closely related to *M. croceipes*, namely *M. demolitor*. Estimates of parasitism for *M. demolitor* of between 30-50% have been recorded in cotton crops in Australia (Murray *et al.*, 1996; Shepard *et al.*, 1983a) and may even be higher at certain times of the year in

cotton (see Chapter 6). Titmarsh (1981) found that *M. demolitor* was the dominant parasitoid of *Helicoverpa* spp. in tobacco in northern Queensland. Forrester (1981) recorded parasitism of *Helicoverpa* spp. by *M. demolitor* in sunflower over the season of 16% for December, 3% for January, 5% for February, 16% for March, 12% for April and 6% for May. Broadley (1984) studied parasitism of *Helicoverpa* spp. by *M. demolitor* in sunflowers in southeast Queensland. He found over 90% total parasitism late season, with *M. demolitor* the dominant parasitoid. Broadley (1984) found a distinct seasonal build-up in parasitism of *Heliothis* spp. He suggests that this indicates that early season augmentation may be very productive. Forrester (1981) recorded no pronounced seasonality in parasitism by *M. demolitor*, however he collected only a relatively small number of larvae (502).

The effects of parasitism on *Helicoverpa* spp. are often underestimated. In addition to direct larval mortality, parasitised larvae often cause less damage to the crop than unparasitised larvae. Feeding experiments have shown that *Helicoverpa* spp. larva parasitised by either *M. demolitor* or *M. croceipes* during the first through fourth larval instars consumed approximately 10% of that consumed by a healthy larva (Powell, 1989; Cobb *et al.*, 1985). Parasitised larvae, at the end of the parasitoid's larval stage, weighed about 10% of healthy larvae (Murray and Rynne, 1992; Seymour, 1991). This will be discussed in more detail in Chapter 5.

Managing beneficials

Rarely are beneficials considered when making *Helicoverpa* spp. control decisions. The economic value of indigenous predators in the Mississippi delta has been calculated at $43.47 per ha. and this value does not consider the action of parasitoids (Parvin *et al.*, 1988, cited in King and Coleman, 1989). It may be assumed that the value of indigenous predators is similar for Australian cotton fields. This resource is largely ignored, perhaps because no guidelines exist for sampling or decision making for their conservation.

Mensah *et al.* (1996) published predator-prey ratios that have been used to manage cotton without insecticides, which have produced yields similar to conventionally managed cotton. A threshold of 0.5 to 1 predator per *Helicoverpa* spp. egg or larvae has been suggested as a necessary level for control (Johnson *et al.*, 1986). If accurate thresholds can be determined, then

the impact of beneficials based on their number may be accurately predicted. Beneficial thresholds are only useful if there is an accurate and reliable method of sampling populations and the role of any beneficial species is well understood. Relative estimates of predator densities have been determined using visual assessments, shake sheets, sweep nets and suction machines (Murray and Mensah, 1996). Assessment of beneficial insects will be an additional burden on crop managers. However, if savings from reduced insecticide application can be passed on to crop consultants, then smaller areas of crops may be managed more satisfactorily.

Conservation of natural enemies requires knowledge of their ecology. With this knowledge, crop protection strategies can be modified to avoid natural enemy destruction. Methods used to conserve natural enemies may include:

- reducing, or eliminating insecticide use;
- delaying insecticide application early season;
- using least disruptive insecticides;
- application of insecticides to coincide with least susceptible life stages of beneficials;
- maintaining habitat;
- maintaining alternate food sources or hosts;
- use of insecticide resistant beneficials;
- avoidance of harmful cultural techniques;
- use of nursery crops; and
- provision of artificial food supplements.

The expectations of growers about beneficial insects must be realistic. According to Murray and Mensah (1996), season long benefits should not be expected from predators. Instead, early to mid season contributions can greatly reduce insecticide use. Beneficials must be viewed as a major piece of the IPM mix. Once it is established that naturally occurring beneficials are present at sufficient densities, every effort should be made to conserve them. As the use of disruptive insecticides is reduced with the implementation of true IPM, the true impact of beneficials will be realised, and it may be greater than previously envisioned.

Conclusions

After a review of the literature on *Helicoverpa* spp. management in Australia, it is easy to be cynical about the progress made by the Australian cotton industry. The same conclusions are drawn, the same recommendations are made, and the same plea for reduced insecticide use through promotion of natural enemies is made. It is disheartening that the main push for IPM comes not in the light of reducing insecticide use, but as a last resort, as resistance to many insecticides increases.

A simple IPM package for cotton production has only recently been published. However, many of the components, such as economic thresholds, pupae destruction, trap crops, nursery crops and food sprays, have long been developed and have the potential to reduce insecticide use and manage insecticide resistance. These components are not being widely implemented by growers (Shaw and Browne, 1995). The long term goal of research in cotton is to implement IPM systems where insecticide inputs are relatively minor. However, until this is realised, industry should aim to reduce insecticide use, even if only by a few sprays per season. Hopefully the role of natural enemies and pathogens, although not fully understood, is at least appreciated and the days of regular blanket application of broad-spectrum insecticides are over. Often biological control measures are unfairly compared against broad-spectrum insecticides. Biological methods must be used as a package with all factors contributing a little. Effective, economical, and environmentally responsible control of *Helicoverpa* spp. will be achieved only through the integration of biological control with other pest management approaches (Knutson and Nagarkatti, 1985).

Hopefully, recommendations such as those made by the USDA Yearbook of 1952, quoted at the beginning of this chapter, are nowadays a source of amusement. Early farmers cannot be blamed for using insecticides recklessly, because they knew no better. Hopefully we have learned something over the years. Farmers are in the business of growing cotton for profit. The bigger the yields the bigger the profit. Farmers cannot, for the most part, be blamed for the slow adoption of IPM practices; chemicals reduce risk and are tried and tested. IPM in comparison is not, and in the end it is the farmer who takes the ultimate risk. Once IPM becomes accepted, adoption throughout the industry will, I believe, be rapid.

Chapter 4

M. demolitor: A key component of IPM in Australian cotton

A key component of IPM is the conservation and maximum use of indigenous natural enemies (King and Coleman, 1989).

Introduction

M. demolitor is the key parasitoid of *Helicoverpa* spp. in many cotton producing areas of Australia, including southeast Queensland (Murray, 1994). *M. demolitor* is a solitary parasitoid, belonging to the subfamily Microgasterinae, family Braconidae, subfamily Ichneumonoidea. The genus *Microplitis* Foerster contains more than 130 described species worldwide, all of which are endoparasitoids of moth larvae, mostly from the family Noctuidae (Austin *et al.*, 1993). The genus *Microplitis* is characterised by characteristic propodeal sculpturing, the shape of the fore wing areolet and first metasomal tergite, and the absence of a prepectal carina (Austin and Dangerfield, 1992; Mason, 1981). There are three described species of *Microplitis* recorded from Australia. These are *M. demolitor*, *M. basalis* (Bingham), and *M. perelegans* (Bingham). This genus is poorly studied and more species certainly occur in Australia (Austin *et al.*, 1993).

This chapter will examine the biology and ecology of *M. demolitor*, including host range, life-cycle, sex determination, mate location, host location, superparasitism, effects on the host, ascovirus, and diurnal behaviour. Estimations of populations in the field, monitoring techniques, and effects of pesticides with be briefly discussed. *M. croceipes*, a closely related species indigenous to the USA, has been studied extensively, and is the predominant parasitoid of *H. zea* and *H. virescens*, native to the USA (King *et al.*, 1982; Lewis and Brazzel, 1968). Work carried out on *M. croceipes* can often be extrapolated to *M. demolitor*.

Biology and ecology of *M. demolitor*

Host range

M. demolitor is indigenous to Australia (Austin *et al.*, 1993) and ubiquitous to areas where cotton is grown. It attacks several species of Noctuid larvae, including *H. armigera* and *H. punctigera* in several field crops. *M. demolitor* has been reared from *H. armigera*, *H. punctigera*, *Spodoptera litura* (F) (Hafez, 1951) and *Laelia obsoleta* (F) (Austin and Dangerfield, 1992) in Australia. In the USA, *M. demolitor* has been reared from *H. zea*, *H. virescens*, *Pseudoplusia includens* (soybean looper) (Walker) and *Trichoplusia ni* (Hübner) (Shepard *et al.*, 1983b; Yanes and Boethel, 1983). Shepard *et al.* (1983b) showed that *M. demolitor* readily attacked *Spodoptera exigua* (Hübner), *S. frugiperda* (J.E. Smith) and *Anticarsia gemmatalis* (Hübner), however, parasitoids did not develop successfully in these hosts.

Life-cycle

M. demolitor females attack *Helicoverpa* spp. larvae early in their development, usually the second instar. Third and fourth instar larvae are suitable as hosts but these larvae vigorously defend themselves, often resulting in injury or death of the adult female (Shepard *et al.*, 1983b). Up to fifth instar larvae are suitable for development by *M. croceipes*. The life cycle of *M. demolitor* (Figure 4-1) takes about 12 days at 25°C, with 7 days from egg lay to pupation and 5 days for pupal development (Murray and Rynne, 1992). Developmental times of *M. demolitor* from egg to pupation in *H. zea* of 9 days at 26°C (Shepard *et al.*, 1983b) and 27°C (Cobb, 1983 cited in Cobb *et al.*, 1985) have been recorded when hosts were fed artificial diet and 8.7 days when fed cotton foliage (Culin and Dubose, 1987).

The life cycle of *M. demolitor* can be summarised as follows: the female usually oviposits 1-3 (average = 1.4 ± 0.7) eggs (Strand *et al.*, 1988) through the integument into the haemocoel of the host. Oviposition lasts 1 to 2 seconds; the host is held with the anterior pair of legs while the parasitoid folds her wings above her body and lifts the middle and posterior pairs of legs as a form of defence (Shepard *et al.*, 1983b). Approximately 5 minutes is required between ovipositions, with this time increasing with repeated ovipositions. The pre-oviposition period in *M. croceipes* is

extremely short, with successful oviposition possible about 24 hours after adult emergence (Bryan *et al.*, 1969).

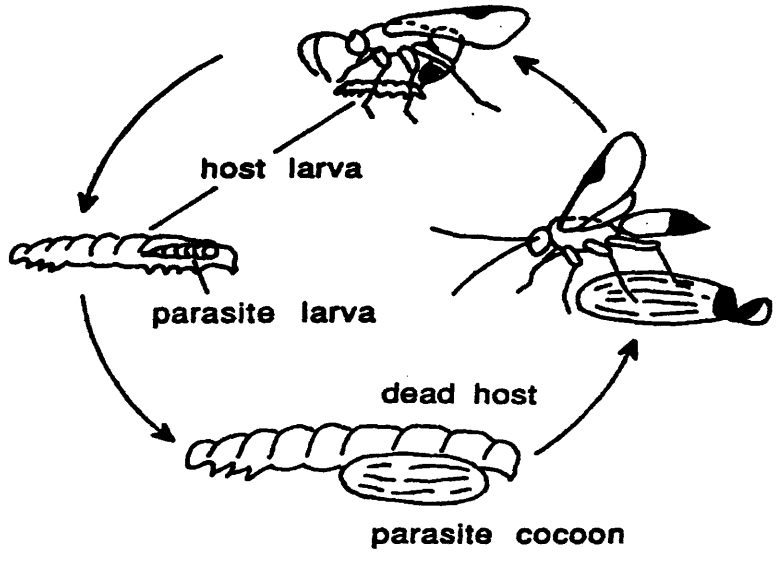

Figure 4-1. Life cycle of *M. demolitor* (drawn by Dr. D. Murray).

After hatching the parasitoid larva consumes the host internally. The larval parasitoid goes through 4 or 5 instars (Strand, unpublished data, cited in Strand *et al.*, 1988) before it exits from the host, spins a cocoon and pupates next to the host. Host larvae always complete two instars before the parasitoid completes larval development (Seymour, 1991). Development from pupation to adult emergence takes about 5 days, with males developing significantly faster than females (Shepard *et al.*, 1983b). Occasionally twin *M. demolitor* are produced, often depending on the size of the host (Shepard *et al.*, 1983b). Strand *et al.* (1988) found twin parasitoids in 6% of hosts (n = 33). Titmarsh (1985) estimated that 1% of parasitised *H. armigera* completed development and pupated successfully after the parasitoid larva had emerged. Host larvae parasitised by *M. crociepes* successfully pupate much more commonly (Shepard *et al.*, 1983). Whether host pupae produce viable adults requires investigation. Strand *et al.* (1988) found that 10% of hosts showed characteristics associated with parasitism but no parasitoids emerged. This may be termed pseudo-parasitism, and of such cases, half of the parasitoid larvae developed to final instar and half died as unencapsulated larvae at first instar. Emergence of larval parasitoids is determined

photoperiodically. Strand *et al.* (1988) showed that parasitoids only emerged from their host 1-3 hours after exposure to light. Adult males usually emerge slightly sooner than the females.

Mating occurs soon after adult emergence. As the male approaches the female, they become stimulated and extend and flutter their wings. The male then mounts the female and extends the tip of his abdomen down. Copulation usually lasts only a few seconds.

Adult *M. demolitor* live for approximately 8 days at 26.7°C, with males living for approximately 8.3 days and females approximately 7.6 days. This difference was not statistically significant (Shepard *et al.*, 1983b). *M. demolitor* readily diapause, over-wintering in the prepupal stage in the cocoon. Adults emerge from late June to mid December, with peak emergence in August (Kay, 1981). This cycle is synchronised with its host. The cues inducing diapause are unknown, although Bryan *et al.* (1969) suggested that exposing larval *M. croceipes* to low temperatures induced diapause.

Sex determination

Adults can be readily sexed with the naked eye. Males are distinguished from females as they have longer antennae. Females also have a larger darker region at the tip of the abdomen and the ovipositor may also be readily seen, although confirmation of the presence of the ovipositor often must be done under the stereo microscope. Conversely, males have a smaller dark region at the tip of the abdomen which is more rounded and lacks an ovipositor (Figure 4-2).

Shepard *et al.* (1983b) showed that the sex ratio of *M. demolitor* progeny in a laboratory culture was 1:1 where males and females were allowed to emerge and mate *ad libitum*. In a culture of *H. zea* and *H. virescens*, Tillman *et al.* (1993) found that 61% of progeny were female when reared in *H. armigera*, however only 21% were female when *H. zea* were used as host.

The sex ratio of laboratory reared wasps is affected by temperature. When laboratory wasps were held at lower temperatures, their progeny were predominantly males. These effects were observed when cultured adult wasps were stored at 15°C (Herard *et al.*, 1988a). Bryan *et al.* (1969) also observed a similar temperature effect on *M. croceipes*. Herard *et al.* (1988a) showed

that when *M. demolitor* pupae were held at 13°C for 7 and 13 days, 94 % of the emerged progeny were males. *M. demolitor* exhibits arrhenotokous parthenogenicity. Virgin females produce male progeny (Shepard *et al.*, 1983b; Hafez, 1951), this suggests that low temperatures may be interfering with reproduction, possibly harming the sperm or egg. This is important when culturing *M. demolitor* in the laboratory.

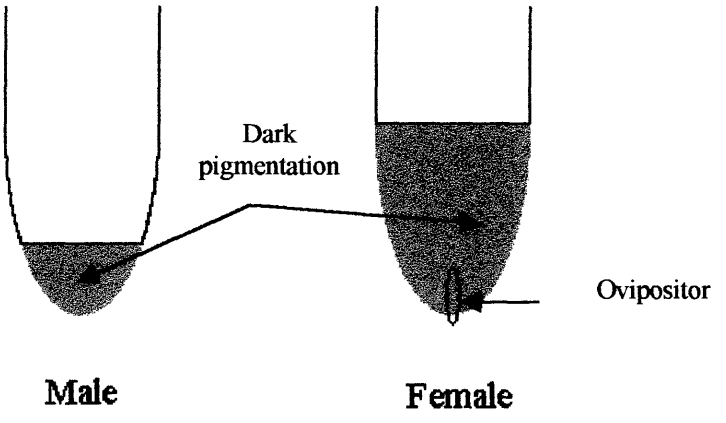

Figure 4-2. Diagrammatic representation distinguishing the sexes of *M. demolitor*. Abdomen of male and female *M. demolitor*, showing ovipositor protruding in the female.

Mate location

Powell and King (1984) studied the activity of *M. croceipes* males in response to sticky traps baited with virgin females. They found that the use of sticky traps baited with virgin females may be an important monitoring tool. Powell and King (1984) found the earliest catch of male wasps was at 0830. 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. Herard *et al.* (1988a) found that females which had been stored at 13°C as adults or pupae produced pheromones normally and male wasps stored likewise, responded positively to calling by females. This means that insects, which are chilled to slow development, can still be used in reproductive studies.

Host location

Female parasitoids use short range chemical cues from fecal material excreted by a potential host as an aid in host location. Sight does not appear to be involved even at very close range (Lewis, 1970a). Sheehan *et al.* (1993) showed that *M. croceipes* adults spent less time searching sites previously searched by themselves or by other adults than unsearched sites. Sheehan *et al.* (1993) suggested that adults use chemical marking and spatial memory to avoid previously searched sites. Observations in the field show that females are frequently observed in the crop searching for potential hosts and can be readily followed to larvae in the crop.

Many studies have shown that insect parasitoids use semiochemicals from the caterpillars or their food plants to locate potential hosts. Norlund and Lewis (1985) showed that *M. demolitor* females were stimulated by kairomones (including 13-methylhentriacontane) in the frass of *H. zea* larvae. Larval diet of hosts affects *M. demolitor* ability to responded to plant volatiles. *M. demolitor* reared from host *H. zea* larvae fed artificial diet did not respond to these volatile semiochemicals (Whitman and Eller, 1992; Herard *et al.*, 1998b; Norlund and Lewis, 1985). Whitman and Eller (1992) found that if *M. demolitor* females had pre-flight contact with frass from *H. zea* fed cowpea, they were stimulated to respond to the plant volitiles. Whitman and Eller (1992) also found that chilling parasitoid pupae rendered most of the emerging females unresponsive to volatile semiochemicals. This has implications for inundative and augmentative release programs.

Many studies have demonstrated the occurrence of associative learning in the host finding process in *M. croceipes* (Takasu and Lewis, 1993; Kaas *et al.*, 1990; Lewis and Tumlinson, 1988; Drost *et al.*, 1986) and *M. demolitor* (Whitman and Eller, 1992; Herard *et al.*, 1988b). Olfactory cues have been shown to be important (Lewis and Tumlinson, 1988). Prior experience influences the searching behaviour with an increased response and increased activity of wasps after previous contact with host frass, or plant material, on which the parasitoid had previously found a host. Kaas *et al.* (1990) showed that the conditioning response decreased with time and, when offered alternate stimuli, wasps remember their initial training. The ecological advantages of learning are that wasps show an increased response to a successful oviposition and limit their searching to rewarding host plants. The decrease in conditioning over time means that wasps which have little

success can change their searching pattern. It is unclear how remembering initial conditioning can benefit wasps. Learning ability is thought to be more important in oligophagous parasitoids than in monophagous parasitoids, enabling these insects to behave more flexibility in variable environments (Vinson, 1976). Semiochemicals may be used in pest management to attract parasitoids to a crop or to stop naturally occurring ones, dispersing. If the volatiles associated in host location can be identified and synthesised, they would be a useful tool in IPM. Some aspects of host location will be discussed in Chapter 6.

Superparasitism

Superparasitism by larval parasitoids has been studied in depth. Vinson and Guillot (1972) showed that the braconid parasitoids, *C. nigriceps* and *M. croceipes*, readily parasitised *H. virescens* hosts which had been stung only once or had been stung by the alternate species. However, hosts which had been stung more than once were attacked significantly less often by the same species. Vinson and Guillot (1972) marked hosts with extracts of parts of the female reproductive organ of the parasitoid and showed that extracts from the alkaline gland were responsible for marking hosts. Vinson and Guillot (1972) suggest that in larval parasitoids, parasitisation more than once may be an evolutionary adaptation to ensure that aggressive host larvae are successfully stung, compared to the egg parasitoids, *T. evanescens*, which do not readily superparasitise their hosts (Salt, 1937).

M. demolitor readily superparasitises host larvae. Shepard *et al.* (1983b) found that *M. demolitor* females readily accepted parasitised hosts, but a single parasitoid normally develops from each host even after super-parasitism. Avoidance of super-parasitism may be important to *M. demolitor* in the field as energy is wasted searching for and stinging larvae already parasitised, and in laboratory cultures as the full reproductive potential of each female may not be realised. Shepard *et al.* (1983b) demonstrated that there was no increase in host mortality even after repeated attacks. The occurrence of superparasitism in *M. demolitor*, no doubt, contributes to the transmission of ascovirus in the field (see below).

Effects on the host

Endoparasitoids induce a variety of physiological and behavioral effects on their host, including juvenilizing, arrested or accelerated development, inhibition of metamorphosis and disruption of diapause. Most parasitoids are adapted to a specific life stage in a specific host and are unable to successfully parasitise other stages or hosts. This indicates that there are complex specific endocrine interactions between the host and parasitoid which must be exactly synchronised in order for a parasitoid to complete development. Endoparasitiods and hosts often display remarkable developmental synchrony.

Some aspects of the effects of *M. demolitor* on host *H. armigera* larvae will be discussed in greater detail in Chapter 5.

Ascoviruses

Ascoviruses are a relatively newly discovered group of DNA viruses reported from noctuid larvae. Ascoviruses cause chronic and fatal disease, with the haemolymph of the host becoming a characteristic milky white colour (Govindarajan and Federici, 1990). Ascoviruses have been described in *T. ni* (Browning *et al.*, 1986), *H. virescens* and *H. zea* (Carner and Hudson, 1983), and *S. frugiperda* (Hamm *et al.*, 1986). In these species, infected larvae lost their appetite, did not gain weight; they grew and developed slowly before succumbing to the virus. Symptoms of ascovirus infection in *Helicoverpa* spp. are pale coloration and slower growth and development than healthy larvae. Infected larvae had extended development times, but did not pupate. Govindarajan and Federici (1990), studying ascoviruses isolated from *H. zea*, *S. frugiperda*, and *T. ni*, showed that, although infection through ingestion was possible, it was erratic and at very high doses of the viruses (10^6 viral vesicles resulted in about 30% infection). The most likely and the most ready form of infection was through introduction of the virus into the haemolymph (10 to 10^5 viral vesicles per larva, resulted in ≥90% infection). This suggests that ascoviruses in the field are most likely vectored by insect parasites and parasitoids.

From an evolutionary perspective, how a virus using a parasitoid as a vector for its transmission would evolve is unclear, because the parasitoid larva would consume and kill the host before the virus would have the opportunity to become transmitted to a new host and therefore

would not proliferate. However, Hamm *et al.* (1985) showed that *S. frugiperda* larvae infected with ascovirus by its parasitoid, *C. marginiventris*, at the time of oviposition or during the period of parasitoid larval development, failed to complete development, even though the host survived much longer than the period required for the parasitoid to complete development. It is unclear how the virus is inhibiting the growth and development of the parasitoid larvae.

Prevalence of ascoviruses in the field vary from about 10-25% in *T. ni* (Browning *et al.*, 1986), *Heliothis* spp. (Carner and Hudson, 1983) and *S. frugipera* (Hamm *et al.*, 1986). Murray (1995b) reported infection of *Helicoverpa* spp. larvae collected from cotton in southeast Queensland of between 20-80% ascovirus infection of larvae and 100% ascovirus infection of *M. demolitor* adults collected from the same plot (determined by successful infection of healthy larvae in the laboratory by ovipositing *M. demolitor* females) during late January and February. Murray (1995b) states that larvae expressing ascovirus symptoms were most likely initially parasitised by *M. demolitor*.

It is unclear where the initial inoculum of ascovirus is originating. There are many possibilities including alternate host larvae present in the cropping region (*M. demolitor* is known to parasitise several species of noctuids). Another possible source is from overwintering wasps, which carry the virus. This has been shown for braconids but not *M. demolitor*. Overwintering *Helicoverpa* spp. larvae may carry the virus, but are asymptomatic (Ian Newton, *pers. com.*), however, Hamm *et al.* (1986) reported ascovirus from *S. frugipera* was not transmitted transovarialy (moth to progeny). *H. punctigera* migrating from central Australia (see p. 10 of this thesis) may be importing inoculum from inland populations. This question of where the initial ascovirus inocullum is originating requires future study. The interaction between *M. demolitor* and ascovirus will be examined further in Chapter 6.

Diurnal Behavior

The diurnal behaviour of *M. demolitor* in the field has not been studied. With information on the diurnal behaviour, recommendations to reduce the impact of disruptive insecticides can be made, such as timing applications when adults are inactive. The diurnal behaviour of adult *M. demolitor* is examined and discussed in Chapter 8.

Estimating populations in the field

Determination of the number of naturally occurring *M. demolitor* adults in the field has not been attempted. This would be useful in determining the number of adult females required in an inundative or augmentative release program. This is examined and discussed in Chapter 7.

Monitoring in the field

Monitoring of *M. demolitor* in the field has been poorly studied. Techniques for successfully monitoring parasitoid populations in the field would be very useful tools for IPM. Techniques for monitoring *M. demolitor* in the field are a major focus of this thesis and will be examined in Chapter 6.

Percent parasitism

Larval parasitism by *M. demolitor* of between 30-50% has been recorded in cotton crops in Australia (Murray *et al.*, 1996; Shepard *et al.*, 1983a) and may even be much higher at certain times of the year (see Chapter 3, p. 23). Distinct seasonal patterns of the abundance of *M. demolitor* have been identified, but the actual level of control *M. demolitor* exerts on *Helicoverpa* spp. in the field is not understood fully. This is a major focus of this thesis and is examined in Chapter 6.

Effects of pesticides

The effects of insecticides on parasitoids, including *M. croceipes*, have been studied in detail in the USA. However, there has been little work investigating the effect of insecticides on *M. demolitor*. Data from studies indicates that some parasitoids are tolerant to selected insecticides, and are relatively more tolerant at particular times in their life-cycle. Circumstantial evidence, such as high populations of *M. demolitor* adults in heavily sprayed fields (D. Murray, *pers. com.*) suggests that *M. demolitor* is tolerant to certain insecticides or relatively tolerant at certain times in its life-cycle. This topic is a major focus of this thesis and is examined in Chapter 11 and Chapter 12.

Conclusions

It is critical to an IPM strategy in Australian cotton that indigenous populations of natural enemies such as *M. demolitor* are conserved. In order to protect indigenous natural enemies, their life cycle and ecology must be understood and any possibility of reducing harm to these beneficials exploited. Many aspects of the biology and ecology of *M. demolitor* have not been studied in any detail, including:

- monitoring techniques;
- the time in the season when *M. demolitor* occur in the crop;
- estimates of the impact on *Helicoverpa* spp. populations;
- diurnal behavior of adults;
- population estimates; and
- effects of pesticides on all of the parasitoid's life stages.

With more detailed information on the ecology of *M. demolitor*, attempts can be made to conserve indigenous populations in the field which, as King and Coleman (1989) point out, is a key component of successful IPM.

Chapter 5

Food consumption and weight gain of *H. armigera* larvae after parasitisation by *M. demolitor*

Abstract

The aim of this trial was to examine the effects on feeding behaviour and weight gain of *H. armigera* larvae following parasitisation by *M. demolitor*. *H. armigera* larvae parasitised by *M. demolitor* consumed significantly less artificial diet than unparasitised larvae throughout parasitoid larval development on all days except day 2. Parasitised larva consumed on average each day, after parasitisation: day 0, 40%; day 1, 86%; day 2, 124%; day 3, 36%; day 4, 7%; day 5, 9%; day 6, 34%, day 7, 4%, day 8, 0.1% the amount consumed by an unparasitised larva over the period of larval parasite development. Unparasitised larvae weighed significantly more than parasitised larvae on all days. Upon parasite emergence (day 9), parasitised larvae weighed only 9.4% of unparasitised larvae. Larval *M. demolitor* took an average of 10.4± 0.1 days to complete development from oviposition when *H. armigera* hosts were fed artificial diet at 25°C, 60-70% relative humidity. The implications of this work to IPM in Australian cotton and the endocrine effects that induce effects in host by *M. demolitor* are discussed.

Introduction

Larval parasitoids have been demonstrated to affect the feeding behavior of their hosts. Food consumption may be increased (Brewer and King, 1981; Hunter and Stoner, 1975; Rahman, 1970), reduced (Parkman and Shepard, 1981; Rahman, 1970) or variable depending on the stage of the parasitised host or the number of parasitoids per host (Brewer and King, 1980). Specific studies have examined the effects of parasitisation by *M. demolitor* or *M. croceipes* on their hosts' food consumption. Lewis (1970c) found that feeding by *Heliothis* spp. larvae parasitised by *M. croceipes* was reduced although larvae continued to moult normally. Feeding experiments have determined that *H. virescens* or *H. zea* larvae parasitised by either *M. demolitor* or *M. croceipes* during the first through fourth larval instars consumed significantly less than unparasitised larvae (Powell, 1989; Strand *et al.*, 1988; Cobb *et al.*, 1985). Powell (1989) estimated that a larva parasitised at second instar consumed only 10% of the amount consumed by a healthy larva.

Larvae parasitised by *M. demolitor* consumed less than those parasitised by *M. croceipes* (Powell, 1989). Seymour (1991) and Strand *et al.* (1988) found that host larvae stop feeding and move off diet one to two days prior to parasitoid emergence. These results do not concur with the results of Powell (1989), who showed that parasitised larvae feed until parasitoid emergence, although at a reduced rate. Reduced feeding by parasitised larvae should result in less damage to the crop compared to unparasitised larvae.

Parasitoids have been demonstrated to affect host weight. Murray and Rynne (1992), Seymour (1991) and Strand *et al.* (1988) showed that the weight of a larva parasitised by *M. demolitor* at the end of the parasitoid's larval stage was only about 10% that of an unparasitised larva. Whether reduced weight is due to decreased feeding or internal feeding by the parasitoid on the host or both is unclear.

This study examines the feeding behavior of *H. armigera* larvae parasitised by *M. demolitor*. The timing of when feeding slows or stops in parasitised *H. armigera* larvae will be investigated. These data will be useful in the analysis of results from a study of the effects of stomach insecticides on larval parasitoids (Chapter 12). They also imply reduced crop losses due to reduced feeding by parasitised larvae. Data of the development times of larval *M. demolitor* will also be provided.

Materials and Methods

Insects

M. demolitor adults and *H. armigera* larvae used in this trial were reared using the methods outlined in Appendix 1.

Trial

H. armigera larvae were separated from the main culture and reared on stainless steel trays three-quarter filled with standard *Heliothis* diet (Appendix 1), with stainless-steel grids pushed into the diet, thereby separating larvae and reducing cannibalism. *H. armigera* eggs were sprinkled onto

these trays and larvae developed normally till the required size. This method usually allowed many more larvae to develop than were needed, so only healthy larvae of the desired size were used. Tests were initiated when larvae were early second instar larvae, weighing 4-5 mg.

150 larvae of similar size and age were used in this trial. Approximately 100 larvae were exposed to a mixture of approximately 200 male and female *M. demolitor* adults in a 1.5 litre plastic container for about 4 hours. Larvae were provided with a small quantity of diet. Adult parasitoids were provided with cotton dental wicks (see Appendix 3) soaked in water and 1% honey solution and a small amount of honey smeared on the side of the cage. Larvae, which had been exposed to adult parasitoids, were placed individually in Falcon® (see Appendix 3) dishes, about half filled with standard *Heliothis* diet. Fifty larvae were weighed and placed directly into Falcon® dishes without exposure to parasitoids and 51 Falcon® dishes were approximately half filled with *Heliothis* diet as controls.

Food consumption by the parasitised and unparasitised larvae was determined by measuring the weight lost from each Falcon® dish daily. Falcon® dishes were weighed (Sartorius balance, see Appendix 3) to the nearest 0.001g, after the larva and any frass were removed. Extreme care was taken to remove only frass and not diet from each dish. After weighing, larvae were returned to their original dish. Weighing was carried out at approximately the same time each day until parasitoid emergence. The control dishes were weighed to measure moisture loss. Due to natural variation, parasites started emerging on day 8 and finished on day 12. In order to standardise results, only larvae with a parasitoid emerging on day 10, (which was the peak day of emergence) were considered, the rest were discarded leaving 57 larvae. Only larvae developing normally were considered in the unparasitised group, making a total of 36 larvae.

A *t*-test was carried out to determine if feeding by parasitised and unparasitised larvae were significantly different and whether the weights of parasitised and unparasitised larvae were significantly different. Data were analysed using the analysis package incorporated in Microsoft Excel®

Results

Unparasitised larvae consumed significantly more diet ($P< 0.05$) than parasitised larvae on all days except day 2 (Figure 5-1). Weight loss of diet from trays containing unparasitised larvae was significantly greater than from the controls (no larvae) on all days ($P< 0.05$). Feeding by parasitised larvae slowed a few days after parasitisation. Parasitised larva consumed on average each day after parasitisation: day 0, 40%; day 1, 86%; day 2, 124%; day 3, 36%; day 4, 7%; day 5, 9%; day 6, 34%, day 7, 4%, day 8, 0.1% of the amount consumed by a unparasitised larva over the period of larval parasite development. Weight loss of diet was greater in trays containing parasitised larvae compared to the control trays on all days ($P< 0.05$) except day 5, 8 and 9. This indicates that feeding by parasitised larvae had effectively stopped during the last two days of parasite development. The reduced feeding on day 5 was probably due to moulting by the host larva. Unparasitised larvae weighed significantly more than parasitised larvae on all days ($P< 0.05$) (Figure 5-2). Upon parasite emergence, parasitised larvae were only 9.4% of the weight of unparasitised larvae. Larval *M. demolitor* took an average of 10.4 ± 0.1 days (Figure 5-3) to complete development from oviposition when *H. armigera* hosts were fed artificial diet at 25°C, 60-70% relative humidity.

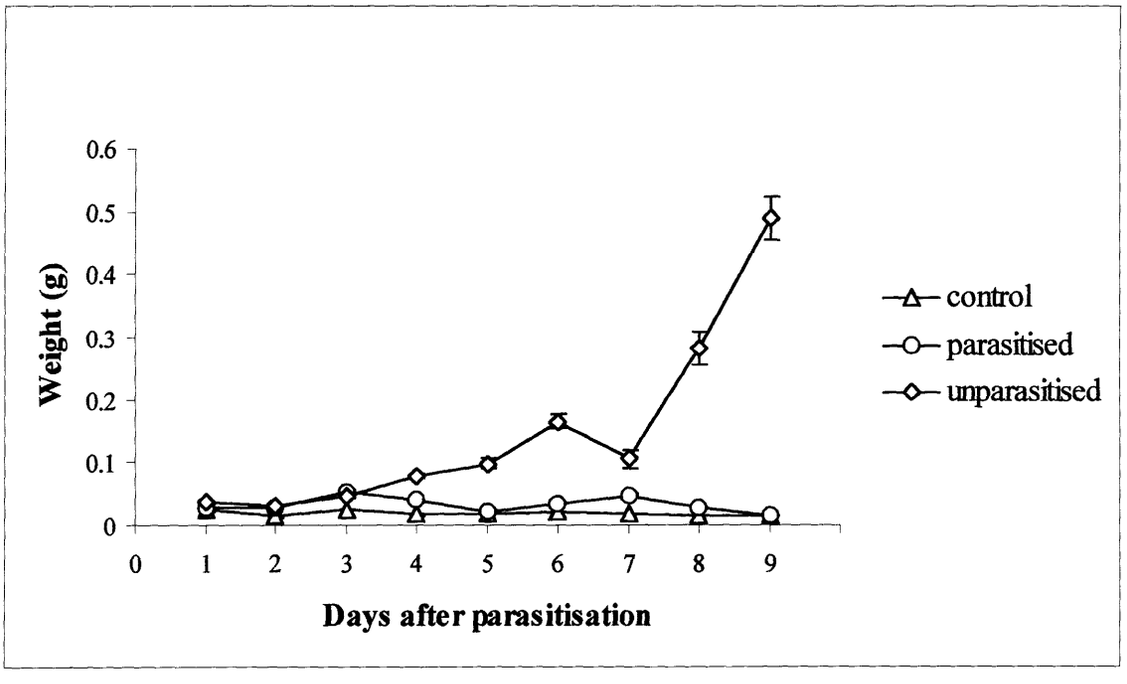

Figure 5-1. Diet consumption by second instar (at day 0) *H. armigera* larvae unparasitised and parasitised by *M. demolitor* (error bars are standard errors of the means).

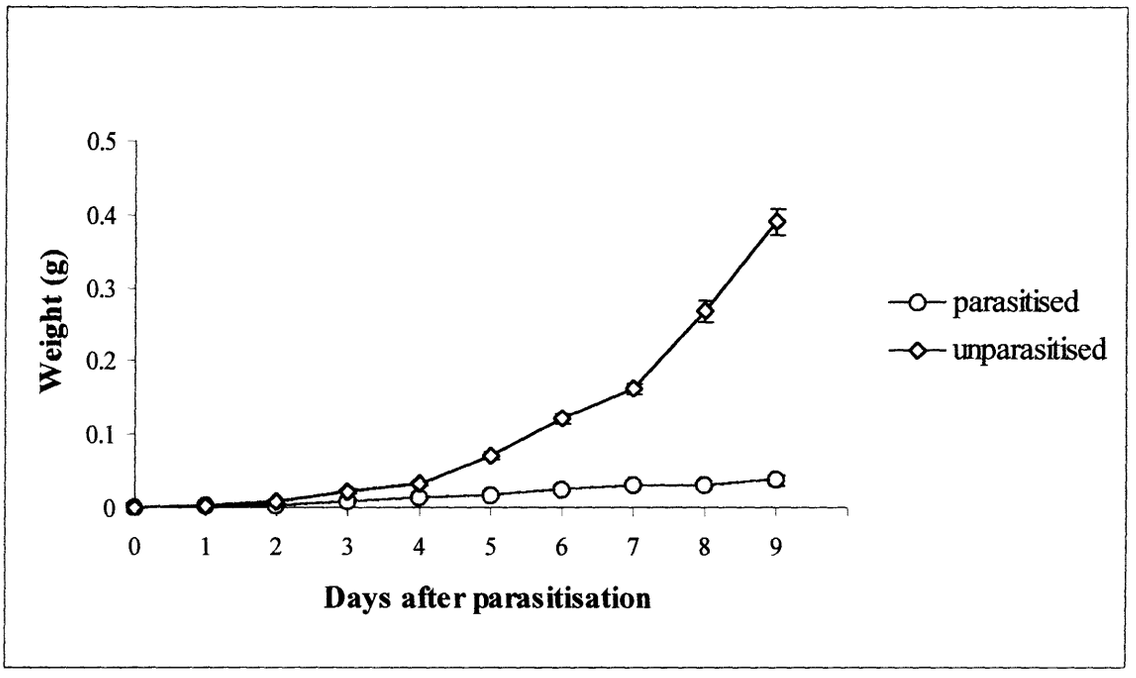

Figure 5-2. *H. armigera* larval weights, larvae unparasitised and parasitised by *M. demolitor* (error bars are standard errors of the mean).

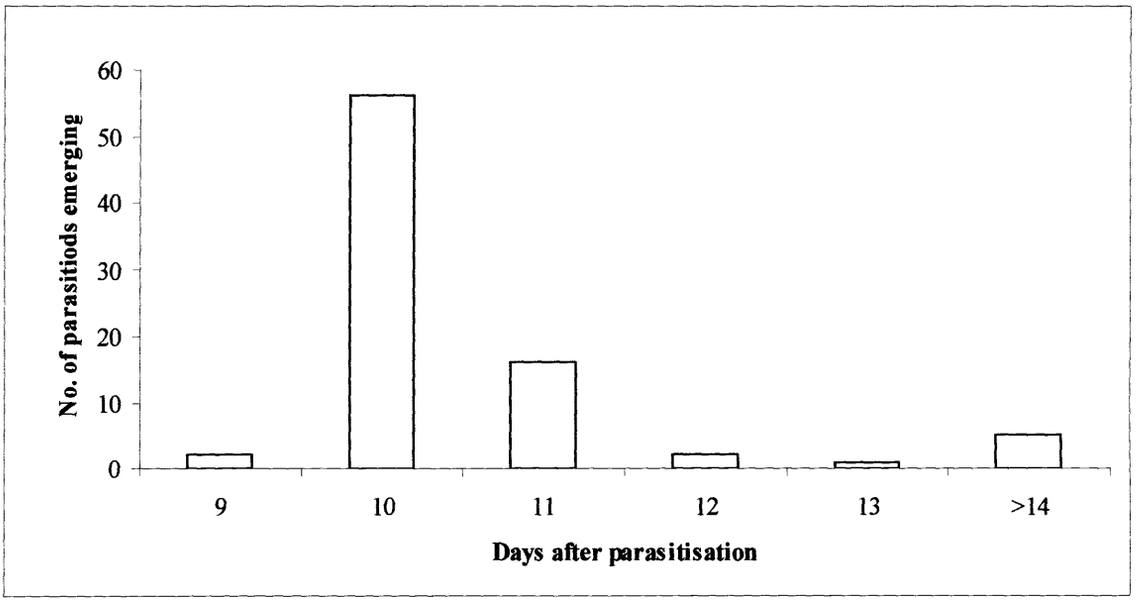

Figure 5-3. Numbers of *M. demolitor* larvae emerging from *H. armigera* hosts (days after parasitisation).

Discussion

H. armigera larvae parasitised by *M. demolitor* consumed in total only 11.5% of the amount of diet consumed by unparasitised larvae throughout the duration of the parasitoids larval development. This figure does not include the amount of diet unparasitised larvae would consume in completing development. According to King (1981), over 50% of the total food consumption by *H. virescens* larvae occurred from the fifth instar to the second day before pupation. Unparasitised larvae were fourth and fifth instar when the larval parasitoid emerged, and the trial was terminated. This means that the host larva had consumed less than half the potential amount it would consume over its life-time. Therefore, parasitised larvae consume in the order of only about 5% of unparasitised larvae.

The lack of significant difference between weight loss of control trays and trays containing parasitised larvae on day 5 and unparasitised larvae on day 7 was most probably due to reduced feeding during larval moulting. Examination of Figure 5.1 shows that there was a slight decrease in diet consumption by unparasitised larvae on day 5. This suggests that both unparasitised and parasitised larvae moulted at day 5. However, on day 7 parasitised larvae did not molt, indicating that parasitised larvae missed a molt, due most probably to the parasitoid larvae interfering with the endocrine functioning of its host. Data on head capsule widths was not collected; perhaps these data would confirm this hypothesis. Moulting by insects is a very complex process. During moulting *Helicoverpa* spp. larvae cease feeding and feeding is reduced before and after this time. This agrees with the results of Lewis (1970c), who found that moulting by *Heliothis* spp. larvae parasitised by *M. croceipes* was unaffected by parasitism until the final instar.

Reduced feeding alone does not necessarily mean reduced yield loss in the field. Often cotton plants shed squares and bolls due to superficial grazing. Hopper and King (1984a) found that 47% of damaged squares, 100% of damaged blooms and 86% of damaged bolls were shed by the plant due to grazing, compared to 8% of undamaged squares and 11% of undamaged bolls. This means that the total biomass consumed is irrelevant. It must be demonstrated that yield losses caused by superficial grazing of larvae do not compensate for reduced food consumption, and that parasitised larvae damage fewer fruit because they attacked fewer fruit per day, not because they

spent less time on the plant. Larvae parasitised by *M. croceipes* were observed to rest more frequently, while crawling and feeding less frequently than unparasitised *Heliothis* spp. larvae (Hopper and King, 1984a; 1984b). This indicates that less fruit would be visited and damaged by parasitised larvae. Reduced movement is readily observed in *Helicoverpa* spp. larvae parasitised by *M. demolitor*. Parasitised larvae often become inactive at the top of the canopy (D. Murray, *pers. com.*), indicating that parasitised larvae graze less than unparasitised larvae. This reduced grazing, in addition to food consumption in the order of 5% of unparasitised larvae, indicates that parasitised larvae cause negligible damage in the cotton crop. Parasitised larvae may even be an important food source for predators.

Parasitised larvae cease feeding and move off the diet about 24 hours before *M. demolitor* emergence. This behavior is associated with moulting to another instar rather than preparing for metamorphosis. Larval moulting of the parasitoid and host is partially synchronised, at least with *H. virescens* (Lewis, 1970c). Head capsule widths of parasitised larvae were the same up until the emergence instar, however parasitised larvae had significantly smaller head capsules compared to unparasitised larvae at the emergence instar.

The effects of larval *M. demolitor* on its host have been demonstrated in this study to be a juvenalising effect, reduced weight gain, reduce feeding, reduced host movement and characteristic "wandering" just prior to parasitoid emergence. These disruptions in the development of the host have been attributed to endocrine effects caused by the injection of factors, include polydnavirus (MdPDV) and proteinaceous venom into the host at oviposition by female *M. demolitor* (Jones and Lewis, 1971). The injected venoms and MdPDV are thought to be crucial in overcoming the host's immune system (Gullan and Cranston 1994). Effects on hosts could also be due to the parasitoid feeding on host tissue, although Strand *et al.* (1988) state that *M. demolitor* larvae fed only on the host's hemolymph. Although venom itself does not affect the host, calyx fluid, MdPDV and/or teratocytes induce host responses similar to parasitisation.

Endoparasites and their hosts display remarkable developmental synchrony (Beckage, 1985). Suitable endocrine interactions may limit a parasitoid's host range. The uncommon pseudoparasitism, where the host appears parasitised, but no parasite emerges, may be due to the

female injecting all of the factors associated with normal oviposition but not an egg, or if the larval parasite becomes encapsulated and dies. It is also possible that the parasitoid completes development but does not get the cue from the host to emerge and pupate.

Conclusions

This study showed that *Helicoverpa* spp. larvae parasitised by *M. demolitor* consume approximately 5% of the diet consumed by unparasitised larvae. *M. demolitor* took 10 days to complete larval development at 25°C, 60-70% relative humidity and a 14:10 light: dark photoperiod. *M. demolitor* injects substances into its host which disrupt normal behavior and development.

The implications for IPM of this study are that *Helicoverpa* spp. larvae parasitised by *M. demolitor* cause negligible damage to the cotton crop, although these larvae will be present in the crop for 10 days before the parasitoid larvae emerge and pupate. These larvae should be ignored in counts to determine spray decisions and may be a useful food source for predators within the crop. In the field, *Helicoverpa* spp. larvae parasitised by *M. demolitor* can be readily identified by gently squeezing the hind portion of the larvae until it "pops". The developing larval parasitoid larvae can be readily identified as a small white larva, approximately 2-5 mm, which will wriggle slightly for a few minutes before it dies (Unpublished observations).