Aspects of the ecology of *Microgaster demolitor*, a larval parasitoid of *Helicoverpa punctigera* and *Helicoverpa armigera* in Australia.

Thesis submitted by
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Dedication.

To Freddo,

this is as much yours as it is mine.

Abstract.

M. demolitor (Wilkinson), a solitary braconid parasitoid of *Heliocoverpa* spp in Australia, displays a humidity related diapause strategy. At low humidities, diapause is more likely to be initiated and is maintained longer than at high humidities. This strategy is effective in areas where decreases in rainfall, resulting in a decrease in the number of hosts, due to increased mortality and/or migration, leave the parasitoid without suitable oviposition sites. Diapause initiation and maintenance were not affected by photoperiod or temperature.

The incidence of diapause in *M. demolitor* increased with latitude in both hosts, being lowest at Mareeba, (the most northern sampling site), and highest at Grafton, (the most southern site). This correlates with a similar pattern in the diapause distribution of the host, that is, with increasing latitude the incidence of diapause in the host also increases.

At Mareeba, host species affected diapause incidence: parasitoids reared in *H. armigera* never entered diapause, while those reared in *H. punctigera* did. This is thought to be related to the differences in seasonal distributions of the hosts. That is, *H. armigera* is present all year round in Mareeba but *H. punctigera* is not. The higher migratory tendencies of *H. punctigera* result in periods of the year when, although conditions may be suitable for it, it may migrate from the area. Thus the possibility of some of the parasitoids entering a

diapause state if they emerge from *H. punctigera* would then safeguard against the possibility of the hosts migrating from the area and leaving the parasitoid population to perish due to a lack of hosts.

Parasitoids that entered diapause were significantly heavier as prepupae. The increase in weight of the diapausing prepupae may result from increased fat or water reserves which enable the parasitoid to survive its dormancy period. The cocoons of diapausing parasitoids were heavier and had a ribbed appearance, with a tighter silken weave. This increase in the closeness of the weave may reduce evaporation and hence decrease the desiccation rate of the diapausing prepupae. The cocoon is a vital component in the maintenance of diapause, as partial removal of the cocoon results in termination of diapause. The duration of diapause was unaffected by the sex of the parasitoid, the host it was reared in, the weight of the prepupae or the weight of the cocoon.

Parasitoids reared in field based cultures of *Heliocoverpa* spp. developed slower than those reared from a laboratory based culture. This result may be due to a decrease in the genetic fitness of the laboratory maintained culture, as no field animals have been added to this population in over 14 years. Hosts in which parasitoids were reared were always smaller than non parasitised hosts of the same age, with 100% mortality occurring in all parasitised hosts once the parasitoid emerged.

The larval developmental thresholds of the parasitoid in the three sites studied were all significantly different and were all higher than that of their hosts. Similarly, the pupal developmental thresholds of the parasitoids were similar to, or higher than, their two hosts, however the Mareeba population had a significantly lower pupal threshold than the other two populations.

Longevity of the parasitoid was affected by both temperature and food. At high temperatures, longevity was short and increased as temperature decreased. A quiescence strategy was initiated in the parasitoid when temperatures dropped below 20°C, significantly increasing their longevity. Longevity could also be increased by supplying the parasitoid with a 10% honey and water solution.

Parasitoids showed a preference for 2nd and 3rd instar larvae for oviposition. This preference minimised direct injury of the parasitoid. That is, when later instars were parasitised the probability of physical damage to the parasitoid was greater. Host species preference varied with latitude. In the northern sampling sites a preference for *H. armigera* was displayed, in the southern areas a preference for *H. punctigera*, while at Toowoomba, the two hosts were attacked with equal frequency. This correlates with the relative spatial and temporal distribution of the hosts, that is, *H. armigera* occurs in relatively greater concentrations in the north, while *H. punctigera* occurs in relatively higher concentrations in the south. However, in areas such as Toowoomba, where *M. demolitor* displays no preference, both hosts occur in substantial numbers, but at different times of the year.

These results then suggest that *M. demolitor* in Australia is not a homogeneous species but that perhaps clinal variation or a group of sibling species exists. Genetic work is required to consolidate this theory.

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Chapter 1 Introduction.

In Australian agriculture many lepidopteran larvae cause damage to economically important crops. Of these, species in the genus *Heliocoverpa* (Noctuidae), are possibly Australia's major agricultural pest (Zalucki *et al.* 1986).

There are five species in the genus *Heliocoverpa* in Australia; *H. armigera* (Hubner), *H. punctigera* (Wallengren), *H. assulta* (Gueunee), *H. rubrescens* (Walker), and the recently described *H. prepodes* (Common), (Common 1985). Of these species only the former two are recognised as pests (Wilson 1983).

H. punctigera is endemic to Australia and occurs in all states. It has also been recorded in New Zealand (Fox 1978) and Norfolk Island (Holloway 1977 in Zalucki *et al.* 1986) presummably arriving there as a wind borne migrant. *H. armigera* is a cosmopolitan pest with a broad distribution through Asia, Africa, Southern Europe as well as Australia. Within Australia, it has not been recorded in Tasmania (Wilson 1983), only 3 specimens have been reported from South Australia (Zalucki *et al.* 1986) and it is only of minor importance in Victoria (Wilson 1983).

Both these species of *Heliocoverpa* undergo extensive movements and it appears that although *H. punctigera* is the more mobile of the two species and is possibly a obligate migrant, migrational movement undertaken by *H. armigera*

may still be extensive (Farrow & Daly 1987); (see Zalucki *et al.* 1986 for a comprehensive review of the current literature on *Heliocoverpa* spp in Australia).

In 1980, these two species alone cost Queensland agriculture approximately 16 million dollars, split evenly between the cost of spraying and the residual cost to crops from those individuals that either escaped spraying or had a sufficiently high level of insecticide resistance to survive (Alcock & Twine 1981). Because of this increasing resistance to pesticides, biological control of *Heliocoverpa* is becoming increasing important.

There are several potential biological control agents for *Heliocoverpa* present in Australia but perhaps the most promising, due to its wide distribution and ease of rearing, is a small solitary braconid parasitoid; *Microgaster (=Microplitis) demolitor* (Wilkinson).

Several workers, (Titmarsh 1981, 1985; Broadley 1981, 1984; Forrester 1981; Wilson 1983), have found *M. demolitor* in Australian *Heliocoverpa* larvae in the field. However, no work has been undertaken that investigates the parasitoid's ecology and how this relates to the bionomics of its native hosts, *H. punctigera* and *H. armigera* in Australia. This type of information is imperative if the parasitoid is to be used against these pests with any success. Similarly, no definitive work has been carried out to determine if the parasitoid has a preference between these hosts, although Titmarsh (1985), suggested that

M. demolitor in tobacco fields in Mareeba may be displaying a preference for *H. armigera.*

This study then aims to determine

i) the natural history of *M. demolitor*, including longevity, attack sequence and physical effect the developing parasitoid has on its host;

ii) the developmental biology of the parasitoid, from three different geographic locations, for both the larval and pupal stages;

iii) factors which initiate diapause in the parasitoid and whether the incidence of diapause varies between geographically separated populations;

iv) structural and physical differences between parasitoids that enter diapause compared to those that do not;

v) the factors which affect diapause termination in the parasitoid and

vi) differences in preferences shown by geographically separated populations of the parasitoid for *H. armigera* and *H. punctigera.*

Chapter 2 General Biology of Braconid parasitoids.

2.1 Introduction

Braconids, a family of hymenopterans, are all insectivorous parasitoids laying their eggs in or on the larvae, pupae or adults of other insects (Matthews 1974). Like other parasitoids, they are separated from true parasites by several important features:

i) the developing wasp almost always causes mortality to its host;

ii) they are parasitic as larvae only;

iii) they do not exhibit heteroecism and

iv) their influence on the population dynamics of the host resembles that of a predator rather than that of a parasite (Doutt 1959).

Braconids commonly utilise lepidopteran larvae as hosts, and as such they are an important component of many biological control programs (Matthews 1974). Perhaps because of this, the amount of literature on this family of hymenopterans is quite substantial with more than 8000 publications up to 1959 (Shenefelt 1965 in Matthews 1974). However only in the last two to three decades, with the advent of controlled environment chambers, has the temperature dependent rate of development of these organisms been accurately determined.

One of the objectives of this review is to examine selected aspects of the biology of braconids, (except *M. demolitor* which is reviewed in chapter 4), with particular emphasis on diapause. The effects of temperature, photoperiod and hosts on developmental and diapause biology will be covered as well as factors influencing longevity of the parasitoid. All these areas are examined with respect to *M. demolitor* and its hosts, *H. punctigera* and *H. armigera*, in subsequent chapters.

2.2 Developmental Biology

Reviews on developmental biology of insects usually include diapause, but, as there is such an obvious distinction between diapause and non-diapause development in braconids, diapause biology will be treated as a separate section later in this review.

2.2.1 Temperature effects.

Braconid development, like development in other insects, is temperature dependent, with increasing temperature producing increases in developmental rate until an upper threshold is reached (Butler *et al.* 1983; Nealis, Jones & Wellington 1984; Obrycki & Tauber 1979; Johnson & Smith 1980; Lee & Chippendale 1985; Nealis & Fraser 1988). A number of workers have examined the effects of ambient temperature on braconids by rearing groups of individuals at a range of constant, and in some circumstances, varying temperatures.

2.2.1.1 Constant temperatures.

Jackson *et al.* (1978) and Jackson and Butler (1984), showed that *Chelonus blackburni* and *Bracon* sp. both developed faster at higher constant temperatures but that the greatest reproductive success occurred at lower temperatures. Madar and Miller (1983) perceived a similar effect in *Apanteles yakutatensis*. Campbell *et al.* (1974) found similar temperature effects and also that thermal constants, that is, the amount of heat required over time for an insect to complete a particular stage of development, may vary between different geographic populations of parasitoids.

2.2.1.2 Fluctuating temperatures.

There have been only a few studies looking at the differences between fluctuating and constant temperatures and the effect these have on the developmental rate of braconid parasitoids. They have, however, all come to similar conclusions; that is, that fluctuating temperature regimes around a mean temperature do not significantly change the developmental rate in comparison to a constant regime with the same mean temperature, providing that temperatures do not drop below the parasitoid's developmental threshold. Laing & Heraty (1987), found that the rates of development in larval stages of *Pholetesor ornigis* and *Pholetesor pedias* at fluctuating temperatures, with means of 15 and 21.9°C and temperture steps of 10°C, did not differ from those at constant temperatures

of 15 and 22°C respectively. Similar results were found for larval and pupal developmental rates in *Chelonus texanus* (Butler 1966) and *Cardiochiles nigriceps* (Butler, Hamilton & Lopez 1983).

2.2.1.3 Developmental thresholds.

Developmental thresholds, or the theoretical temperature at which no development of a particular insect occurs, are usually calculated by extrapolation of the relationship between rate of development and temperature. These relationships are often assumed to be linear (Danks 1987), when many are in fact sigmoidal (Davidson 1944) or non linear. However, the majority of the literature on braconid development has linear relationships fitted and developmental thresholds calculated accordingly.

There are numerous reports of braconid parasitoids with thresholds higher than their hosts (Herbert & McRae 1983; Johnson, Trottier & Laing 1979; Laing & Heraty 1987; Hutchinson, Butler & Martin 1986; Nealis, Jones & Wellington 1984) though there are exceptions. *Microplitis rufiventris* reared in *Spodoptera exigua* (Hutchinson, Butler & Martin 1986), *Apanteles ornigis* reared in *Phyllonorycter blancardella* (Herbert & McRae 1983, Johnson, Trottier & Laing 1979) and *Pholetesor ornigis* and *Pholetesor pedias* reared in *Phyllonorycter blancardella* (Laing & Heraty 1987), all have larval thresholds significantly lower than their hosts. *Chelonus texanus* reared in *Spodoptera exigua* (Butler 1966),

Dacnus dryas reared in *Agromyza frontella* (Guppy, Meloche & Harcourt 1988) and *Perilitus coccinella* reared in *Coleomegilla maculata* (Obrycki & Tauber 1979) all have similar larval thresholds to those of their hosts.

Where the data do exist, it appears that parasitoids with higher thresholds than their hosts as larvae also have higher thresholds as pupae, while those parasitoids with similar larval thresholds as their hosts have similar pupal thresholds as well. Exceptions to this are three parasitoids of *Pieris rapae*, namely *Apanteles rubecula*, *Apanteles glomeratus* and *Pteromalus puparum* all of which have similar larval thresholds to their host in two locations, Australia and Canada, but in Australia the pupal thresholds of the parasitoids are substantially higher than the host (Nealis, Jones & Wellington 1984). This difference results from a difference in the host's threshold between the two locations.

2.2.2 Photoperiod.

Hegazi (1986) found that photoperiod directly affected the rate of development of *Microplitis rufiventris*, with total darkness and 6L:18D producing quicker developmental rates at 20°C, than photoperiods of 12L:12D or 18L:6D at the same temperature. This effect decreased with increasing temperatures.

2.2.3 Host effects.

Host instar, (or age), and host species may often significantly affect the developmental biology of braconid parasitoids. Hooper and King (1984), working with *Microplitis croceipes*, Sato (1980) with *Apanteles glomeratus* and Nealis *et al.* (1984) with *Apanteles rubecula*, found that developmental rate was influenced by the age of the host, being faster in older, larger hosts. Similarly in *Hyposter exiguae*, a parasitoid of *Trichoplusia ni*, the developmental rate was correlated with host age, that is, fastest in the 5th instar and slowest in first instar larvae (Smilowitz & Iwantsch 1973). The age of the host was not found to affect the developmental times of either *Apanteles yakutatensis* or *Orgilus elasmopalpi* (Madar & Miller 1983, Johnson & Smith 1980).

Fisher (1971) suggests that development of *Apanteles glomeratus* is partially dependent on its host, *Pieris brassicae*. Here, if *Pieris brassicae* is super-parasitised at different times by different adult females then all the larval parasitoids of different parentage emerge from the one host simultaneously, strongly suggesting that parasitoid developmental time is regulated by the host's physiological state.

Nealis, Jones & Wellington (1984) found that parasitoid densities in gregarious parasitoids also affect developmental rate. In the larval parasitoid *Apanteles glomeratus*, high parasitoid densities within the host, *Pieris rapae*,

resulted in slower developmental times for the parasitoids, while in the pupal parasitoid, *Pteromalus puparum*, developmental times were reduced with increased larval parasitoid densities.

2.2.4 Sex effects

Sex of the parasitoid may also influence developmental times. In *Meteorus autographae* the time from egg deposition to pupation was shorter for female parasitoids but pupation to adult times were shorter in the males. Overall, females of this species had longer developmental times than males (Grant & Shepard 1984). Similar results have been found for *Microplitis croceipes* and *Cotesia margiventris* (Hamm *et al.* 1983), *Microplitis rufiventris* (Hegazi 1986) and *Rhaconotus roslinensis* (Hawkins & Smith 1986). Grant & Shepard (1984), found that male *Meteorus autographae* parasitoids developed slower than females in the egg to prepupae stages, faster in the pupal to adult stages and had a shorter development time overall, while Nealis & Fraser (1988) found males of *Apanteles fumiferanae* developed quicker than females due to a shorter larval developmental time. In *Apanteles rubecula*, females take longer to develop in the pupal stage but the development of the larval stages of the sexes is similar (Nealis, Jones & Wellington 1984).

2.3 Synchrony of generations

In order for either introduced or native braconid parasitoids to maintain themselves in a host population (if the host's or parasitoid's generations do not overlap and the parasitoid is not migatory) they must be synchronous with the host (Fisher 1971; Matthews 1974; Fye & Jackson 1973; Huffaker & Messenger 1976). The overall precision with which the parasitoid matches the host's generations is determined primarily by environmental factors to which the host and parasitoid may respond, either independently or by a physiological interaction between them (Fisher 1971; Matthews 1974; Huffaker & Messenger 1976). However, if the parasitoid can attack a number of host species then synchrony with one host is not as important (Fye & Jackson 1973).

The majority of the literature on braconids shows that the host and the parasitoid have similar generation times resulting in distinct synchrony between the two, such as *Chelonus blackburni* parasitising *Pectinophora gossypiella* (Jackson, Delph & Neeman 1978), *Chelonus texanus* parasitising *Spodoptera exigua* (Butler 1966), *Dacnusa dryas* attacking *Agromyza frontella* (Guppy, Meloche & Harcourt 1988).

However Jackson & Butler (1984) showed that *Bracon greeni*, *Bracon hebetor* and *Bracon brevicornis* all developed more quickly than their host *Pectinophora gossypiella*, resulting in 2 to 3 generations of the wasp to 1

generation of the host. Similarly Obrycki & Tauber (1979) found that *Perilitus coccinellae*, a parasitoid of *Coleomegilla maculata*, was also capable of producing more generations per year than its host.

Nealis, Jones & Wellington (1984) reported that Canadian populations of *Apanteles glomeratus*, *Apanteles rubeula* and *Pteromalus puparum* all had shorter generation times than their host *Pieris rapae*, but that Australian populations of the same wasps had longer generation times than the host at low temperatures and shorter generation times at high temperatures.

Synchrony may be most striking when either the host insect has a facultative diapause (Fisher 1971) or when the parasitoid can attack two species that differ in voltinism (Fisher 1971; Huffaker & Messenger 1976). For example, *Chelonus annulipes*, which parasitises the single and multibrooded strains of *Pyrausta nubilalis* (corn borer moth) attacks the first generation of the bivoltine strain of its host, completing its development in two months, and emerges to attack the second brood of its host which enters diapause in autumn as mature larvae and pupates in the following spring. The parasitoids then enter diapause with their hosts and emerge ten months later to attack the next generation (Fisher 1971).

2.4 Diapause.

Diapause is often essential in some stage of the life cycle if synchronisation of development between the host and its parasitoid is to occur (Doutt 1959; Huffaker & Messenger 1976). Braconids enter diapause either within the host larvae (Subba Rao *et al.* 1969; Wylie 1980, Guppy, Meloche & Harcourt 1988), external to the larvae within a cocoon as a prepupa or pupa (Anderson & Kaya 1974; Bryan *et al.* 1969; Giron 1978; Hegazi, Kolaib & Abd-el Fattah 1980; Herbert & McRae 1983; Ingram 1981; Saunders 1965; Schneiderman & Horwitz 1957; Thuston 1976; Wallner & Grinberg 1984) or as an adult (Lee & Chippendale 1985). Not all braconids enter diapause, for example *Rhaconotus roslinenis* (Hawkins & Smith 1986). Many factors are believed to induce diapause in braconids but perhaps the best known are temperature and photoperiod.

2.4.1 Diapause induction.

Temperature, photoperiod and changes in the physiological state of the host have all been implicated as factors which, independently or combined, may induce diapause in braconids.

2.4.1.1 Temperature effects.

Temperature can induce diapause in offspring both directly and indirectly, that is, by affecting the parents and thus the offspring, or by acting directly on the offspring (Doutt 1959; Saunders 1965; Schneiderman & Horwitz 1957). In many species high temperatures prevent diapause induction (Thuston 1976; Bryan *et al.* 1969). For example, the perccentage of the population in diapause in *Microplitis croceipes* is directly proportional to the temperature the larvae are held at, that is, as the temperature increases the percentage of larvae entering diapause decreases (Bryan *et al.* 1969). However, in *Nasonia vitripennis*, diapause is induced in the last larval instar of offspring by exposing the females to low temperatures, usually less than 15°C, during ovigenesis (Saunders 1965).

Butler, Hamilton & Lopez (1983) isolated temperature as the factor initiating diapause in *Cardiochiles nigriceps*. Exposure of the developing larval parasitoids to temperatures below 25°C initiated diapause however the mechanism for diapause release was not found. Similarly, Hegazi Kolaib & Abd-el Fattah (1980) suggested that exposure of the developing parasitoid *Microplitis rufiventris* to temperatures below 15°C initiates a diapause response. By contrast, Thuston (1976), found that low temperatures were not important in inducing diapause in *Manduca sexta* and *Apanteles congregatus* but that high temperatures would inhibit larvae entering diapause.

2.4.1.2 Photoperiod effects.

Photoperiod effects on diapause in braconids often act on the parents, thus producing diapause in the offspring by indirect means (Fisher 1971; Anderson & Kaya 1974). This is exemplified in *Coleoides brunneri*, where short photoperiods applied to developing larvae had no effect on the incidence of diapause in that generation, but the majority of their offspring entered diapause (Fisher 1971). However, the possibility of producing diapausing larvae by exposing them to decreased photoperiods does exist (Wylie 1980; Wallner & Grinberg 1984; Butler *et al.* 1983; Giron 1978).

Diapause in *Microtonus vittatae* (Wylie 1980) and *Rogas indiscretus* (Wallner & Greinberg 1984) was found to be produced by exposing parasitised larvae to photoperiods below 14:10 (light:dark) hours, and at 11:13 hours almost 100% of both these species went into diapause. Similarly, Van Steenwyk & Stern (1976), found that all individuals of *Peristenus stygicus* entered diapause when reared at photoperiods of 12.75L:11.25D or less but that the proportion decreased with increasing light intervals.

Photoperiod and temperature can act together to initiate diapause, such as in *Microctonus vittatae*, which requires both short days and low temperatures for diapause to be induced (Wylie 1980). Similar results were found for *Cotesia rubecula* = *Apanteles rubecula*, a parasitoid of *Pieris rapae* (Nealis 1985). Zinov'yeva (1985), found that when *Alysia manducator* was kept below 18°C it

showed a weak long-day photoperiod-induced diapause, but at temperatures between 18 and 23°C diapause could not be invoked, regardless of the photoperiod. This situation was then reversed when the parasitoid was reared at temperatures above 23°C, as here it showed a short day photoperiod induced diapause.

2.4.1.3 Physiological effects.

Other factors may affect the percentage of offspring entering diapause, including the physiological state of the adult female prior to and at the time of oviposition (Doutt 1959, Huffaker & Messenger 1976). Depriving the adult female parasitoid of hosts can also affect the incidence of diapause. In *Mosmoniella vitripennis* (Schneiderman & Horwitz 1957) and *Nasonia vitripennis* (Saunders 1965), increasing deprivation time also increased the percentage of diapausing offspring.

Host physiology also affects the incidence of diapause in braconids, that is, the percentage of diapausing parasitoids often increases when the host itself is entering diapause (Doutt 1959, Huffaker & Messenger 1976). Nealis & Frazer (1988) highlighted a similar situation in *Apanteles fumiferae*, this parasitoid only entering diapause when the host in which it is developing does so.

Nutrition has been shown by Saunders (1965) and Doutt (1959) to influence diapause in *Nasonia vitripennis* and *Cryptus inornatus*. In the latter the incidence of larval diapause was increased if raisins were added to its sugar cane diet (Doutt 1959).

2.4.2 Diapause termination.

Termination of diapause in braconids has not been well documented. In *Pholetesor ornigis*, diapause is terminated by long photoperiods (> 16L:8D). When shorter photoperiods (12L:12D) were encountered this parasitoid required exposure to temperatures below 2°C for a minimum of 16 weeks before diapause was terminated (Laing & Heraty 1987). This parasitoid uses different cues than either its host, *Phyllonorycter blancardella*, or a second parasitoid, *Pholetesor pedias*, which uses the same host during the same time of the year. *Pholetesor pedias* breaks diapause as temperatures rise once it has been exposed to at least 10-12 weeks of temperatures below 2°C (Laing & Heraty 1987).

Obrycki & Tauber (1979) found that in *Perilitus coccinella* diapause was maintained by short day lengths and that low temperatures were not important to the parasitoid in determining dormancy periods, but they were unable to isolate any diapause terminating stimuli. Nealis (1985), found that once diapause was induced in *Cotesia rubecula*, cool temperatures kept the parasitoid in diapause and that the rate of diapause termination was temperature dependent.

2.5 Longevity.

Several factors have been implicated in affecting the longevity of braconids, ranging from the exposure time of the adult female to hosts to differences in diet, but perhaps the major factor determining braconid longevity is temperature (Gifford & Mann 1967).

2.5.1 Temperature effects.

With an increase in temperature the longevity of parasitoids decreases (Rachav 1978, Gifford & Mann 1967, Calkins & Sutter 1976), but their physiological lifespan, (in terms of day degrees), may not necessarily increase. Unfortunately, few definitive studies on the effects of temperature on longevity of braconid parasitoids have been conducted, and little evidence exists for quiescence strategies, (which result in increases in physiological time), occurring at low or high temperatures.

2.5.2 Sex effects.

Longevity may also vary between the sexes; Rachav (1978) showed that in *Chelonus inanities* females live longer than males. Nealis & Fraser (1988) and Hawkins and Smith (1986) found similar results in *Apanteles fumiferanae* and *Rhaconotus roslinensis* respectively. Butler *et al.* (1983) reported no substantial differences between the longevity of either sex of *Cardiochiles nigriceps* with

Jackson & Butler (1984), Hutchinson, Butler & Martin (1986), Grant & Shepard (1984), Lee & Chippendale (1985) and Johnson & Smith (1980) finding similar results in *Bracon* spp., *Microplitis rufiventris, Meteorus autographae, Iphiaulax kimballi* and *Orgilus elasmopalpi* respectively.

2.5.3 Interaction effects.

The longevity of *Apanteles flavips* males was also influenced by the number of matings; longevity decreasing with increased number of matings (Gifford & Mann 1967). Females of *Meteorus autographae*, an indigenous parasitoid of soybean loopers, were found to be affected by host exposure; increased exposure, (pressummably with increased oviposition), to hosts led to decreased longevity (Grant & Shepard 1984).

Encounters with older hosts also reduces the longevity of *Peristenus stygicus* adult females (Van Steenwyk & Stern 1976).

2.5.4 Diet effects.

Diet also affects the longevity of braconids. The addition of high concentrations of carbohydrates to artificial diets increased the longevity of *Peristenus stygicus* (Van Steenwky and Stern 1976), *Chelonus inanitus* (Rachav 1978) and *Apanteles yakutatensis* (Madar and Miller 1983). The addition of protein to the artificial diet also increased the longevity of *Apanteles militaris* (Calkins & Sutter 1976) and *Microplitis rufiventris* (Hegazi &

El-Minshawy 1981). Similarly, the addition of honey to the water source of some species of braconids also significantly increases their longevity (Johnson & Smith 1980, Lee & Chippendale 1985).

2.6 Conclusion.

As braconids are often used in biological control programs, and as temperature, photoperiod and to a lesser extent, host physiology all appear to affect the basic biology of parasitoids, it is important that an understanding of how these factors affect individual braconid species is obtained. This type of information is not only essential for classical biological control programs but also should also form an integral part of other types of programs, such as inundative and augmentative control programs. The following study then aims to collect and analyse this type of data for the parasitoid *M. demolitor* and two of its native hosts, *H. armigera* and *H. punctigera*.

Chapter 3 Sampling sites

Five sites along the east coast of Australia, (see appendix 1), were sampled at various times for *Heliocoverpa* spp. Sites at Toowoomba, Emerald and Mareeba, where selected as Department of Primary Industry staff stationed there where able to sample and ship parasitoids to Townsville. Grafton was chosen as a further site as again sampling personnel were readily available in this region while Townsville was selected due to its proximity to the university.

3.1.1 Site 1: Mareeba.

Mareeba lies on the Atherton Tablelands in Northern Queensland at an elevation of 335 metres and at latitude 17°S, longitude 145° 26'E. It is separated from the coastal plain by large mountain ranges to the east and is approximately 40 kms from the coast.

Mean monthly minimum temperatures throughout the year range from 11.1°C to 21.3°C, while monthly maxima range from 25.1°C to 32°C, with the majority of the yearly rainfall occurring from January to March (figure 3.1).

Several types of crops are planted at Mareeba: tobacco and peanuts are the major crops, while corn and *Lablab purpurea* are also grown. All these crops are suitable hosts for *Heliocoverpa* spp.

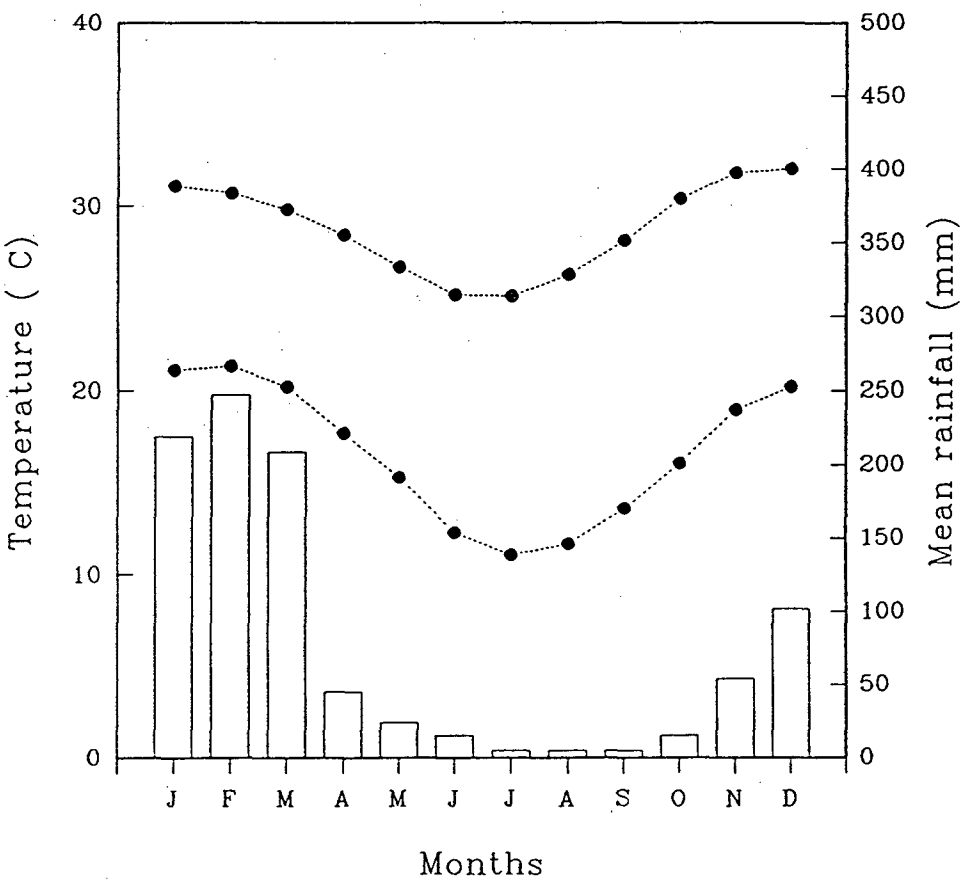


Figure 3:1. Mean monthly maximum and minimum temperatures with associated mean monthly rainfall for Mareeba (Source -Bureau of Meteorology 1988). Temperature = ● , Rainfall = □.

3.1.2 Site 2. Grafton.

Grafton lies on the flood plains of the Clarence river, approximately 40kms inland on the east coast of New South Wales at an elevation of 6.5 metres and latitude 29° 41'S and longitude 152° 56'E. It is characterised by a temperate Australian climate with mean monthly maximum temperatures ranging from 20.1°C to 29.7°C. Mean monthly minimum temperatures range from 5.8°C to 19.6°C Most of this area's rainfall occurs during December, January, February and March (figure 3.2).

The predominant crops are corn, planted from September to December and harvested from December to August, soybeans, planted from November to January and harvested late April to May and Triticale sp., a hybrid wheat/rye crop, planted in May and harvested in November, all of which are subject to *Heliocoverpa* spp, probably *H. armigera* (Fitt pers. comms 1991) damage (Grafton experimental Agricultural Farm Staff, pers. comms 1985).

3.1.3 Site 3: Darling Downs; Toowoomba.

Toowoomba is situated in the south eastern corner of Queensland at an elevation of 675 metres and at latitude 27° 35'S and longitude 151° 56'E. The region experiences mean monthly maximum temperatures ranging from 16 to 27.1°C and is significantly colder than the other sampling sites throughout the entire year, with mean monthly minimum temperatures ranging between 5 and

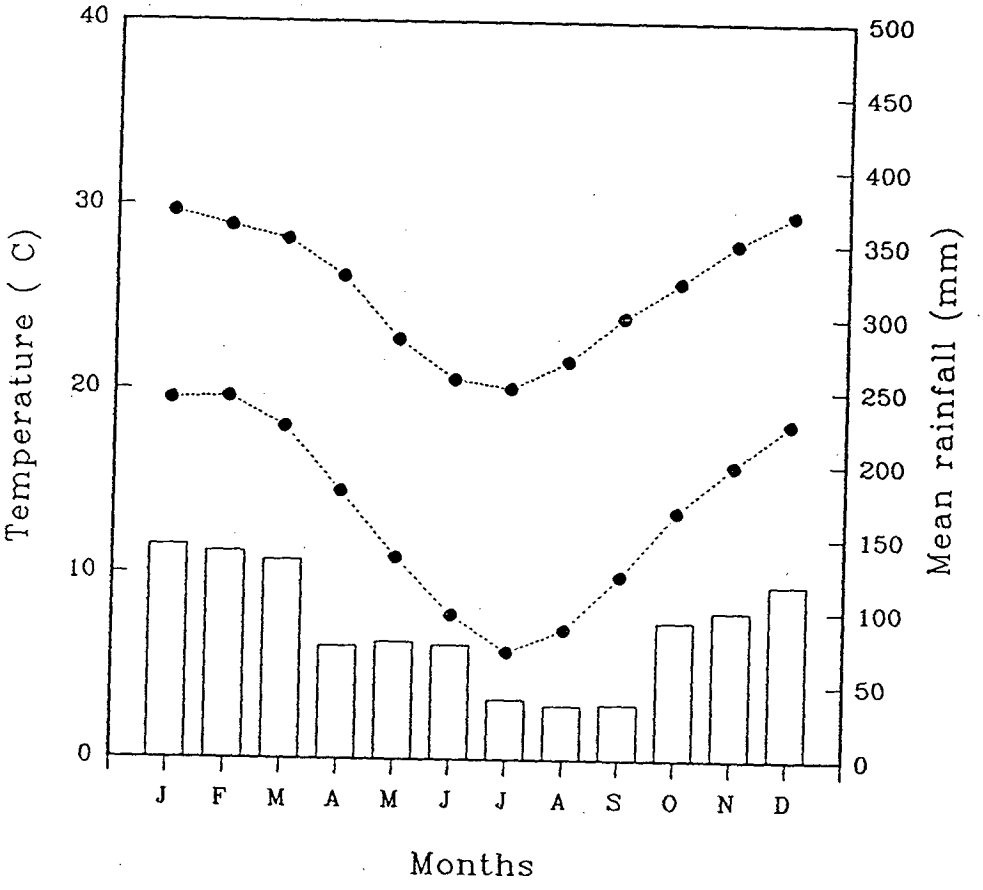


Figure 3:2. Mean monthly maximum and minimum temperatures with associated mean monthly rainfall for Grafton (Source -Bureau of Meteorology 1988). Temperature = ● , Rainfall = □.

16.7°C (figure 3.3). The rainfall regime of the area is similar to that of Grafton, with peaks occurring in December and January.

The main crops affected by *Heliocoverpa* sp damage are wheat, barley, chickpeas, sorghum, soybeans, sunflower and cotton. Wheat and barley are planted from late May to July with barley being cropped before wheat, which is harvested in November. Soybeans and cotton are both planted in October/November/December with cotton being harvested in May/June. Sorghum is planted in October, chickpeas in late April to mid June and sunflowers from late August to January (Murray pers. comm 1991).

3.1.4 Site 4. Townsville

Townsville is located on the East coast of Northern Queensland at Latitude 19° 15'S and Longitude 146° 46'E at an elevation of 4 metres. Mean monthly maximum temperatures range from 31.3°C in December to 24.8°C in July while mean monthly minimums range from 13.4°C in July to 24.0°C in January . The predominant rainfall is monsoonal in origin and falls during January, February and March (figure 3.4).

Although the area does not have a large agricultural base, there are a number of hobby farms in the region which suffer from *Heliocoverpa* attack. The local prison farm also regularly plants crops throughout the year and this was the major source of *Heliocoverpa* larvae from Townsville for this study.

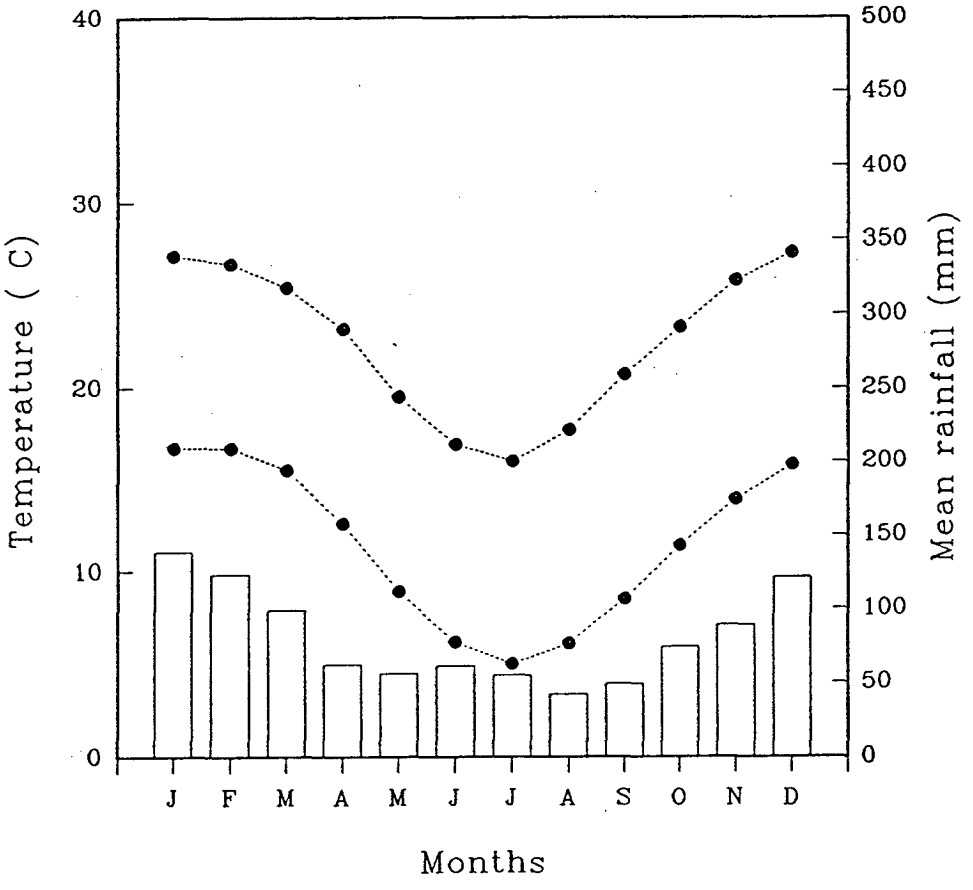


Figure 3:3. Mean monthly maximum and minimum temperatures with associated mean monthly rainfall for Toowoomba (Source -Bureau of Meteorology 1988). Temperature = ● , Rainfall = □.

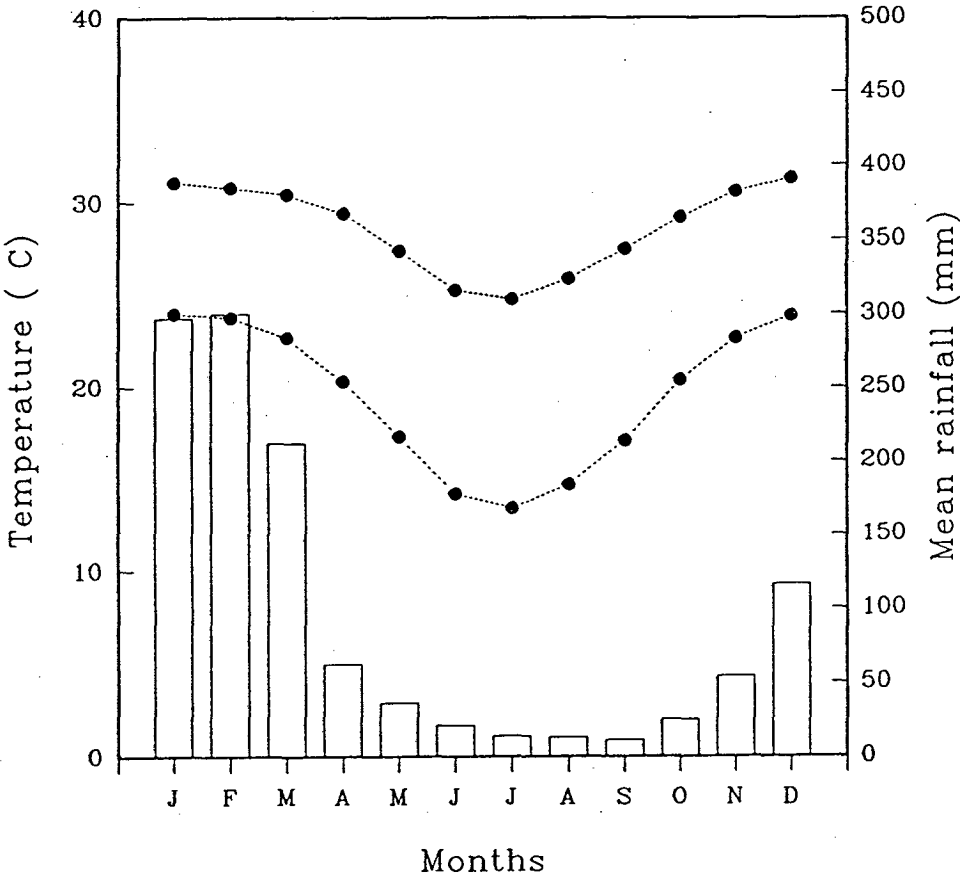


Figure 3:4. Mean monthly maximum and minimum temperatures with associated mean monthly rainfall for Townsville (Source -Bureau of Meteorology 1988). Temperature = ● , Rainfall = □.

3.1.5 Site 5: Emerald

Emerald is situated at latitude 23° 32'E and longitude 148° 10'S at an elevation of 179 m, approximately 300 km from the coast. The mean maximum monthly temperature of 33.8°C occurs in January and falls to a low of 22.4°C in July. The mean minimum monthly temperatures range from 7.3°C in July through to 21.5°C in January. Mean monthly rainfall in this area is low with peaks occurring during January, February and December (figure 3.5).

The main crops of the area are cotton, sown in October/November, soybeans sown in December to mid February, sorghum, sown during January to mid February , sunflower from late January to mid-march and wheat planted in May/June.

3.2 Populations of *Microgaster demolitor* collected.

Separate colonies of *Microgaster demolitor* from each of the above sites were collected and reared . *Heliocoverpa* larvae were collected from Mareeba during September 1987 off tobacco; from Townsville in late September, 1987, off cabbage; from Toowoomba in March 1987 off sunflower; and from Grafton off *Triticale* in October, 1986. They were also collected from Emerald in 1987 from sunflower. The *Heliocoverpa* larvae collected from these locations were of

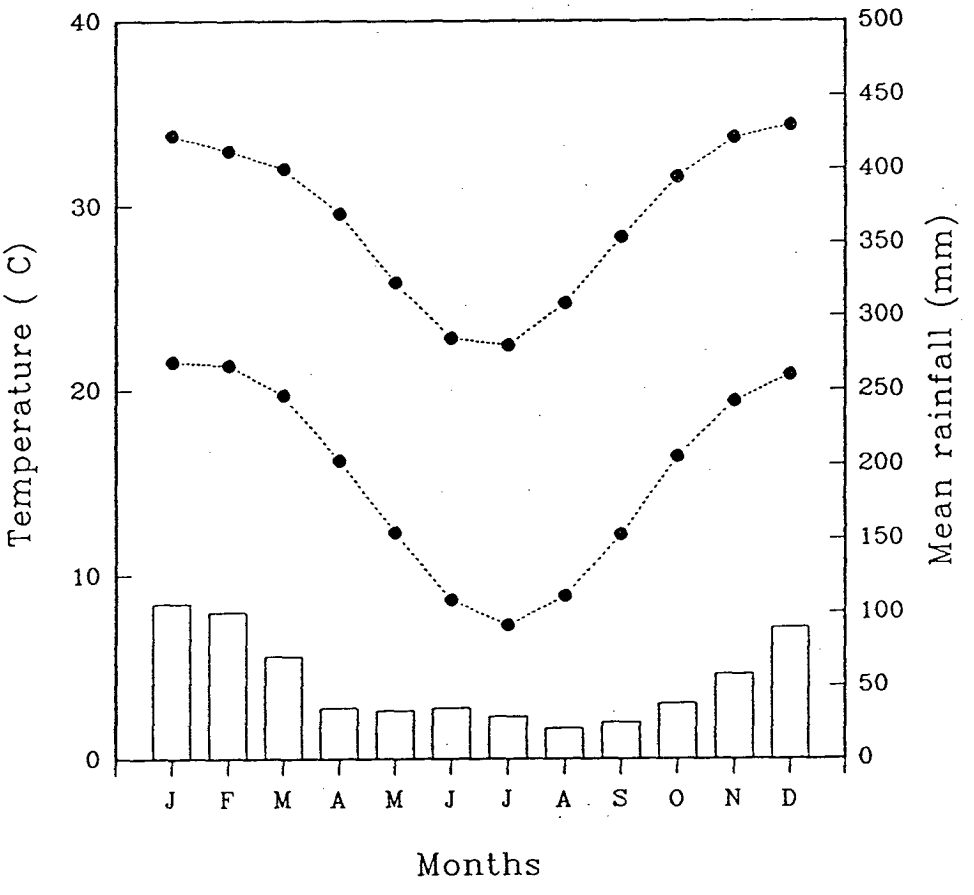


Figure 3:5. Mean monthly maximum and minimum temperatures with associated mean monthly rainfall for Emerald (Source -Bureau of Meteorology 1988). Temperature = ● , Rainfall = □.

unknown species, except those from Toowoomba where the host was known to be *H. armigera* (D. Murray pers comms 1987). However, the population collected from Grafton was almost certainly *H. armigera* (Fitt pers. comms 1991).

At the time of the study, no reliable key to *Heliocoverpa* spp larvae existed. Thus, as some of the colonies of *M. demolitor* were founded from unknown host species, interpopulation differences between parasitoid colonies caused by different hosts is possible. Knowing that this possiblity exists, differences between populations of the parasitoids found in this study should then be viewed with this in mind.

All *Heliocoverpa* were collected from the crops by hand, brought back into the laboratory and reared in 29.6 ml opaque Solo plastic souffle cups containing 10mls of artificial diet, (see appendix 2), and capped with a plastic lid in which a cotton plug was placed, to allow for air flow, until the emergence of any parasitoids or until pupation of the host occurred.

Chapter 4 Natural History

4.1 Introduction

Microplitis demolitor is endemic to Australia and was first described by Wilkinson (1934) from two male and two female specimens collected from *Heliocoverpa armigera* from Stanthorpe, in Southern Queensland, Australia. However, Achterberg (1982) found a synonymy existed for this genus and consequently it was renamed as *Microgaster demolitor.*

The parasitoid was imported into Egypt in 1940 and 1941 (Hafez 1951) and into America in 1981 (Shepard *et al.* 1983a), in an unsuccessful attempt to establish it as a biological control agent. Both these importations came from southern Queensland, Australia. This species of parasitoid has been the subject of a range of biological investigations, both within Australia and elsewhere, as outlined below.

M. demolitor is a solitary internal parasitoid of several species of noctuid larvae (Hafez 1951, Shepard *et al.* 1983a). The adult female may deposit up to 4 eggs into any one host, but only one of these eggs will survive (Johnson 1987, Strand, Johnson & Culin 1988). Strand *et al.* (1988) showed that the size of the emerging parasitoid was positively correlated with the size of the host but that the number of eggs deposited in the host was not.

4.1.1 Host range of the parasitoid.

The parasitoid can successfully parasitise *Prodenia litura, Laphygma exigua* (Hafez 1951), *Heliocoverpa zea, Pseudoplusia includens, Trichoplusia ni* and *Heliocoverpa virescens* (Shepard *et al.* 1983a, Cobb 1983, Herard, Keller & Lewis 1988). Two noctuid species present in Australia, *H. armigera* and *H. punctigera,* are also recorded as hosts for this parasitoid (Titmarsh 1985, Broadley 1984, Room 1979, Smith 1945, Kay 1982b). *Spodoptera exigua, Spodoptera frugiperda, Anticarsia gemmatalis, Pieris rapae, Plathypena scabra* and *Galleria mellonella* were all found to be unsuitable hosts by Shepard *et al.* (1983a), though Salama *et al.* (1982), found *Spodoptera exigua* and *Spodoptera littoralis* were favourable hosts for an imported population of *M. demolitor* from Australia.

4.1.2 Distribution and parasitism rates.

M. demolitor has been recorded in Australia from *Heliocoverpa spp* on cotton in the Namoi valley (Room 1979), soybean at Coominya, (west Morton district, south east Queensland) and pigeon pea from Lockyer valley (Shepard, Lawn & Schneider 1983). It has also been found in larvae collected from maize and sunflower in the Darling Downs district (Kay 1982b), from sunflowers in Southern Queensland (Broadley 1984), from tobacco around Mareeba in North Queensland (Titmarsh 1985), and from sunflowers in Northern New South Wales (Forrester 1981). Recently it has also been recorded from *Heliocoverpa* from

central Australia (Gregg pers. comm. 1990). The current geographic range of *M. demolitor* does not correspond to that of its hosts, that is, it has not been recorded in all locations that its hosts have. Whether this is a true indication of the parasitoid's distribution, or whether this just reflects the areas in which intensive searches for it have been carried out, has not yet been determined.

The parasitism rates attributed to *M. demolitor* in the field vary considerably, depending on host species, instar and the plant the host is associated with. Broadley (1984) estimated percentage parasitism rates of *Heliocoverpa spp.* on sunflower of 36.5, 25.0 and 21.8 percent over three seasons by three major parasitoids, one being *M. demolitor.* Kay (1982b) found differential parasitism rates between crops with *M. demolitor* parasitising 0 to 4% of *H. armigera* on maize, but 12 to 16% on sunflower. Titmarsh (1985) demonstrated that the host instar and time of the year affected parasitism rates of *Heliocoverpa spp.* on tobacco. Rates ranged from 0% parasitism in February and March in all instars to almost 100% parasitism of 5th instar larvae in September/October.

4.1.3 Developmental biology.

The duration of the immature stages varies inversely with temperature, ranging from 15 to 18 days at 20.5°C to 7 to 8 days at 28°C for the egg to prepupal stage for parasitoids reared from *Prodenia litura* or *Laphygma exigua* (Hafez 1951). Similar times were found when *Heliocoverpa virescens* (8.1 days), *Heliocoverpa zea* (9.0 days) and *Pseudoplusia includens* (8.0 days) were used as

the host at 26.7°C (Shepard *et al.* 1983a). Culin & Dubose (1987) and Cobbs (1983) confirmed these developmental times for *M. demolitor* reared in *Heliocoverpa zea*. Unfortunately, no similar data exists for the parasitoid when reared from *H. armigera* or *H. punctigera*.

The duration of the larval stages usually corresponds to 2 instars of the host: that is, if the parasitoid is deposited in a 2nd instar host it will normally emerge from the fourth instar, and if deposited in the 3rd it will emerge from the 5th instar (Strand *et al.* 1988).

Once the parasitoid emerges from the host it spins a silken cocoon within which it then pupates. Hafez (1951) described the cocoon of this parasitoid as being orange yellow in colour while Smith (1945) describes the cocoon as having a brown felt-like appearance. After emergence of the parasitoid the host usually dies (Hafez 1951, Shepard *et al.* 1983a, Smith 1945 and Cobb 1983) but some individual hosts may be able to survive this experience, pupating and emerging successfully as adults (Titmarsh 1985). Parasitised host larvae display a significantly reduced feeding rate (Cobb 1983, Yanes & Boethel 1983, Johnson 1987).

Pupal duration for the parasitoid is substantially shorter than the larval duration, ranging from 6 to 8 days at 20.5°C to 3 to 5 days at 28°C when *Prodenia litura* or *Laphygma exigua* were used as hosts (Hafez 1951). Shepard *et al.* (1983a) found that when three different hosts were used, *Heliocoverpa zea,*

Heliocoverpa virescens or *Pseudoplusia includens,* the pupal times at 26.7°C ranged from 4 to 5 days and that males developed faster than females.

M. demolitor has a true diapause lasting from 3 to 6 months (Hafez 1951 & Kay 1982b) which is synchronous with diapause in one of its hosts, *H. armigera,* at one southern Queensland site (Kay 1982b).

The longevity of adult *M. demolitor* varies with temperature and food availability. At 22°C males lived 19 days when fed honey and water or 16 days if fed honey alone, while females lived 23 days when fed honey and water and 18 days if fed honey alone (Hafez 1951). Similar longevity was reported by Shepard *et al.* (1983a), though they found no difference between the sexes. An extended lifespan has been noted in *M. demolitor* at temperatures below 20 degrees, where the parasitoids live for 55-70 days (Hafez 1951). Herard *et al.* (1988), were also able to increase the longevity of the wasps by maintaining them at high humidities (approx 90%). They also showed that the longevity of female parasitoids was reduced when she was allowed to continually oviposit.

Hafez (1951) reported considerable difficulty in obtaining successful matings between parasitoids but reduced the problem by separating the parasitoid pupae before emergence from the cocoon and then reintroducing the emerged parasitoids to each other to ensure mating occurred. Similar problems were encounted by Herard *et al.* (1988) when *H. zea* was used as a host, though the problem was reduced by keeping the male and female parasitoid together for

24hrs before allowing the female to oviposit. Other authors have not reported difficulties in obtaining successful matings of the parasitoid (Shepard *et al.* 1983a, Cobbs 1987, Horosko 1988 and Johnson 1987).

4.1.4 Host searching behaviour and preference.

The chemical 13-methylhentriacontane, occurring in the frass of hosts reared on natural diets, stimulates host searching behaviour in *M. demolitor* (Norland & Lewis 1985). However the frass produced from hosts reared on artificial diet fails to elicit this response (Norland & Lewis 1985, Herard, Keller, Lewis & Tumlinson 1988a). Lewis, Sonnet and Norland (1988) refined this approach and showed that the R and S stereoisomers of this chemical, both separately and as a combination, all invoked host searching behaviour. Antennal contact with the hosts' frass on emergence of the parasitoid is enough to establish the search response, but it can be depressed by exposing the parasitoid prepupae to low temperatures (Herard, Keller, Lewis & Tumlinson 1988a).

When *M. demolitor* is reared in hosts fed on plants, chemicals which appear to supplement 13-methylhentriacontane in producing this searching response are present in the cocoon of the parasitoid, presumably being trapped there when the wasp prepupae spins its cocoon next to its host. This suggests that parasitoids reared on hosts from different plants may have different searching routines (Herard, Keller, Lewis & Tumlinson 1988b).

M. demolitor is capable of distinguishing between parasitised and non parasitised hosts. It can detect parasitism not only by its own species but also when the host has been parasitised by *Microplitis croceipes*, using sensory structures on its ovipositor (Horosko 1988). However it does not discriminate against hosts infected with microsporidian pathogens (Horosko 1988).

Titmarsh (1985) suggested that *M. demolitor* in tobacco in Mareeba may have a preference for *H. armigera* over *H. punctigera*. Cobb (1983), could not demonstrate a preference by the wasp for either *Heliocoverpa zea* or *Pseudoplusia includens* even though parasitoids reared in *Heliocoverpa zea* were significantly larger than those reared in *Pseudoplusia includens*. The parasitoid does exhibit a preference for the 2nd 3rd and 4th instars of its hosts (Cobb 1983, Johnson 1987).

4.1.5 Interactions between the parasitoid, pathogens and insecticides.

Culin & Dubose (1987) found that the insecticides chlordimeform, methyl parathion and fenvalerate when applied to parasitised hosts of *M. demolitor* did not affect the developmental rate of the enclosed parasitoid. The insecticides did decrease the survival rate of the parasitoid, and the survival rate of insecticide treated parasitised larvae was significantly reduced in comparison to unparasitised larvae (Culin & Dubose 1987). Cobb (1983) found that the fungicide Benlate (Benomyl) caused 100% mortality of parasitised *Heliocoverpa zea* when the foliage they were eating was treated, compared to a 100% survival in non-

parasitised larvae under the same feeding regime. A small but significant decrease in the longevity of the adult parasitoids was found when they were reared in *Heliocoverpa virescens* infected with the Microsporidian pathogen *Vairimorpha necatrix* (Horosko 1988). Horosko (1988) also found that *M. demolitor* could successfully transmit this pathogen from host to host.

Larvae of *H. armigera* parasitised by *M. demolitor* are less susceptible to infection by nuclear polyhedrosis virus (Teakle, Jensen & Mulder 1985). Conversely host larvae infection before parasitisation showed a reduction in successful emergence of the parasitoid (Teakle, Jensen & Mulder 1985). Similar effects were found with *Spodoptera littoralis* and *S. exigua* larvae infected with the pathogen *Bacillus thuringiensis*. Here the presence of the pathogen in the hosts caused 85.9% mortality of the developing parasitoid (Salama *et al.* 1982).

4.2 Methods

4.2.1 Taxonomic studies

Several male and female parasitoids from Mareeba and Grafton were sent to Dr I. Galloway, D.P.I., Brisbane, for identification. Similarly specimens from Mareeba, Townsville, Emerald, Toowoomba and Grafton were sent to Dr A. Austin, Waite Institute, S.A..

4.2.2 Host Parasitisation

All experiments described below were conducted with a laboratory strain of parasitoids established from Toowoomba, south east Queensland.

To determine if *M. demolitor* showed different atack sequencces for different instars or differnt species of hosts, ten mated female parasitoids were placed individually in clear plastic containers, 8cm in diameter by 5 cm high, in which ten *Heliocoverpa* spp larvae were housed. Artificial light was supplied via a 75 watt light globe suspended 1 metre above the container. The larvae ranged in instar from 2nd to 6th but only one host species was present within the container at any time. Artificial diet for the hosts was always present in small quantities spread through out the container.

The movements of the parasitoids were then observed before, during and after parasitisation of the hosts. Once a host was parasitised it was removed and replaced with a similar sized larva of the same species.

4.2.3 Mating of the parasitoid

As mating between male and female parasitoids placed together at room temperature was often unsuccessful, female parasitoids were cooled at 15°C while males were warmed at 30°C for several minutes. One female was placed with

five or more males at 26°C degrees in a small transparent 59ml container. The colder female being sluggish was easily mounted by the male resulting in increased mating success.

4.2.4 Egg content of female parasitoids

Seven female parasitoids, which were reared at 26°C and 14L:10D in *H. punctigera*, were preserved in 70% ethanol 24 hours after they had emerged from their cocoon. Their reproductive organs were dissected out, the ovariole walls broken open and the contents stained in a 1% solution of toluene blue in 1% borax for 1 minute. The eggs were stained blue/purple and could be easily seen and counted.

4.2.5 Damage to internal organs of host

Six 2nd instar *H. punctigera* and six 2nd instar *H. armigera* larvae were parasitised and then held at 26°C at a photoperiod of 14L:10D. Two individuals of each host species were killed and fixed in a solution of 70% alcohol and 3% propylene glycol 4 days after being parasitised. Another two of each species were killed at 7 days and a further two when the parasitoid had emerged. These individual hosts were then embedded in paraffin and longitudinally sectioned at a thickness of 10μm and stained in mallory trichome. The resulting sections were then examined under a high power microscope to determine the position of the growing parasitoid and to examine damage to internal organs.

4.2.6 Weights of parasitised and non parasitised larvae

Sixty *H. punctigera* larvae were reared on artificial diet at 26°C and a photoperiod of 14L:10D to the 2nd instar. Half the larvae were then parasitised by two *M. demolitor* and allowed to develop. The two groups of larvae (unparasitised and parasitised) were then weighed on a Cahn microbalance (to within 0.05mg) seven days later.

The weights of the unparasitised and parasitised larvae were then analysed with a one way analysis of variance.

4.2.7 Longevity

4.2.7.1 Food effects

Thirty two parasitoids, originating from three different female parents, reared in *H. punctigera* at 26°C and a photoperiod of 14L:10D were used to determine the effects of adult nutrition on longevity. The parasitoids were either starved (n=6), fed water alone (n=9), a 10% honey and water solution (n=10) or a 10% solution of fructose, glucose and sucrose (2:2:1) (n=7) until death occurred. The parasitoids were housed in small (7.5 cms in diameter by 5.5 cms deep), opaque containers with cotton mesh lids to allow for air movement. Food

was supplied daily on small cotton buds soaked in the desired food. These cotton buds were left in the containers for a maximum of 6 hours. The differences in longevity between groups fed the different food types were then analysed using a one way analysis of variance.

4.2.7.2 Temperature effects

The effects of temperature on longevity of parasitoids reared in *H. punctigera* at a photoperiod of 14L:10D and fed a 10% honey with water solution was investigated. Parasitoids were housed in opaque containers with food supplied on soaked cotton plugs. Groups of parasitoids were placed at 15°C (n=11), 20°C (n=18), 24°C (n=11), 27°C (n=6) and 30°C (n=13) and fed daily, until death occurred.

The relationship between temperature and longevity was then determined using regression analysis.

4.3 Results

4.3.1 Taxonomy

M. demolitor from Grafton, Toowoomba, Emerald, Townsville and Mareeba appear morphologically similar (Galloway pers comm 1985, Austin pers comm 1988). They are approximately three millimetres long from the head to the

tip of the abdomen. The antennae of the males are slightly longer than the body whereas female antennae are slightly shorter than the body. Male antennae are also thicker than those of the female. Females have predominantly black abdomens and a short black ovipositor, while males have a small area of black pigmentation on the rear of an otherwise yellow/orange abdomen. These differences allow the parasitoids to be sexed. The thorax and head of both sexes are black and the appendages are yellow/orange.

4.3.2 Host attack

M. demolitor attacked both *H. punctigera* and *H. armigera* in a similar way. The parasitoid would first preen itself while some distance from the host/s, starting with the antennae, then the legs and finally the wings. This procedure took between 1 and 10 minutes depending on previous attacks. For example, an attack on a 5th instar larvae usually resulted in the wasp being showered in digestive fluid from the host's mouth. The parasitoid would then spend up to 10 minutes preening itself. However an attack on a 2nd instar larvae usually resulted in no fluid being ejected by the host and preening would only take 1 to 2 minutes. Any contact made by the wasp with the artificial diet also caused preening.

Once preening was complete the parasitoid would then travel around the container antennating the substrate until it came in contact with either a suitable

host or frass from the host species. If frass was encountered the parasitoid would search predominantly around the area of the frass.

Once a host was selected, which occurred after the parasitoid palpated the host with its antennae, the parasitoid raised its wings and curved its abdomen under its body, jumping on the host at the same instant. She then lifted her posterior pair of legs off the host and inserted her ovipositor. As the ovipositor of *M. demolitor* is small, approximately 0.2mm, and is not extendable, the parasitoid was always close to the host during oviposition. This often resulted in larger larvae, 4th, 5th and 6th instars, being able to bend around and attack the parasitoid, often fatally. This entire procedure from palpating the host with the antennae to withdrawal of the ovipositor took between one and five seconds. There appeared to be no preferred area on the host for oviposition as the female normally oviposited in the area of initial contact with host.

4.3.3 Egg content of female parasitoids

The eggs of *M. demolitor* are hymenopteriform (Clausen 1940) and approximately 0.25 mm long and 0.05 mm wide when mature. They become progressively smaller and less developed with distance along the ovaries. Each of the ovarioles contained approximately half of the total egg content, which averaged 118 eggs per female (S.E. = 3.6, n = 7).

4.3.4 Weights of parasitised and unparasitised larvae

Parasitised hosts weighed significantly less than unparasitised hosts, 27mg vs 287mg ($F=248$, 1*58 df, $p<0.001$). Although the parasitised hosts were significantly smaller, they still passed through two instars before the parasitoid emerges. That is, a egg of the parasitoid laid in a 2nd instar host, emerges as a prepupae in the hosts 4th instar, similarly, one inserted in a 3rd instar emerges from the 5th instar of the host. Of a total of 1453 parasitised larvae, 100% mortality was recorded within 1 to 6 days after the parasitoid had emerged.

4.3.5 Internal damage to host

As the parasitoid developed it moved from its initial place of deposition to the rear of the host. After approximately 4 days, at 26°C, the parasitoid occupied approximately 10 percent of the body cavity of its host, (plate 1). One to two days prior to the parasitoid emerging the host stopped feeding and became immobile. At the time of emergence the parasitoid occupied approximately 30 percent of the body cavity and had totally occluded the lower digestive tract, (plate 2).

The parasitoid emerged as a prepupa by burrowing out of the side of the host. The prepupa was light brown to tan in colour and began to spin a cocoon

around itself while the posterior end of its body was still within the host. It did not draw itself totally out of the host until the anterior portion of the cocoon was well developed.

The cocoon was three to four millimetres long and 1 to 1.5 millimetres wide. It was usually attached to the substrate and not to the host (c.f. Smith 1945 who found that the cocoon was often attached to the host as well). The parasitoid prepupa then either pupated or entered diapause as a prepupa within the cocoon. There was a distinct difference between cocoons of diapausing and non diapausing prepupae (see chapter 6).

Upon reaching maturity the fully developed wasp cut an operculum in one end of the cocoon with its mandibles and took 1.95 hours (S.D. = 0.32 hours, n=8) to emerge. At this stage its wings were dry and set and it was capable of flight.

3.3.6 Mating of the parasitoids.

Upon introduction of the female to the male, the male vibrated its wings, and tapped the substrate with its antennae, and often dragged its abdomen along the floor of the container. This behaviour occurred when a pair met and often for several seconds afterwards. This continued until copulation occurred, whereby the male mounted the female, bent his abdomen ventrally and anteriorly, and inserted his intromittent organ into the female's genital opening. Once in

copula the pair remained stationary for a mean period of 24 seconds (SE=1.5, n=9) after which the male left the female. Males, during their lifespan, were capable of mating with and fertilising several females, however it is not known if females are capable of mating more than once.

4.3.7 Longevity

4.3.7.1 Food effects

Average longevity at 27°C was significantly affected by feeding regime ($F=14.86$, 3*28 Df, $p < 0.01$). Wasps fed a 10 percent honey solution survived an average of 11.30 days, while if starved, their longevity was reduced to an average of 3.68 days. A mixture of sugars and water produced a mean life span less than that if the parasitoids were fed honey and water (figure 4.1).

4.3.7.2 Temperature effects

Adult longevity decreased with increasing temperature for *M. demolitor* (figure 4.2). However, longevity at 15°C was exceptionally high, increasing from 14.83 days at 20°C to 57.45 days. Transforming the data by plotting longevity as a rate, that is, 1/longevity, failed to linearize the relationship over the entire

Plate 1. Longitudinal section of *H. punctigera* with developing larval *M. demolitor* , 4 days after parasitism.
H = head, G = gut, P = developing parasitoid, (bar = 2mm).

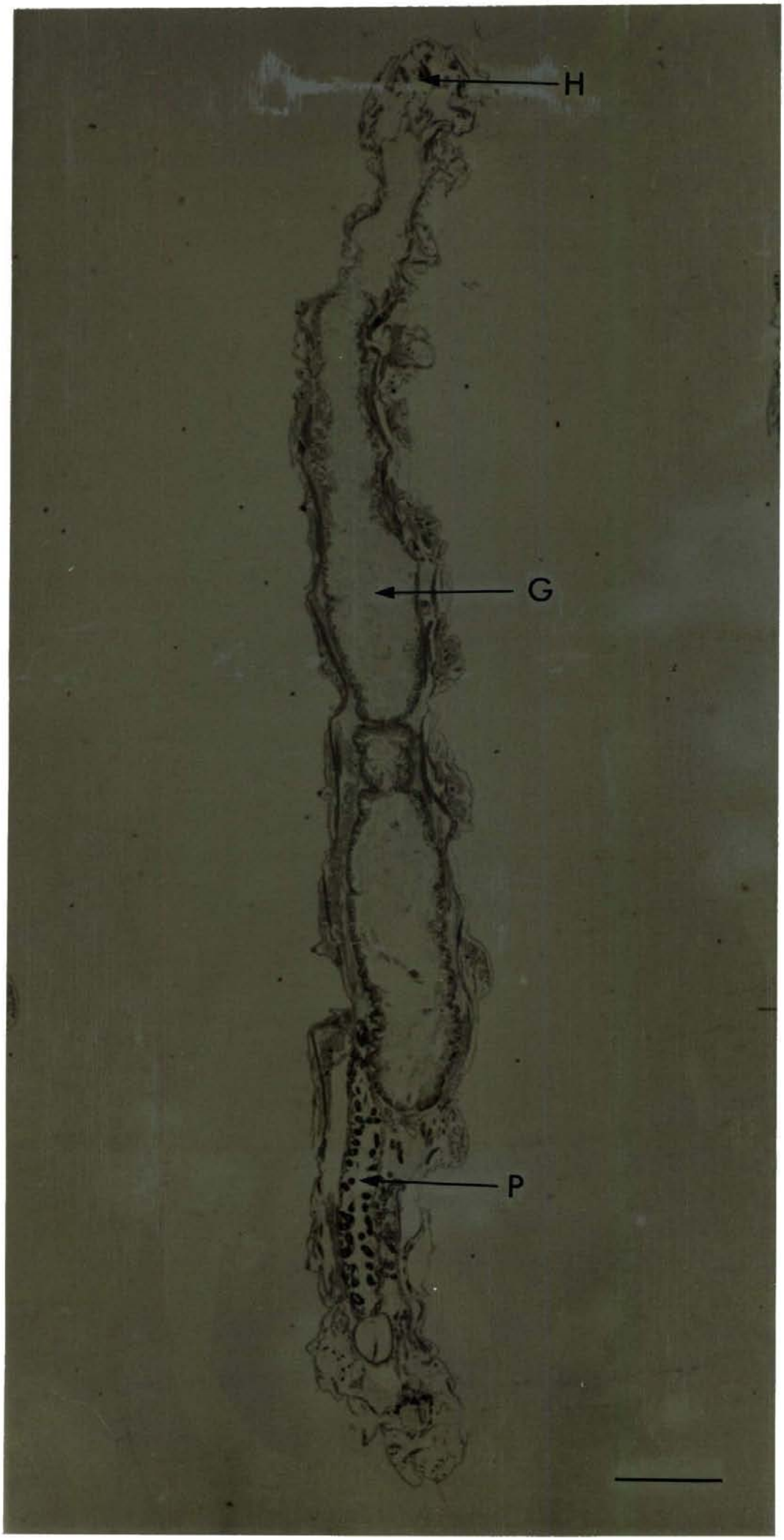
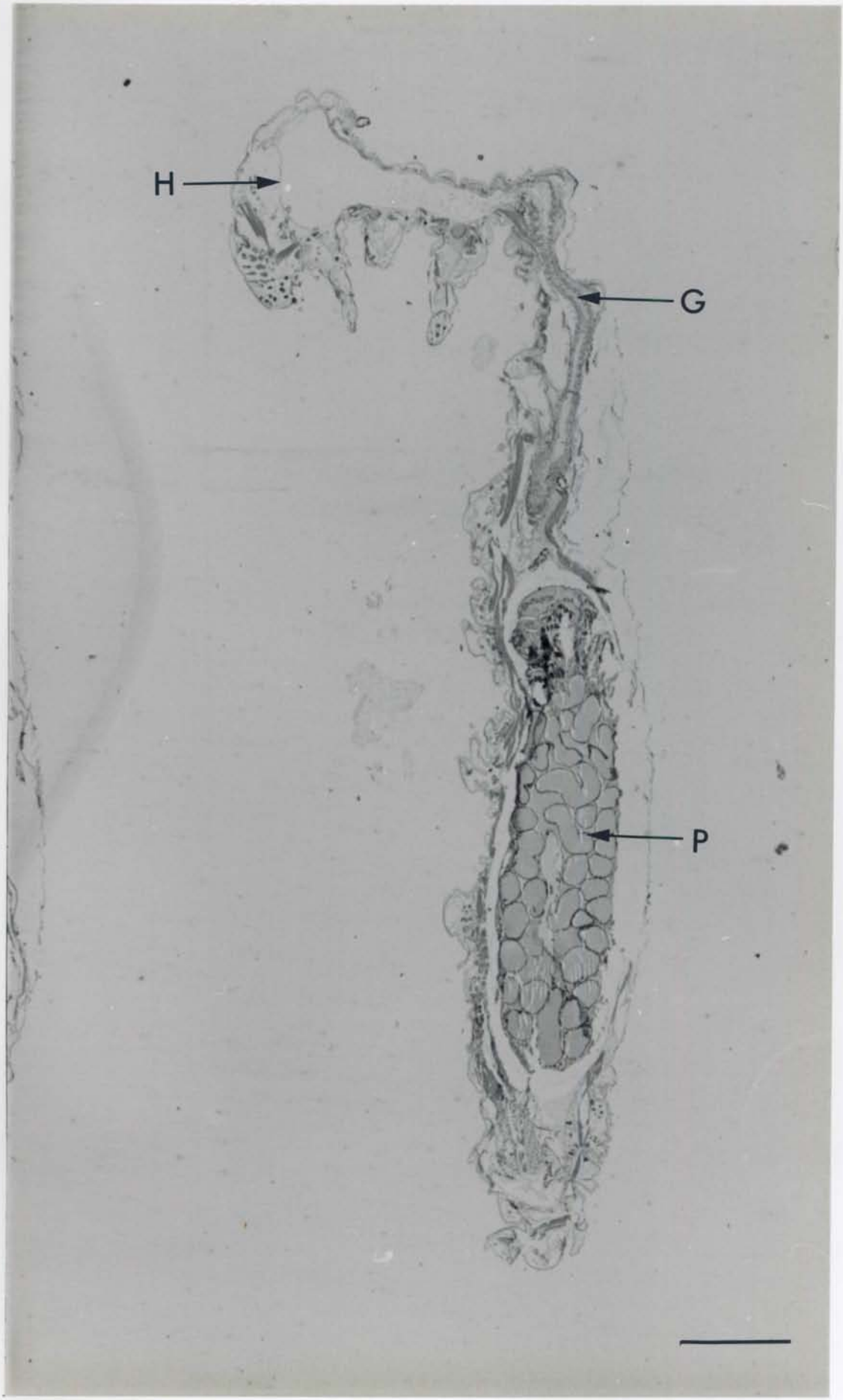

H
G
P

Plate 2. Longitudinal section of *H. punctigera* with developing larval *M. demolitor* , 7 days after parasitism.
H = head, G = gut, P = developing parasitoid, (bar = 2mm).


H
G
P

range. Between 20°C and 30°C there was a significant linear relationship between temperature and longevity;

Longevity(in days) = 26.82 - 0.57*temperature (in °C);

(r^2 =0.27, F=16.62, df=46, $p < 0.01$).

Parasitoids placed at 15°C were often immobile but were still capable of moving around the container to collect food from the cotton plugs. When removed and placed at higher temperatures the parasitoids became highly active within several minutes.

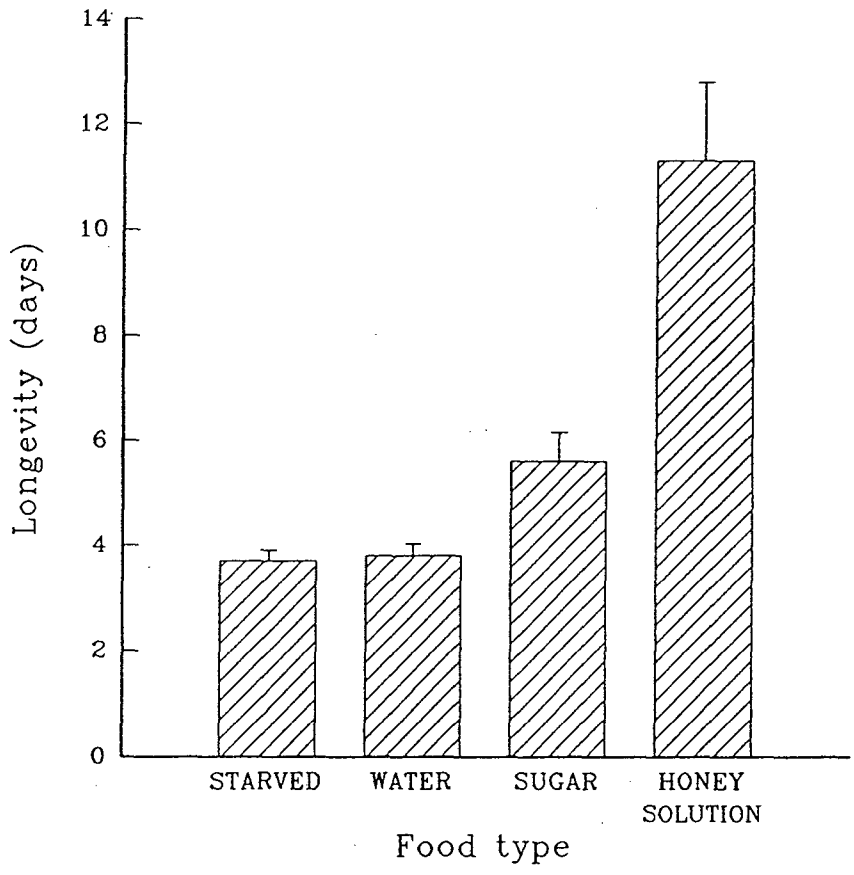


Figure 4:1. The effect of different food types on the longevity of *M. demolitor* from Toowoomba, reared in *H. punctigera*, at 26°C.

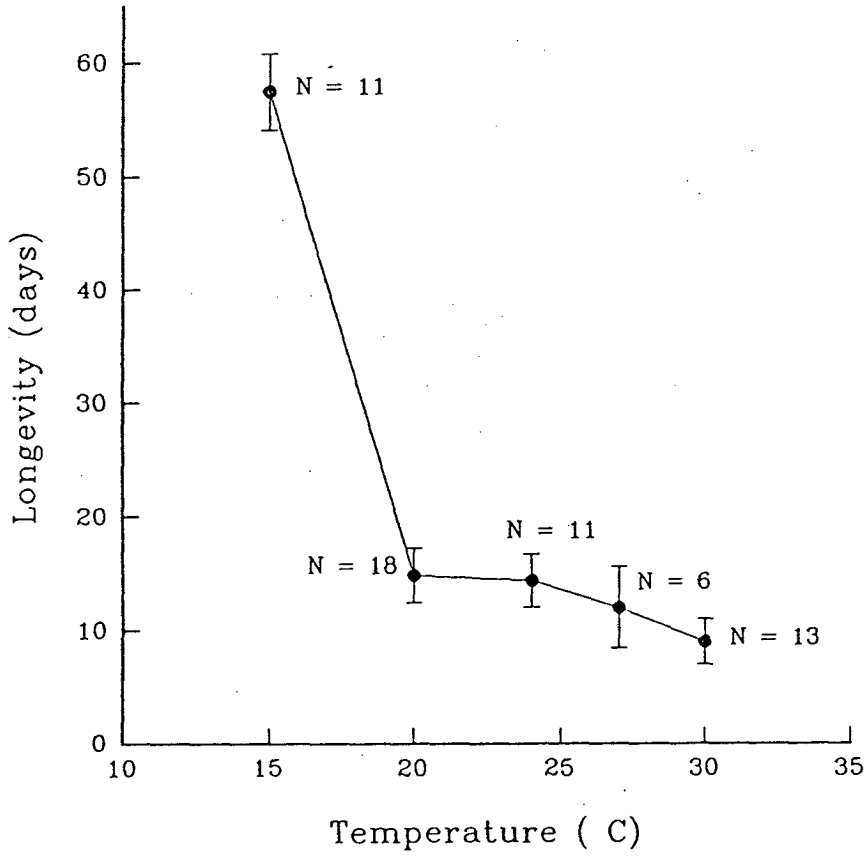


Figure 4:2. The longevity of *M. demolitor* from Toowoomba, reared in *H. punctigera*, for various temperatures.

4.4 Discussion

Shepard *et al.* (1983a) found that adult longevity of *M. demolitor* reared from *H. virescens* was approximately 8 days at 26.7 degrees Celsius. This is lower than the 12 days found here at similar temperatures. This difference may be caused by the difference in adult food. Shepard *et al.* (1983a), used concentrated honey in contrast to the 10% solution used in this study. This higher concentration may cause dehydration of the parasitoid, resulting in premature death due to increased water stress. This effect has been shown in other braconids, with the longevity of *Microplitis rufiventris* decreasing when pure honey was used in comparison to a 50% solution (Altahtawy, Hammad & Hegazi 1976, Yazi & El-Minshawy 1981).

The decrease in longevity caused when only sugars were used as food suggests that additional substances present in honey, for example proteins and amino acids, may have a positive effect on longevity. Similar effects have been shown in other braconids, such as *Microplitis rufiventris* (Altahtawy, Hammad & Hegazi 1976, Yazi & El-Minshawy 1981) and *Apanteles militaris* (Calkins & Sutter 1976).

The decrease in longevity in the absence of food corresponds with the findings by Hafez (1951) for the imported population of *M. demolitor* from Queensland. This suggests that the parasitoid may feed once it emerges, possibly on nectar in flowers. Similar decreases in longevity have been shown in other

parasitoids such as *Microplitis rufiventris* (Hegazi & Minshawy 1981), *Cardiohiles nigriceps* (Butler *et al.* 1983) and *Apanteles militaris* (Calkins & Sutter 1976). Similarly, decreases in longevity with increasing temperature have also been commonly found (Gifford & Mann 1967, Rachav 1968, Calkins & Sutter 1976).

The significant increase in longevity found for the adult wasps at 15°C may be a form of quiescence. That is, at low temperatures the metabolic rate of the parasitoids may decrease in a non linear relationship so that they become motionless, but their longevity is enhanced, until they are exposed to warmer temperatures, where upon they become mobile and active. Hafez (1951) found a similar effect in *M. demolitor*, (originally collected from Stanthorpe in Queensland), with longevity of 55-70 days when the wasps were held at 13.5°C and removed twice weekly and allowed to feed at 22°C.

The quiescence stage employed by the parasitoid may be a bet hedging strategy, that is, a gamble on the possibility of early immigrations of *Heliocoverpa* spp. A parasitoid, emerging from diapause two or three weeks before the start of the season may, by virtue of this quiescence strategy, be able to exploit any early immigrating *Heliocoverpa* populations from central Australia brought on by early rains. However, it may not be fatal to the parasitoid if temperatures remain low and the migration does not eventuate. Temperatures, at least in Toowoomba (see chapter 3), are often low enough at the start of the season to induce this quiescence effect.

This lengthening of the life span of the parasitoid may result in a greater probability of encountering hosts from immigrating populations. However, this strategy will only be effective if temperatures rise, if only for a few days, to allow the parasitoids to become mobile and exploit their hosts.

The colour of the parasitoid's cocoon does not correspond with that found by Hafez (1951) but is similar to that reported by Shepard *et al.* (1983a, 1983b). It is possible that different hosts have different effects on the pigmentation of the cocoon silk as Hafez (1951) reared *M. demolitor* from *Prodenia litura* and *Laphygma exigua* while Shepard (1983a, 1983b) extracted *M. demolitor* from *Heliocoverpa zea, Heliocoverpa virescens* and *Pseudoplusia includens.*

The attack sequence displayed by *M. demolitor* towards both *H. punctigera* and *H. armigera* is similar to that recorded by Shepard *et al.* (1983a) when *Heliocoverpa virescens* was used as the host species. However the 'feigning of death' behaviour reported by Shepard *et al.* (1983a) was never observed.

The recorded decrease in size between parasitised and normal hosts may indicate that parasitised hosts have a decreased feeding rate. This is supported by Cobb (1983), who found that foliage consumption of *Heliocoverpa virescens* larvae parasitised by *M. demolitor* was significantly lower than that by unparasitised larvae.

The 100% mortality rate of hosts after parasitoid emergence does not correspond to that of Titmarsh (1985) who found that 1.01% of parasitised *H. armigera* were able to continue development and pupate to moths. The laboratory reared hosts used in this study are almost certainly genetically different due to inbreeding (supported by the quicker development rates for the parasitoids reared in these hosts, see chapter 5) and hence may not be able to cope with the stress associated with producing a parasitoid. As the proportion surviving is, in any case, small, and it is not known if hosts which survived were capable of reproducing, *M. demolitor* could affect the size of *Heliocoverpa* populations in Australia if parasitism rates were sufficiently high.

Chapter 5 Developmental biology.

5.1 Introduction

The developmental rate of all insects is dependent on temperature, that is, growth is slower at lower temperatures. This relationship between temperature and development is usually sigmoidal in shape with the fastest developmental temperature occurring at the upper point of inflection of the curve (Davidson 1944). This temperature is not necessarily the optimum temperature for the insect, as fecundity and/or survival may vary with temperature and are often higher at lower temperatures than those at which the quickest development occurs (Danks 1987).

The relationship between the upper and lower limits is usually linear and from this, the thermal constant, or the amount of heat required over time for an insect to complete development, can be derived (Campbell *et al.* 1974). At the lower end of the relationship, the curve maybe extrapolated as a straight line to determine the developmental threshold, that is, the temperature at which no development takes place. However, these estimated thresholds are usually higher than the true developmental zeros (Danks 1987). Estimations of the thermal thresholds and constants, as well as their associated standard errors were carried out using the methods outlined in Campbell *et al.* (1974).

Usually, in tropical species, both the lower and upper limits of the developmental relationship are correspondingly higher than those in temperate regions, with individuals outside this temperature range often becoming quiescent (Danks 1987). Developmental thresholds and thermal constants then often reflect the environmental regime to which the insect is exposed (Danks 1987).

If the environmental temperatures experienced by an insect are seasonally unfavourable for development, then it must have some means of ensuring its survival or that of its offspring during the adverse season. Several solutions are possible, ranging from migration from the area, to the entering of dormancy periods such as quiescence or diapause until the environment returns to a state in which the insect can successfully continue development.

Parasitoid/host relationships in temperate studies have usually, but not always, found that the parasitoids' lower developmental thresholds are higher than that of their hosts (Herbert & McRae 1983, Johnson, Trottier & Laing 1979 Obrycki & Tauber 1979, Nealis *et al.* 1984), presumably to allow the hosts to become established before the parasitoids themselves start to develop (Campbell *et al.* 1974).

There are no studies on the developmental rates of *M. demolitor* reared from its natural hosts. For this parasitoid to be considered for a biological control agent in Australia, this type of fundamental data is required.

This study examines the relationships between temperature and developmental attributes of several populations of *Microgaster demolitor*. Only data from non-diapausing larval and pupal parasitoids were used while both laboratory and field strains of the hosts were examined for effects on the developmental rate of the parasitoids. From this data, the lower developmental thresholds and thermal constants for three different populations of *M. demolitor* are determined. These results are then considered in the light of the developmental biology of two of the parasitoid's hosts, *H. armigera* and *H. punctigera*.

5.2 Methods.

5.2.1 Effects of laboratory and field strains of hosts on development.

To determine if different cultures of host affected the developmental rate of the parasitoid, *M. demolitor*, originating from Mareeba, was reared in field and laboratory strains of *H. punctigera* at 24°C.

5.2.1.1 Laboratory host strain

The laboratory cultures were maintained by fortnightly supplies of both *H. punctigera* and *H. armigera* eggs from the laboratory based culture run by Dr B. Teakle at the Long Pocket branch of the Department of Primary Industries at Brisbane. Eggs were sent via mail to Townsville where they were hatched at

room temperature. These larvae were then placed in 175ml plastic containers at a density of approximately 20 per container. Each of the containers held 4 sticks of an artificial diet (appendix 2), each stick being 1cm by 1cm by 4 cm long. This provided a larger surface area of diet than if only 1 piece of diet was added and helped reduce cannibalism between the larvae. Once the larvae had reached 2nd instar they were removed and used for oviposition by the parasitoids. A total of 357 parasitoids were reared from these hosts for this experiment.

5.2.1.2 Field host strain

As all attempts to establish a culture of field *H. armigera* were unsuccessful, only *H. punctigera* could be used to examine differences between field and laboratory strains. The culture of field *H. punctigera* was collected from Townsville, North Queensland, from sweet corn and maize during November, 1987. Field collected larvae were reared in opaque plastic solo p100 containers, approximately 29ml in volume, on foliage and fruit of the host plant from which they were collected, until pupation occurred. Offspring from this field culture were used for 2 generations of the host, both of which were reared on artificial diet (appendix 2). *H. punctigera* pupae were then placed in clear plastic breeding containers, 20cm in diameter and 30cm high, at room temperature and a photoperiod of 12L:12D. They were allowed to mate and lay eggs on paper towelling which was placed around the inner surface of the cage. The paper towelling containing the eggs was then removed and placed in plastic

bags at a constant temperature of 24 $\pm$ 1 degree until hatching occurred. A total of 66 parasitoids were reared from these field larvae using the same technique as the laboratory strain.

5.2.2 Oviposition of the hosts

Oviposition by the parasitoid in the host was obtained by placing groups of twelve 2nd instar larvae of one host species into clear plastic petri dishes, 9cms in diameter and 1.5 cms high with a 0.5cm hole in the lid, sealed with a cotton plug. The dish was then sealed using plasticine between the base and the lid. Parasitoids were anaesthetised in their rearing containers with carbon dioxide which was introduced until the wasp had been incapacitated for approximately 30 seconds. The parasitoid was introduced into the oviposition container via the hole in the lid. The parasitoid was then left with the larvae for 2 to 4 hours under constant light supplied from a 50 watt light globe suspended 0.5 metres above the containers. Care was taken never to mix species of *Heliocoverpa* in the one container and containers were washed and sterilised in 10% bleach before they were reused.

At the end of the oviposition session the female parasitoid was then anaesthetised with carbon dioxide, removed and returned to her original container at 26 degrees and supplied with a 10% water honey solution until used for further oviposition. A total of 31 different female parasitoids were used. Parasitised

larvae were then placed separately in wells of a 12 cell limbro tissue culture plate which contained artificial diet (see appendix 2) and reared under various regimes as described below.

5.2.3 Developmental rates

The effects of temperature, (monitored via a thermohygrograph), host species, sex of the parasitoid and site (or population) effects on the development of the parasitoid were investigated by rearing hosts parasitised by *M. demolitor* at 17, 20, 24, 26 and 30 degrees celsius, ($\pm$1.0°C). Both *H. punctigera* and *H. armigera* were used as hosts and oviposition was initiated in 2nd instar larvae. All photoperiods were constant at 14L:10D. However, humidity was not measured and may have varied between cabinets. Table 5.1 shows the numbers of parasitoids reared for each of the temperatures for larval development while table 5.2 shows that for pupal development. Due to severe mortality of hosts at higher temperatures by fungal disease and the differential rates of diapause in each population, the number of larval and pupal parasitoids in each treatment were different. For the Grafton population, a total of 12 different female parasitoids were used in the production of offspring for the experiment, for Toowoomba a total of 64 and for Mareeba a total of 8.

Table 5.1: Sample sizes of parasitoids used to determine larval developmental rates for different sites.

	Site		
	Grafton	Toowoomba	Mareeba
Temperature			
17	17	--	24
20	23	29	--
24	25	49	--
26	26	514	27
30	--	--	17

Table 5.2: Sample sizes of parasitoids used to determine pupal developmental rate for different sites.

	Site		
	Grafton	Toowoomba	Mareeba
Temperature			
17	8	--	11
20	6	16	--
24	5	49	--
26	--	449	9
30	--	--	61

Larval and pupal thresholds and thermal constants of the parasitoids were calculated using the method outlined in Campbell *et al.* (1974). Hosts and cocoons were checked twice daily for emergence of parasitoids. After emergence the parasitoids were anaesthetised with carbon dioxide and sexed.

All statistical analysis was conducted using the procedure GLM for analysis of covariance and procedure REG for regression analysis in the computer program "SAS". Type III sums of squares were used to allow for unbalanced designs.

5.3 Results.

5.3.1 Field and laboratory host effects.

Parasitoids reared in field hosts at 26°C had a mean larval developmental time of 7.50 days which was significantly longer than that for parasitoids reared in the laboratory based culture of hosts of 7.03 days (Kruskal-Wallis statistic $=12.11$, $p < 0.01$). The laboratory based culture had a smaller variance than that of the field based culture (Table 5.3).

Table 5.3: Mean developmental times for *M. demolitor* reared in laboratory and field strains of *H. punctigera* at 26 degrees.

	Mean time	Variance	n
Lab.	7.03	0.50	357
Field	7.50	1.39	66

5.3.2 Larval development.

An analysis of larval developmental rates for parasitoids from different populations (site), and hosts (*H. punctigera* or *H. armigera*), of different sexes, and at different temperatures was conducted via a ANCOVA using temperature as a covariate. Developmental rates differed between sites, but not between host species or between different sexes of the parasitoid (table 5.4).

Regression lines were therefore fitted to pooled data of sex and host from each of the sites and developmental thresholds and thermal constants were calculated (table 5.5 and Figure 5.1). The temperature by site interaction (table 5.4) indicates that the regression lines had different slopes, that is, the rate of development varies between sites. The Grafton population had the lowest larval threshold (9.5°C), the Toowoomba population had the highest (13.6°C). These thresholds and thermal constants were all significantly different (table 5.7) .

5.3.3 Pupal development.

Analysis of pupal developmental times again showed that site (or population) influenced pupal development time but that the sex of the parasitoid or the species of host in which it was reared did not (see table 5.6). Regressions of developmental rate on temperature for each site are shown in table 5.7 and figure 5.2. Diapausing parasitoids were not included in these calculations (see chapter 8).

The pupal developmental threshold was lowest at Mareeba (9.0°C) and highest at Toowoomba and Grafton (15.0 & 15.2°C respectively). The Mareeba threshold was significantly different from the Toowoomba and Grafton thresholds (table 5.7).

Table 5.4: Summary of the results from an ANCOVA on larval development time of *Microgaster demolitor* reared in *H. punctigera* and *H. armigera*.

Source	DF	Mean Square	F value	p
HOST	1	0.00007644	0.35	0.55
SITE	2	0.00075076	3.49	**0.03**
TEMP	1	0.08936643	416.01	**<0.01**
SEX	1	0.00004227	0.20	0.65
HOST*SEX	1	0.00060630	2.82	0.09
SITE*SEX	2	0.00031402	1.46	0.23
HOST*SITE	2	0.00061022	2.84	0.06
TEMP*HOST	1	0.00009238	0.43	0.51
TEMP*SITE	2	0.00096313	4.48	**0.01**
TEMP*SEX	1	0.00007979	0.37	0.54
TEMP*HOST*SITE	2	0.00056323	2.62	0.07
TEMP*HOST*SEX	1	0.00059977	2.79	0.09
TEMP*SITE*SEX	2	0.00028119	1.31	0.27

Table 5.5: Developmental thresholds and equations for the larval development of *Microgaster demolitor* from different sites. Th = threshold(°C), K = thermal constant(degree days).

	Equation	R^2	Th(SE)	K(SE)	N
Grafton	Y = 0.0082X-0.080	0.69	9.7(0.5)	121.9(8.6)	91
Toowoomba	Y = 0.0118X-0.162	0.47	13.6(0.4)	84.7(3.3)	592
Mareeba	Y = 0.0086X-0.101	0.86	11.9(0.6)	116.3(5.8)	68

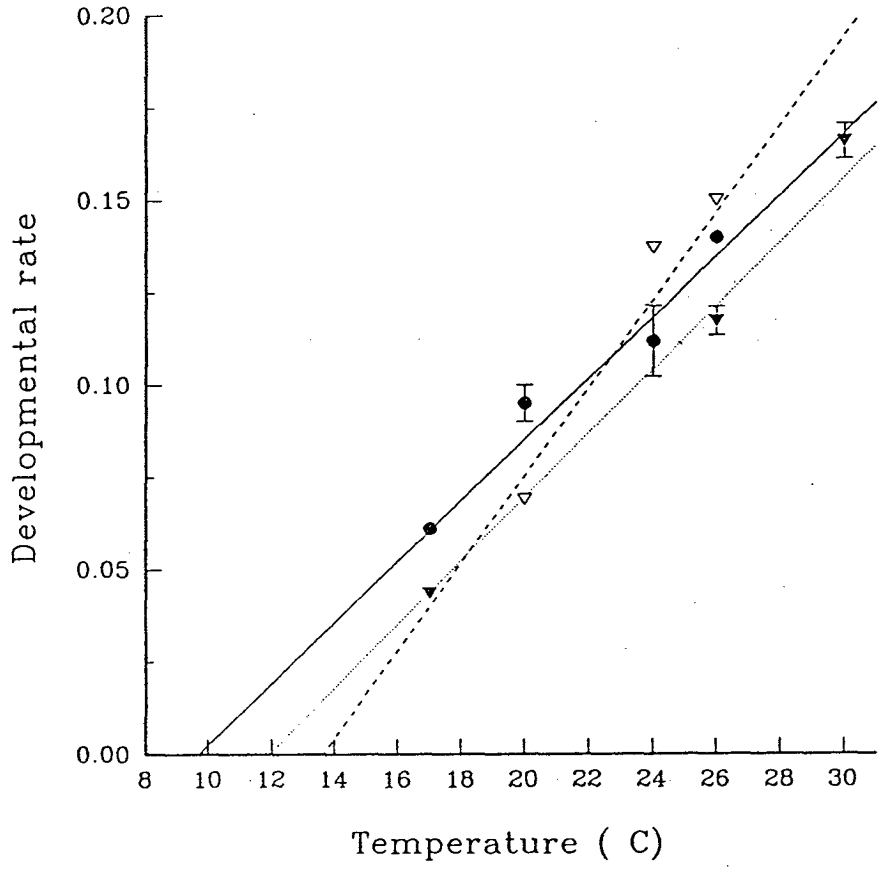


Figure 5:1. Developmental rate for three different populations of *M. demolitor* for egg to prepupal emergence from the host. (Vertical bars are 95% confidence limits).

Mareeba = ▼.....▼

Toowoomba = ▽--▽

Grafton = ●——●

Table 5.6: Summary of the results from an ANCOVA on pupal development times of *Microgaster demolitor* reared in *H. punctigera* and *H. armigera*.

Source	DF	Mean Square	F value	p
HOST	1	0.00341006	3.18	0.07
SITE	2	0.02295887	10.69	**<0.01**
TEMP	1	0.47401098	441.49	**<0.01**
SEX	1	0.00089795	0.84	0.36
HOST*SEX	1	0.00270390	2.52	0.11
SITE*SEX	2	0.00020846	0.19	0.66
HOST*SITE	2	0.00203268	1.89	0.17
TEMP*HOST	1	0.00385924	3.59	0.06
TEMP*SITE	2	0.02929670	13.64	**<0.01**
TEMP*SEX	1	0.00043503	0.41	0.52
TEMP*HOST*SITE	2	0.00217595	2.03	0.15
TEMP*HOST*SEX	1	0.00211154	1.97	0.16
TEMP*SITE*SEX	2	0.00023197	0.22	0.64

Table 5.7: Developmental thresholds and equations for the pupal development of *Microgaster demolitor* from different sites. Th = threshold (°C), K = thermal constant (degree days).

	Equation	R^2	Th(SE)	K(SE)	N
Grafton	Y = 0.0255X-0.376	0.82	14.7(0.5)	39.2(3.5)	19
Toowoomba	Y = 0.0219X-0.330	0.36	15.0(0.6)	45.7(2.7)	514
Mareeba	Y = 0.0132X-0.129	0.57	9.7(1.3)	75.7(6.8)	81

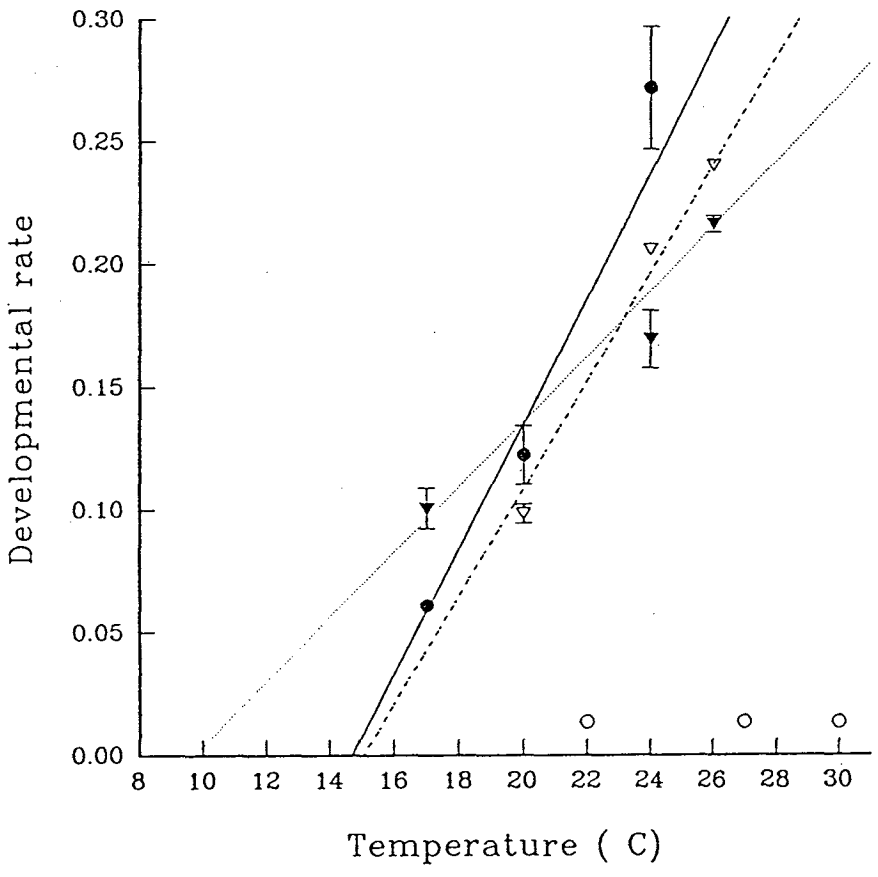


Figure 5:2. Developmental rate for three different populations of *M. demolitor* for prepupal to adult emergence from the cocoon. (Vertical bars are 95% confidence limits).

Mareeba = ▾.....▾

Toowoomba = ▽--▽

Grafton = ●——●

Diapausing parasitoids = ○

5.4 Discussion.

5.4.1 Laboratory/field results.

The significant decrease in the larval developmental time of *Microgaster demolitor* reared in laboratory cultures of *Heliocoverpa spp.* suggests that there is a genetic difference between this culture and the field strain. This is further supported by the differences in the variances between the two cultures. That is the smaller variance of the laboratory culture suggests that this population is more homogeneous than that of the field based culture. This is not surprising since the laboratory cultures have been reared for several years without any new input of genetic material, that is, field hosts into the breeding culture (Teakle pers comm). Since there is generally a bias in laboratory cultures for rearing hosts that develop quicker and have a less aggressive behaviour, and since they are not being exposed to parasitism, the possibility of producing hosts that have a reduced resistance to parasitoids does not seem unlikely. Thus, the results obtained from laboratory hosts should be viewed with caution when attempting to relate them to field conditions.

5.4.2 Developmental thresholds.

Due to the highly unbalanced design of the pupal developmental rate experiments, brought about by differential diapause occurrence in different parasitoid populations, any conclusions made from these results should be viewed with caution.

5.4.2.1 Larval developmental thresholds

Twine (1978) found that the larval threshold for *H. armigera* was 10.6 degrees with a thermal constant of 260 degree days while Zalucki *et al.* (1986) reported a value of 10 degrees for the developmental threshold of *H. punctigera.* Recent studies suggest that developmental thresholds for *H. punctigera* and *H. armigera* are similar (Daglish pers comm 1990). This similarity in the hosts thresholds is not unexpected, as coastal populations are often bolstered by large migrations of *H. punctigera* and *H. armigera* from inland Australia each year (Gregg *et al.* 1990), so that a flow of genetic material between populations is maintained.

In contrast, *M. demolitor* appears to have larval thermal constants and thresholds which vary between populations. This lack of similarity of developmental variables between the populations of the parasitoids suggests that gene flow between populations may not be occurring. That is, parasitoid

populations in different sites have adapted to suit the different type of environmental regimes they are exposed to. This suggests that *Microgaster demolitor* is unlikely to have the migratory characteristics that *Heliocoverpa spp* display, (which may result in the mixing of genes between populations), and in fact may be a quite sedentary organism. To confirm this, migrational and/or genetic studies of the parasitoid would need to be conducted.

Adaptive explanations for differences in thresholds between parasitoid populations are not immediately evident, since there does not appear to be a latitudinal pattern. That is, parasitoids from Mareeba, (which has a more tropical climate than Toowoomba), have a lower larval threshold than expected. This might be explained by differences in the times of year at which the parasitoids are active, but field work on the seasonal phenology of the parasitoids in these areas is needed to clarify this.

5.4.2.2 Pupal developmental thresholds

In contrast, the population differences between pupal developmental thresholds of the parasitoids do show an apparent latitudinal pattern, but in the reverse direction to those identified by Danks (1987); that is, the more tropical population, Mareeba, has the lowest pupal threshold. This again may be explained by differences in the seasonal activities of the different populations of the parasitoids.

Populations of parasitoids in Grafton and Toowoomba experience similar environmental conditions and both populations have high incidences of diapause (chapter 7). That is, during the colder winter months, when rainfall decreases (chapter 3), the majority of the parasitoid population may enter diapause, and no larval or pupal development occurs. Thus a low developmental threshold does not afford the parasitoid any developmental advantage. However, in Mareeba, *Heliocoverpa* spp are probably active all year (Titmarsh 1985), and as the incidence of diapause in this population of the parasitoid is considerably less than the southern areas (chapter 6), *M. demolitor* may produce substantial numbers of larvae which do not diapause during the winter months, and hence would require a lower developmental threshold. Jones *et al.* (1987) found similar results for *Eurema* spp, a genus of tropical pierid butterflies. *Eurema herla*, a species which spends the winter months in an adult diapause, had a higher developmental threshold than other *Eurema* species that produced larvae all year round.

5.4.3 Total developmental time.

Campbell *et al.* (1973) have suggested that parasitoids that possess higher thermal requirements than their hosts are afforded an advantage. That is, as the parasitoids develop slower than their hosts, they may then ensure the continued availability of a minimum host supply, and hence their own survival. This type of strategy does not appear to be functioning in the *Heliocoverpa* - *M. demolitor* relationship. All populations of the parasitoid studied had significantly shorter developmental periods compared to their hosts. *M. demolitor* only requires

approximately 2 instar levels of its host to complete development. This rapid development shown by the parasitoid may be related to the migratory tendencies of its two hosts.

Often, host plants of *H. punctigera* and *H. armigera* are only suitable for one generation of the host(Fitt 1989), with the next generation of adults migrating to another area (often only short distances for *H. armigera* but large distances for *H. punctigera.* In these circumstances, a parasitoid that develops slower than its host may emerge from their pupal cocoons to find no host larvae for oviposition and a departed adult population. In contrast, *M. demolitor* which develops faster than its hosts, may emerge when larvae are still present, allowing them to utilise any later developing hosts or to possibly follow adult hosts moving to nearby developing host crops.

To verify this, information of the migratory tendencies of *M. demolitor* are needed, as it is not known if it is undergoing any short or long distance migrational movements, however these data are suggestive that long distance movement is not occurring.

Chapter 6 Factors affecting diapause initiation.

6.1 Introduction

Debate over a definative definanition of diapause has continued ever since its conception. Depending on the bias of the author, that is if they are physiologically inclined or ecologicaly inclined, the definition varies accordingly. This area is complicated even further by some tropical entomologists attempting to redefine the definition so that tropical diapause will be seen as different when compared to temperate diapause. Danks (1987), reviews the definitions used, and diapause, in this study, will refer to a period of developmental dormancy shown by an insect which is determined by environmental factors which it experiences **before** it enters this state.

Because levels of insect activity in the tropics remain high throughout the year, it used to be thought that tropical insects, unlike their temperate counterparts, rarely used diapause to survive seasonally adverse conditions. A recent review by Denlinger (1986) demonstrates, however, that diapause does occur in many tropical insects.

The environmental cues which initiate diapause have been experimentally established for only a small number of tropical insect species. Cues such as

temperature, photoperiod, rainfall and nutrition have all been suggested as factors operating in the tropics to induce diapause (see Denlinger 1986 for a comprehensive review).

There may be many reasons why a particular time of year may be adverse for a tropical parasitoid, but they are unlikely to include temperatures too low for either the parasitoid or its host to survive. The most common source of adversity in the wet-dry tropics for species dependent on hosts using annual plants is likely to be a dry season. Many studies have shown that insect diversity and abundance in the seasonal tropics are closely tied to the wet season (Frith & Frith 1985, Janzen & Schoener 1968, Lowman 1982, Wolda 1978a, 1978b). It may be expected then, that tropical species may enter diapause in response to environmental cues which signal the onset of dry conditions. In regions where the timing of the wet and dry season is very consistent from year to year, temperature and photoperiod might still be reliable cues, but in tropical Australia, as in many other parts of the tropical world, the timing of the wet season is far from consistent (Danks 1987, Jones 1987). The obvious cue for an insect to use in these circumstances is rainfall, or some other factor correlated with it, such as humidity (Danks 1987). However, several authors believe that moisture levels alone, seldom, if ever, act to induce diapause (Danks 1986, Denlinger 1986, Masaki 1980).

There have been few controlled experimental demonstrations of diapause initiation in response to dry conditions, such as the increase in diapause in the

sawfly *Ertocampa ovata* with decreasing humidity (Mackay & Wellington 1977) and similarly in the moth, *Ephestia elutella* (Bell 1976), although several other studies have reported that the incidence of diapause in the field appeared to be correlated with low humidities or lack of rain, such as in the pierid butterfly, *Eurema laeta* (Jones *et al.* 1987). Because other environmental factors can not be controlled in the field, this latter study is suggestive rather than conclusive.

Diapause is often used by parasitoids to synchronise their life cycles with those of their host. Studies of temperate species have generally found that photoperiod and temperature affect the host and the parasitoid in similar fashions, so that the parasitoid enters diapause at the same time as its host (Fisher 1971). *H. punctigera* and *H. armigera*, which act as hosts for *M. demolitor*, enter a pupal diapause when temperatures decrease and photoperiods shorten (Fitt 1989, Zalucki *et al.* 1986). *H. punctigera* enters diapause in the greatest proportion when reared at 19°C and 12-12.5 hrs photoperiod (Browning 1979, 1981, Cullen & Browning 1978). In general, *Heliocoverpa* larvae reared at temperatures above 23 C with a photoperiod greater than 13 hours do not produce diapausing pupae (Murray pers comm 1990).

Parasitoids which synchronise their activity with the presence of *Heliocoverpa* larvae in Australia must, however, do more than match their hosts' diapause strategy. The two common hosts of the parasitoid *M. demolitor*, namely *H. punctigera* and *H. armigera*, are both opportunistic migrants and can disappear and re-appear for reasons other than entry into diapause. Moreover,

H. punctigera is intrinsically more mobile than *H. armigera* (Farrow & Daly 1987, Morton *et al.* 1981, Wardhaugh *et al.* 1980, Wilson 1983), so that the problem, (for the parasitoid), of predicting when hosts will be available to it depends in part on the species of host it will be attacking.

There is no published work on diapause induction in *M. demolitor*, but there are several references to the presence or absence of diapause in field populations. Kay (1982a,b) showed that *M. demolitor* in the Darling Downs area enter diapause in late April and early May, in synchrony with their local host *H. armigera*. However, Cobb (1983) found no "true" diapause in *M. demolitor* reared from *Heliocoverpa zea* and *Pseudoplusia includens*, from a population of parasitoids introduced into America in 1981, from Stanthorpe, Australia.

This study aimed to elucidate the factors which induce diapause in *M. demolitor* in Australia by examining the effect of the following on diapause incidence:

i) Photoperiod

ii) Temperature

iii) Humidity

iv) Host species (*H. punctigera* & *H. armigera*)

v) Geographic origin of the parasitoid

vi) Variation between the offspring of different parents and

vii) The sex of the parasitoid.

The reasons for studying the first four of these factors are implicit in the previous discussion. Geographic origin is included because it is unlikely that the parasitoids are as mobile as their hosts (see chapter 5) and it is possible that population differentiation with respect to diapause cues could have occurred in response to different climatic conditions. Examination of parental and sex affects aims to determine whether there is substantial genetic variability in this trait within populations and whether both sexes are equally likely to enter diapause in a given set of conditions.

6.2 Methods

The factors affecting diapause initiation were investigated by rearing juvenile parasitoids in different environmental conditions. It was not possible to use a full factorial design, as the number of parasitoids required was excessive. Instead, parasitoids from one location, (Toowoomba), were used to examine the effects of humidity, temperature, photoperiod and parent. Parasitoids from Toowoomba, Mareeba and Townsville were used to assess the effects of geographic origin, sex of the parasitoid and host species from which it was reared. All female parasitoids used for oviposition were kept at a constant temperature of 26°C and a photoperiod of 14L:10D.

Diapausing individuals were diagnosed by the presence of a ribbed cocoon (see chapter 7).

6.2.1 Humidity and Temperature

As humidity could not be controlled directly in the constant temperature cabinets, eight individual glass desiccators were set up within two cabinets, (photoperiod 14L:10D), one at 24°C and one at 30°C. Each of these eight containers held either saturated salt solutions or silica gel so that humidities within the containers could be manipulated. Humidities inside the containers were checked daily with a digital thermotester humidity reader (±5%).

One container at each temperature contained silica gel, resulting in a humidity of 5 ± 5%, one with a saturated solution of potassium carbonate (relative humidity of 46 ± 5%), a third with sodium chloride (relative humidity of 75 ± 5%) and a final with sodium carbonate (relative humidity of 90 ± 5%). Ninety-six *H. punctigera* larvae, parasitised by eight Toowoomba *M. demolitor* (each wasp parasitising twelve larvae), were divided into groups of twelve. Individual hosts from the group were then each placed into a cell of a 12 cell *Limbro*® tissue culture plate which contained a quantity of artificial diet. Each plate was covered with fine cloth gauze to enhance air movement and placed within one of the controlled humidity containers. Due to the mortality of several hosts, the resulting number of parasitoids was less than the original number of parasitised hosts. The effect of humidity and temperature on diapause initiation was analysed via an unweighted logistic regression. The relationship between humidity and the proportion of parasitoids entering diapause was

estimated by linear regression analysis. An exponential transformation, ln(y+1), was applied to the proportion of parasitoids entering diapause to linearize the relationship.

6.2.2 Photoperiod

Photoperiod effects on diapause initiation were examined for Toowoomba parasitoids reared from *H. punctigera* at 24°C in constant temperature cabinets. Light was supplied by five 30cm long fluorescent tubes. Humidity was recorded continuously using a paper chart thermohygrograph for the duration of the experiment and ranged from 34% to 53%. Twenty four parasitised hosts were placed at each of three photoperiods; 14L:10D, 12L:12D and 10L:14D, however mortality of the hosts reduced these figures.

Three female parasitoids were used for parasitisation of the hosts with the parasitised hosts then being randomly placed at one of the three photoperiods. Each adult female parasitoid produced twenty four of the seventy two parasitised hosts used. The data was then analysed using a Chi squared contingency table with diapause and photoperiod as catagories.

6.2.3 Location, sex and host effects

Differences in diapause initiation between three populations of *M. demolitor*, (Toowoomba, Townsville and Mareeba), from both *H. punctigera* and *H. armigera* reared at 26°C, and a photoperiod of 14L:10D were investigated. Relative humidity was monitored with a thermohygrograph and ranged from 38% to 58%.

A total of three female parasitoids from the Mareeba population, four from the Townsville population and twenty three from the Toowoomba population were used to parasitise both host species. All females had been allowed to mate with males from their own location. All parasitoids were sexed upon emergence from their cocoons.

To allow interaction effects between the variables within a location to be considered the data were analysed using a series of separate Chi squared homogeneity tests rather than using a logistic regression.

6.2.4 Parent and Host effects

The female Toowoomba parasitoids and their offspring used in the geographic analysis above were used to determine whether the female parent and/or the host the offspring was reared in affected the proportion of offspring entering diapause.

As no female parasitoids were allowed to oviposit in just *H. punctigera* alone or *H. armigera* alone, parental and host effects were analysed together via a two way analysis of variance without replication. As the data were entered as proportions of offspring entering diapause, an arcsin square root transformation on the proportion of parasitoids entering diapause was performed.

6.3 Results

6.3.1 Humidity and Temperature

The unweighted logistic regression of temperature and humidity against proportion of parasitoids entering diapause revealed that the change in deviance when temperature was removed from the model was not significant but that removal of humidity from the model had a significant effect (table 6.1).

Table 6.1: Summary of unweighted logistic regression for humidity and temperature against proportion of parasitoids from Toowoomba reared in *H. punctigera* entering diapause.

Model	Deviance	Change in Deviance	Df.	P value
Constant	31.110	29.485	1	**<0.001**
Constant + Temp	30.75	0.36	1	0.548
Constant + Hum	1.625	0.104	1	0.747
Constant + Hum + Temp	1.521			

Hum = Humidity
Temp = Temperature

That is, temperature difference did not affect the frequency with which parasitoids entered diapause, but variations in humidity did. The relationship between humidity and proportion of parasitoids entering diapause, (figure 6.1), was estimated as the following relationship;

$$Ln(y+1) = 0.575 - 0.0059x$$

where y = Proportion in diapause and

x = relative humidity (%)

($r^2=0.97$, $p<0.001$, $n=90$)

6.3.2 Photoperiod

No significant effect of photoperiod on the frequency of parasitoids entering diapause was found ($\chi^2=0.40$, $df=2$, $p=0.82$; table 6.2).

Table 6.2: Proportion of *M. demolitor*, (from Toowoomba), entering diapause under different photoperiods.

Photoperiod	Diapause No	Diapause Yes	Proportion in Diapause
10L:14D	9	14	0.39
12L:12D	7	16	0.30
14L:10D	8	16	0.33

6.3.3 Sex and location effects

Among the three populations of *M. demolitor*, males and females were equally likely to enter diapause if reared in *H. armigera* (figure 6.3); (0.18 for males, 0.22 for females, $\chi^2=0.72$, $p=0.39$, $n=324$ for Toowoomba; 0.18 for males, 0.15 for females, $\chi^2=0.07$, $p=0.79$, $n=51$ for Townsville and no animals entered diapause in Mareeba $n=36$). The difference between locations was significant ($\chi^2=7.31$, $p=0.03$, $n=484$), that is, the proportion of parasitoids entering diapause increased with distance from the equator.

For parasitoids reared from *H. punctigera*, (figure 6.2), sex had no significant effect on the proportion of parasitoids entering diapause from the Townsville population (0.17 for males, 0.15 for females, $\chi^2=0.05$, $p=0.82$, $n=56$) or in the Mareeba population (0.11 for males, 0.10 for females, $\chi^2=0.01$, $p=0.937$, $n=19$) but females from Toowoomba entered diapause in greater proportions than males (0.19 for males, 0.43 for females, $\chi^2=23.44$, $p<0.001$, $n=343$). The difference between location for females was significant ($\chi^2=6.45$, $p=0.04$, $n=214$), that is, the proportion of diapausing individuals increased with increasing distance south. A similar trend occurred in the males but this was not significant ($\chi^2=0.27$, $p=0.88$, $n=308$).

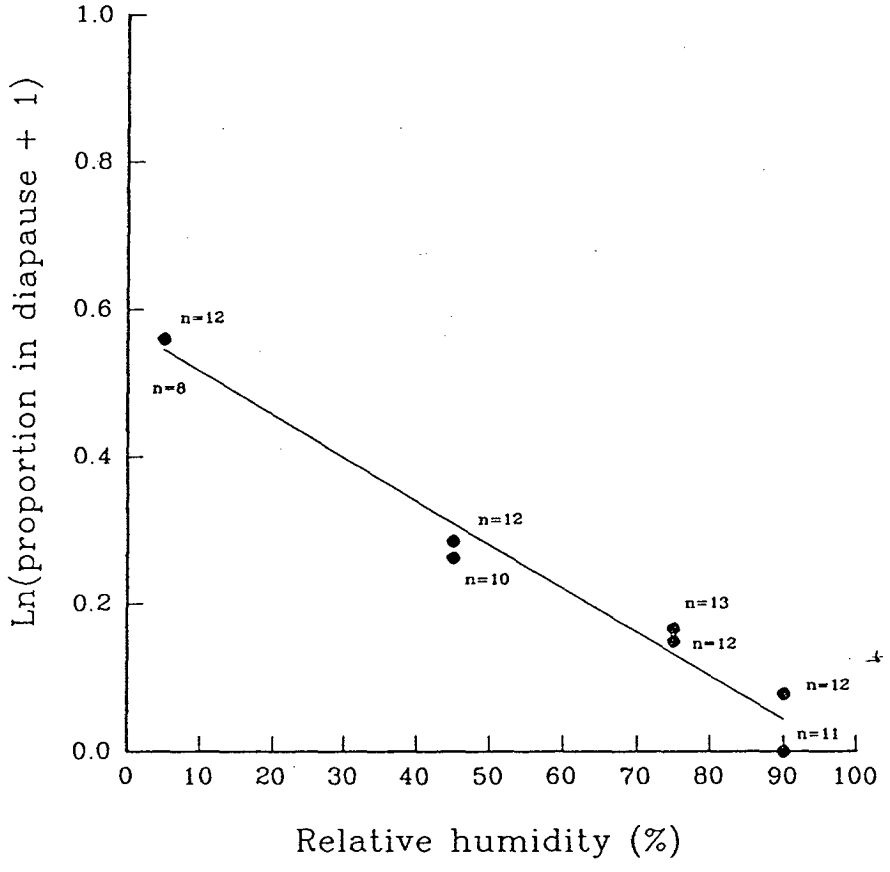


Figure 6:1. The effect of humidity on the incidence of diapause in *M. demolitor*, (from Toowoomba), reared in *H. punctigera* at 14L:10D.

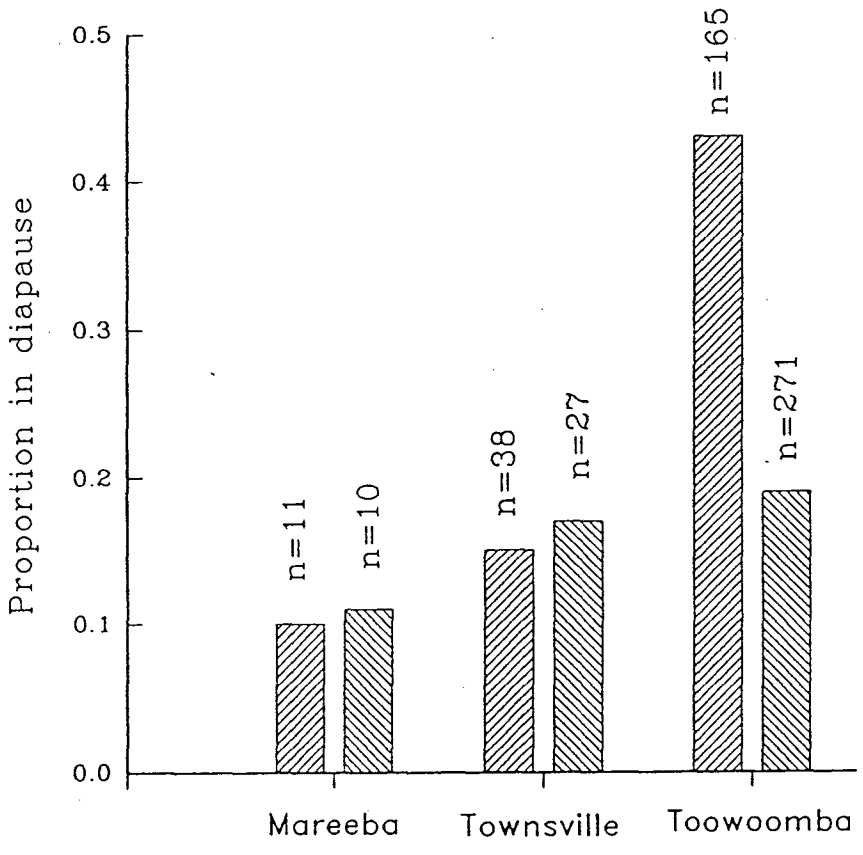


Figure 6:2. The proportion of *M. demolitor* entering diapause, reared in *H. punctigera*, for both males and females, for three separate locations.

Males = ▨

Females = ▨

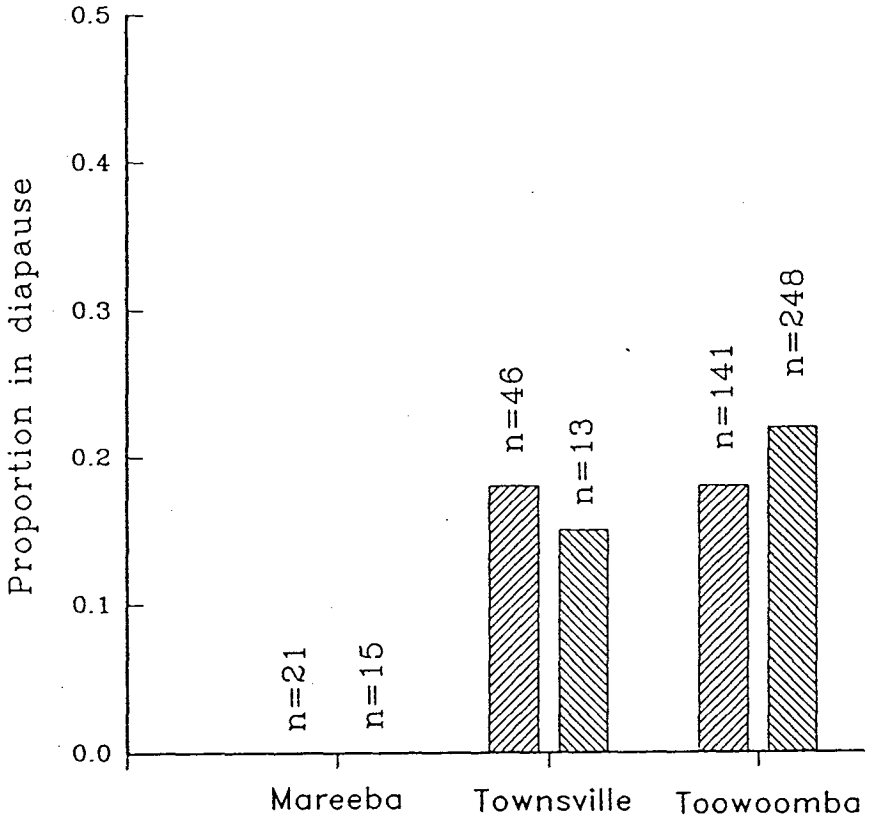


Figure 6:3. The proportion of *M. demolitor* entering diapause, reared in *H. armigera*, for both males and females, for three separate locations.

Males = ▨

Females = ▨

6.3.4 Host effects

The species of host in which the parasitoid was reared did not affect the proportion of prepupae entering diapause for Townsville (χ^2=0.003, p=0.96, n=107) or for males from Toowoomba (χ^2=0.003, p=0.95, n=437). However, for the Mareeba population, parasitoids reared in *H. punctigera* entered diapause in greater proportions than those reared in *H. armigera* (χ^2=3.93, p=0.04, n=55). Female parasitoids from Toowoomba also entered diapause in greater proportions when reared in *H. punctigera* (χ^2=11.32, p=0.001, n=230).

6.3.5 Toowoomba Parental effects.

The effect of parent on the proportion of parasitoids entering diapause for the Toowoomba *M. demolitor* population at 26°C was not significant (F=2.06, df=22,22, p=0.08).

6.4 Discussion.

In temperate insects, photoperiod and temperature are the major cues for diapause initiation (Fisher 1971, Tauber & Tauber 1976, Danks 1987). These factors do not appear to play a major role in the initiation of diapause in *M. demolitor*; it is ambient humidity which is the major factor determining the development strategy of this parasitoid; that is, low humidities result in a higher incidence of diapause.

As all the adult parasitoids were kept at similar temperatures and humidities, and as it was only the parasitised hosts that were exposed to varying humidities, the increased weight of a diapausing individual at emergence from the host (see chapter 7), suggests that the decision to enter diapause is made while the parasitoid is within the host. That is, the sensitive stage for diapause initiation in *M. demolitor* is the larval stage. Thus the developing larvae of the parasitoid must have some means of determining the external humidity of the environment from within the confines of its host. The parasitoid may be monitoring physiological changes in the host caused by external humidity changes or changes in the moisture content of the food the host ingests. It may also be able to determine the humidity via sensory organs or be monitoring the internal physiology of the host which may change with changes in humidity. In this study, however, it cannot be determined if humidity has a direct or indirect effect.

Mortality rates of eggs and first instar larva of *Heliocoverpa* spp. in Mareeba increased with increasing wind speed and temperature (Titmarsh 1985). This suggests that desiccating conditions, although probably not producing diapause in *Heliocoverpa*, may, nonetheless, greatly reduce the availability of hosts for the parasitoid. Consequently, the fact that *M. demolitor* enters diapause when humidities fall may allow it to avoid the possible decrease in numbers of its host brought about by higher mortality rates in drier conditions.

Heliocoverpa spp. in the Toowoomba district enter diapause during March to May (Kay 1982a) with a greater proportion entering each month (D. Murray pers comm 1990). The relative humidity at this location, varies only slightly throughout the year, see chapter 3. However these values represent measurements taken from meteorological weather stations and are unlikely to reflect the true range of humidities experienced by insects on plant surfaces. Variations in microhumidity around the developing larval parasitoids may be better monitored by variations in rainfall: the combination of wet plant surfaces, high frequencies of stomatal openings as plant water balance improves and lower wind effects close to the plant surface, all contribute to higher humidities in the insects microhabitat after rain. Rainfall in Toowoomba decreases during April/May to a low in August and rises steadily to a high in January (see chapter 3). Thus using the criteria established above, it would be expected that at Toowoomba, *M. demolitor* would begin to enter diapause in high numbers in April/May. This agrees with the field results found by Kay (1982b) for diapause initiation in *M. demolitor*. The use of a humidity cue may therefore allow the

parasitoid to enter diapause at approximately the same time as its hosts, as well as allowing it to escape harsh environmental effects which cause population crashes in its host.

The decrease in the proportion of diapause in *M. demolitor* with decreasing latitude corresponds with a similar distribution of diapause frequencies in its hosts. In Northern Australia the percentage of *H. punctigera* entering diapause is significantly lower than that in southern regions (Zalucki *et al.* 1986) and in *H. armigera* a similar pattern can be seen (Fitt 1989, Kay 1982a). Similar patterns have been identified in other insect species (Danks 1987).

In northern regions such as Mareeba where *H. armigera* may be present all year round (Broadley 1977, Currie *et al.* 1982), those wasps that do not enter diapause have the opportunity to exploit the corresponding non-diapausing hosts. The increasing incidence of diapause seen in the parasitoid with increasing latitude may be an adaptive response to a corresponding increase in diapause in the two hosts.

Of the two main species of *Heliocoverpa* in Australia, *H. punctigera* is by far the more mobile (Farrow & Daly 1987, Fitt 1989, Zalucki *et al.* 1986, Gregg *et al.* 1989), however *H. armigera* is a facultative migrant, while *H. punctigera* is probably an obligate migrant (Zalucki *et al.* 1986). Many host crops of *Heliocoverpa* are only suitable for one generation (Fitt 1989), resulting in a forced migration for the moths when they emerge. It is therefore possible for

M. demolitor developing without diapause to emerge from their cocoons to find no new *Heliocoverpa* spp. larvae present and a departing population of adult hosts. A strategy of entering diapause, even under ideal environmental conditions, may help to alleviate the problem of unpredictable host numbers, brought about by a highly migratory host. This is in contrast to many temperate insect species, which may undergo several generations without any incidence of diapause.

Unlike a majority of temperate insect species, it appears that total diapause initiation in *M. demolitor* offspring does not occur. That is, regardless of how low the humidity may fall, there are always some offspring which develop normally. This type of strategy is possibly linked to the distribution of its hosts. For example, in south coast of Queensland both species of the host are present, albeit in small numbers, all year round (Gregg *et al.* 1989, Zalucki pers comm 1990). In harsh conditions the population numbers of the hosts decrease due to diapause or migration but do not disappear entirely. Thus if small numbers of *M. demolitor* do not enter diapause, they will be able to exploit these small numbers of hosts.

Differences between the incidence of diapause in *M. demolitor* reared in the two species of *Heliocoverpa* may also be related to differences in the host's migratory strategies. *H. armigera* often only migrates small distances to neighbouring crops while *H. punctigera* may migrate to a different geographic region. Thus, depending on the parasitoid's movement capabilities, it may be an

advantage to enter diapause in higher proportions when they develop in *H. punctigera*. That is, if the parasitoid is only able to move small distances, it may be able to follow migrating populations of *H. armigera*, and hence would not need a large proportion of offspring entering diapause. However, the parasitoid may be unable to travel the large distances that *H. punctigera* often undertakes. A strategy of placing higher proportions of offspring in diapause when reared in *H. punctigera* may guard against an unsuccessful migratory attempt by the wasp.

This then offers an adaptive explanation for the higher incidence of diapause in *M. demolitor* reared in *H. punctigera*, but without field knowledge of the parasitoid's migratory capabilities, it is at best, speculative.

The sex difference noted in parasitoids entering diapause from *H. punctigera* may be linked to the quiescence which adult *M. demolitor* enter at low temperatures (see chapter 3). At temperatures below 20°C, the parasitoid's longevity increases dramatically from one to two weeks to two to three months. Therefore a male which stays out of diapause has a chance to survive the winter via a quiescence phase and can be present as soon as females emerge from diapause. The risk associated with this option is that if temperatures rise above 20°C for a extended period the longevity can reasonably be expected to decrease to only 2 - 3 weeks. If the reproductive advantage is significant, this risk may none the less be worthwhile taking. There is no comparable reproductive advantage for females, since they can produce offspring whether or not they encounter a male on emergence (see chapter 3).

In conclusion, *M. demolitor* uses different cues than its hosts to enter diapause. Rather than using temperature and photoperiod, the parasitoid is directly or indirectly, sensing changes in environmental humidity. The difference between these two strategies may be due to the unpredictability imposed by the migratory habits of the parasitoid's hosts. Consequently, the parasitoid is engaging in a bet hedging strategy; firstly they commit some of their offspring into diapause at all times of the year, even when conditions are good, ensuring that there will be offspring capable of exploiting next seasons hosts if the local host migrates from the area. Secondly, they do not commit all of their offspring to a diapause strategy, even when conditions are harsh and the host will almost certainly migrate out of the local area, as in some parts of Australia, *Heliocoverpa* spp is present all year round.

Chapter 7 Diapause effects on prepupal weight, cocoon weight and cocoon structure.

7.1 Introduction

Insects in which development has been modified, due to processes such as diapause induction, often differ from those which develop continuously. Perhaps one of the most consistent features of diapausing insects is their increase in body weight (Bennett & Thomas 1964, Madubunyi & Koehler 1977, Wallace 1970, Danks 1987), in comparison to non-diapausing individuals. This increase in size often reflects an increase in the lipids which enable the insects to survive its dormancy period (Danks 1987, Madubunyi & Koehler 1977, Bennett & Thomas 1964, Begon 1976, Downer and Matthews 1976, Harper and Hynes 1970), however other substances may also significantly increase, such as glycogen (Hodek & Cerkasov in Danks 1986). Not all diapausing insects though, are larger than their normal developing counterparts; the field cricket *Teleogryllus commodus*, when diapausing, is smaller and more compact than a normally developing embryo (Hogan 1960).

Another characteristic of many species which diapause either as larvae within cocoons or as pupae, is a more robust cuticle and/or a thicker coating of wax or wax like substances on cocoons or pupal shells in comparison to those that undergo normal development. This increased covering appears to provide additional protection by altering the permeability of the outer covering and hence

decreasing desiccation during these dormant periods in often harsh conditions (Danks 1986). For example, diapausing pupae of *H. armigera*, have a thicker pupal shell than non diapausing individuals, and lose water at a slower rate than normally developing pupae (Roome 1979).

Many diapausing insects have different cocoons compared to other individuals of the same species which undergo normal development. In some overwintering chironomids, cocoons are firmer and are more tightly applied to the body of the animal, compared to cocoons of non-diapausing individuals, presumably in an attempt to minimise damage by ice in frozen habitats (Danks 1971). However, aestivating chironomids that withstand desiccation in temporary habitats build similar cocoons (Hinton 1960).

Distinct differences between the cocoons of diapausing and non diapausing braconids have also been found. Schlinger and Hall (1960) found that the cocoon of diapausing *Praon palitons* was different in colour and thicker than that of the non diapausing individuals. Similarly, the braconid *Trioxys utilis*, which diapauses within its host aphid, has a tougher and thicker cocoon when diapausing (Schlinger & Hall 1961).

This chapter aims to determine the effects of diapause, sex of the parasitoid, and the species of host in which the parasitoid, *M. demolitor*, was reared, on prepupal and cocoon weights. It also highlights differences between the morphology of cocoons of diapausing and non-diapausing parasitoids.

7.2 Methods

7.2.1 Prepupal and cocoon weights

The weights of diapausing (n=100), and non-diapausing (n=333), prepupae were determined by weighing each prepupa within its cocoon on a Cahn microbalance (± 0.005mg) within 24hrs of emergence from the host. The cocoon was then weighed separately when the parasitoid emerged and the prepupal weight was calculated by subtracting the cocoon weight from the combined cocoon and prepupal weight. Before weighing, any extraneous material contained within the cocoon was removed. Both *H. punctigera* and *H. armigera* were used as hosts for parasitoid larvae, parasitised at the 2nd instar stage and reared on artificial diet (appendix 2) at 26°C, 14L:10D photoperiod within a humidity range of 38 to 58%. The adult parasitoids were sexed, by the presence or absence of an ovipositor, when they had emerged from their cocoon. All offspring originated from parasitoids collected from Toowoomba, in southern Queensland.

Data were analysed with fixed factor analyses of variance and covariance, using the General Linear Models option in SAS to allow for unequal sample sizes of each combination of variables.

7.2.2 Structural differences between cocoon types

Structural differences between the cocoons of diapausing and non-diapausing parasitoids were examined using a dissecting stereo microscope and a scanning electron microscope. Cocoons were prepared for electron microscopy by first drying in a desiccating container containing silica gel for 1 week; they were then gold sputter coated. Coated specimens (five diapausing and five non diapausing) were then examined using an ETEC Autoscan scanning electron microscope.

7.3 Results

7.3.1 Cocoon and prepupal weights

Analysis of the effects of sex, host and diapause on cocoon weight, with prepupal weight as a covariate, showed that all three variables had a significant effect (table 7.1) and that there was a weak correlation between prepupal weight and cocoon weight. There were no interaction effects.

Because all three variables had a significant effect, cocoon weights were adjusted for each individual variable in figures 7.1 to 7.3. For example, when plotting cocoon weights for different hosts, the weights were adjusted for sex effects and for diapause effects. These adjustment values were calculated by determining the mean cocoon weight for each level within each variable, for

example, males and females within sex, and then calculating a joint mean for the variable. The difference between the joint mean and the mean for each level was then used as the adjustment factor. Table 7.2 contains the values used for adjusting the cocoon weights for different variables.

Parasitoids reared in *H. punctigera* had cocoons which were heavier than those from *H. armigera* (1.25 mg, s.e.=0.02 from *H. punctigera* and 1.09 mg, s.e.=0.01 from *H. armigera*; (figure 7.1). Male parasitoids also produced lighter cocoons than females (1.13 mg, s.e.=0.01 for males and 1.21 mg, s.e.=0.01 for females; figure 7.2). Cocoons from diapausing parasitoids were significantly heavier than those from non-diapausing parasitoids (1.40 mg, s.e.=0.02 for diapausing and 0.95 mg, s.e.=0.01 for non-diapausing; figure 7.3).

Table 7.1: Summary of the results of analysis of covariance of the effects of sex, host species and diapause state on cocoon weight with prepupal weight as a covariate.

Source	DF	Mean Square	F value	p
Host	1	1.43	27.18	**0.0001**
Sex	1	0.29	5.54	**0.02**
Diapause	1	9.92	188.21	**0.0001**
Prepupal weight	1	0.34	6.46	**0.01**
Host*sex	1	0.03	0.61	0.44
Host*diapause	1	0.002	0.05	0.83
Sex*diapause	1	0.09	1.80	0.18
Sex*diapause*host	1	0.01	0.12	0.68
Error	424	0.05		

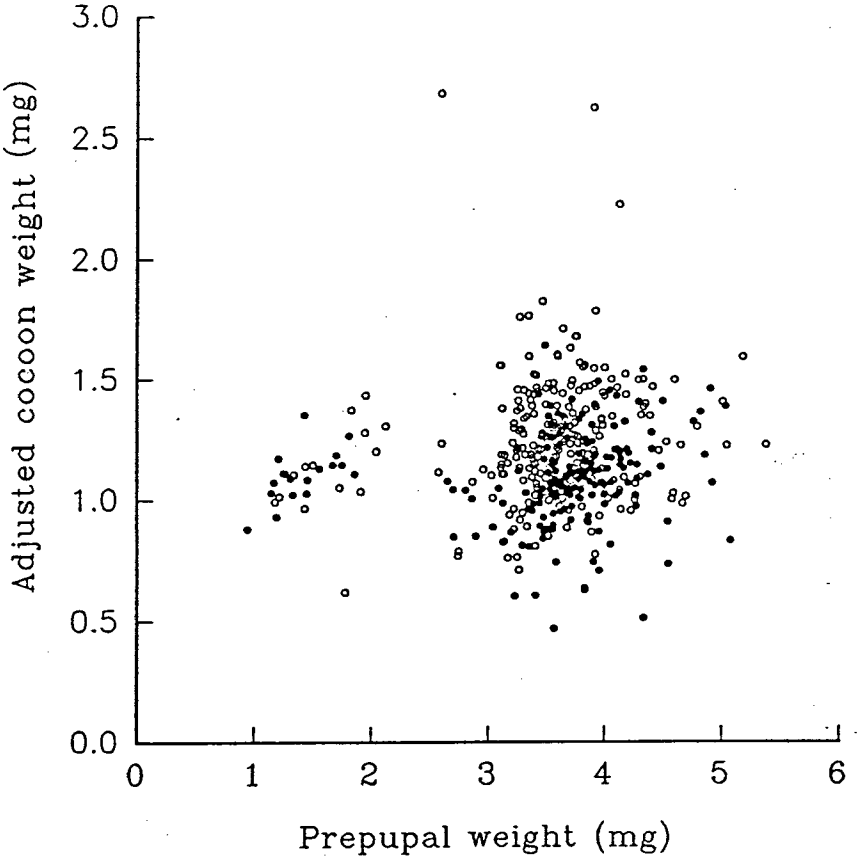


Figure 7:1. Plot of cocoon weight of *M. demolitor*, adjusted for sex and diapause state, against prepupal weight of the parasitoid, reared in two different host species.

H. punctigera = ○

H. armigera = ●

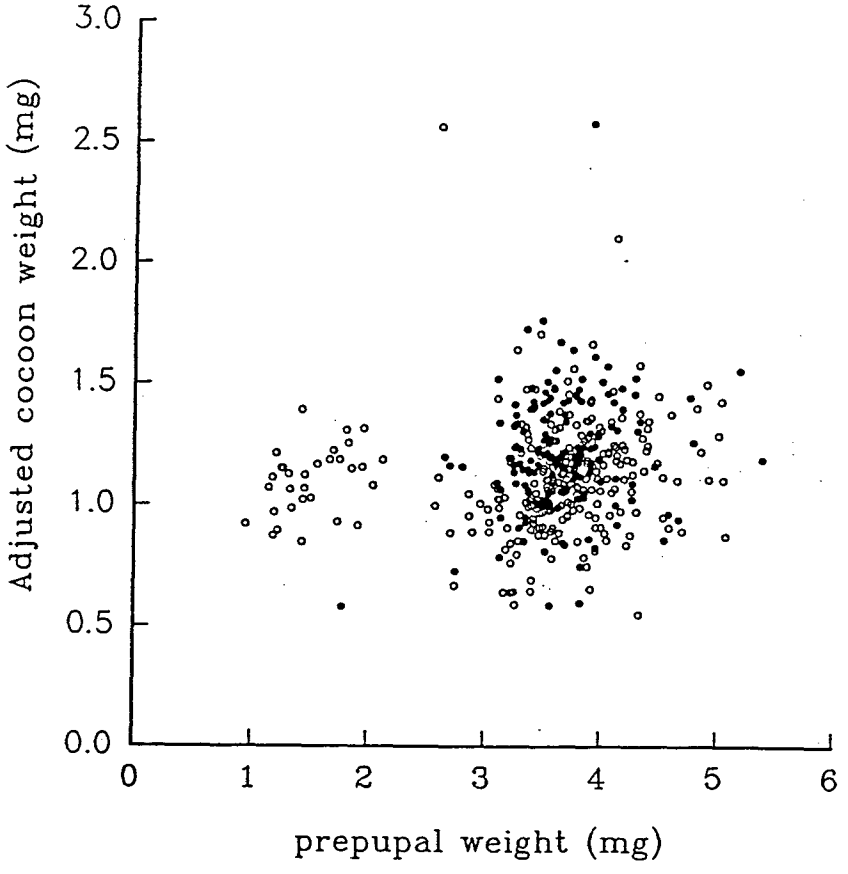


Figure 7:2. Plot of cocoon weight of *M. demolitor*, adjusted for host species reared in and diapause state, against prepupal weight, for both male and female parasitoids.

Female = ●

Male = o

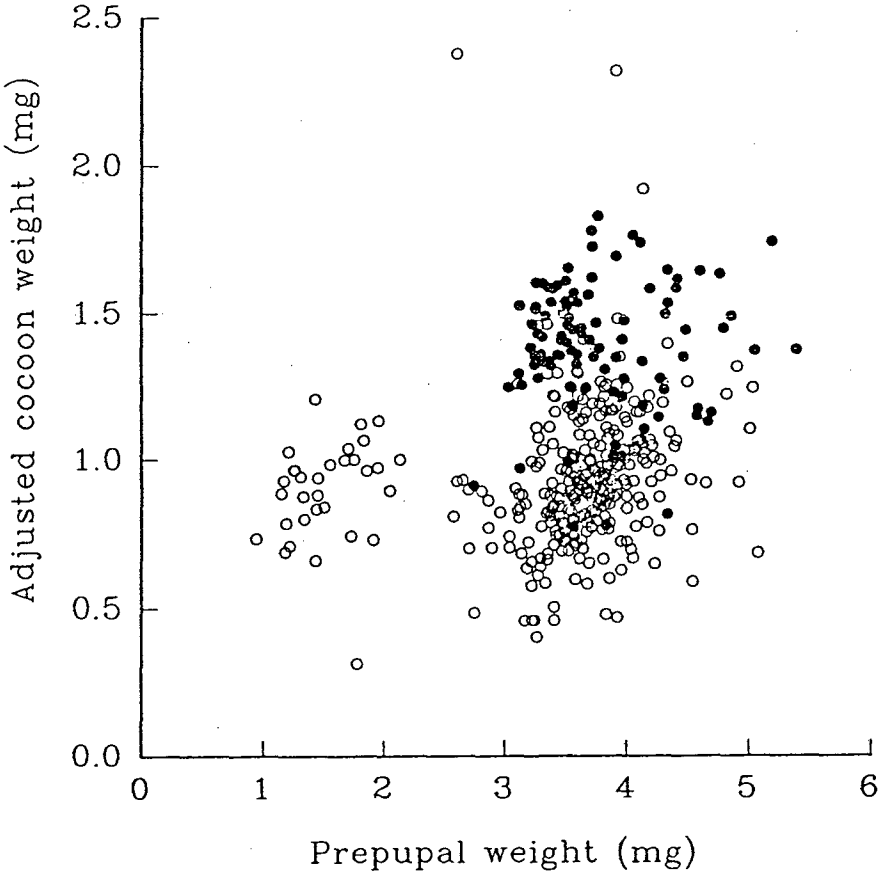


Figure 7:3. Plot of cocoon weight of *M. demolitor*, adjusted for host species reared in and sex of the parasitoid, against prepupal weight, for diapausing and non-diapausing individuals.

Diapausing = ●

Non-Diapausing = ○

Table 7.2 Summary of values used for adjustment of cocoon weight for different variables.

Variable	Mean cocoon weight	Overall mean cocoon weight	Adjustment factor
Female	1.21	1.17	-0.04
Male	1.13	1.17	+0.04
Diapause	1.40	1.17	-0.22
Normal	0.95	1.17	+0.22
H. punctigera	1.23	1.17	-0.08
H. armigera	1.09	1.17	+0.08

7.3.2 Parasitoid prepupal weight

Analysis of the effects of sex, host and diapause condition on prepupal weight of the parasitoid showed that diapause affected prepupal weight, and there was an interaction between diapause state and sex of the parasitoid. Sex of the parasitoid alone and host species it was reared from had no significant affect on prepupal weight (table 7.3). Diapausing male parasitoids were significantly heavier than their non-diapausing counterparts (3.9mg, s.e.=0.04 for diapausing; 3.4mg, s.e.=0.05 for non-diapausing). Similarly, female diapausing prepupae where also heavier than non-diapausing female prepupae (3.7mg, s.e.=0.07 for diapausing; 3.6mg, s.e.=0.08 for non-diapausing). The sex by diapause interaction on prepupal weight revealed that diapausing male parasitoids were heavier than diapausing females but among non-diapausing individuals the female parasitoids were heavier (figure 7.4).

Table 7.3: Summary of the effects of sex, host species and diapause state on prepupal weight.

Source	DF	Mean Square	F value	p
Host	1	0.67	1.37	0.24
Sex	1	0.01	0.001	0.95
Diapause	1	5.85	11.92	**0.001**
Host*sex	1	0.03	0.06	0.81
Host*diapause	1	0.26	0.53	0.47
Sex*diapause	1	2.44	4.97	**0.03**
Sex*diapause*host	1	0.07	0.15	0.70
Error	425	0.49		

7.3.3 Cocoon structure

Examination of the structure of cocoons from diapausing and non-diapausing parasitoids by scanning electron microscope detected a distinct difference. Diapausing parasitoids had cocoons which were conspicuously ribbed and appeared to have a much tighter weave than those on non-diapausing pupae (plate 3a). Cocoons of non-diapausing pupae tended to have a hairy appearance and an open weave without ribbing (plate 3b).

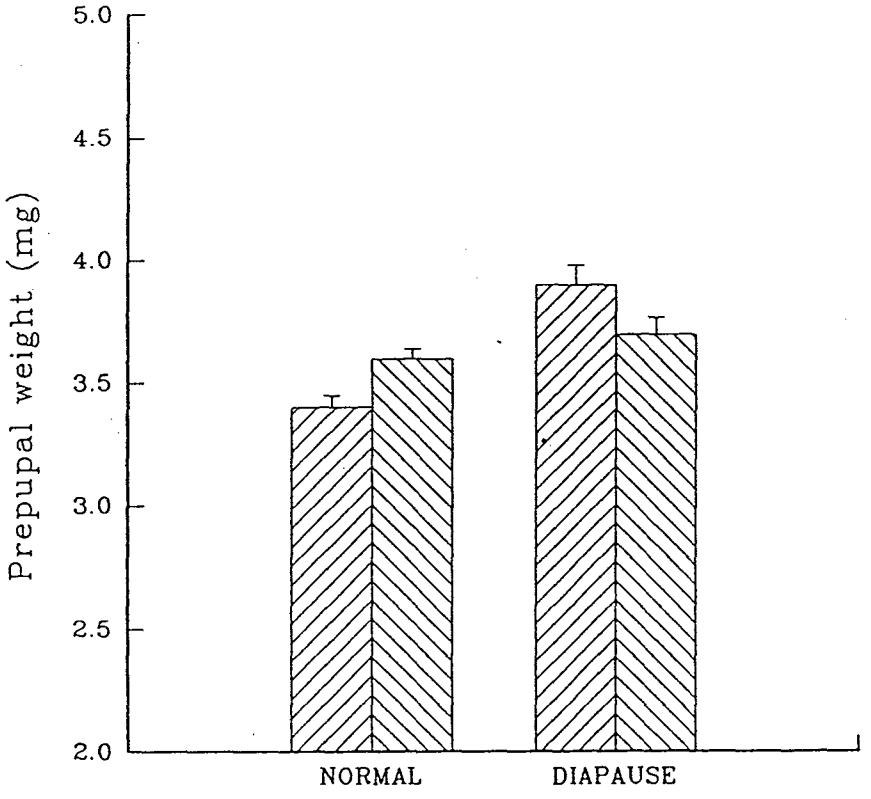


Figure 7:4. Graphic representation of the interaction between sex and diapause state on prepupal weight of the parasitoid. (Vertical bars are 95% confidence limits).

Males = ▨

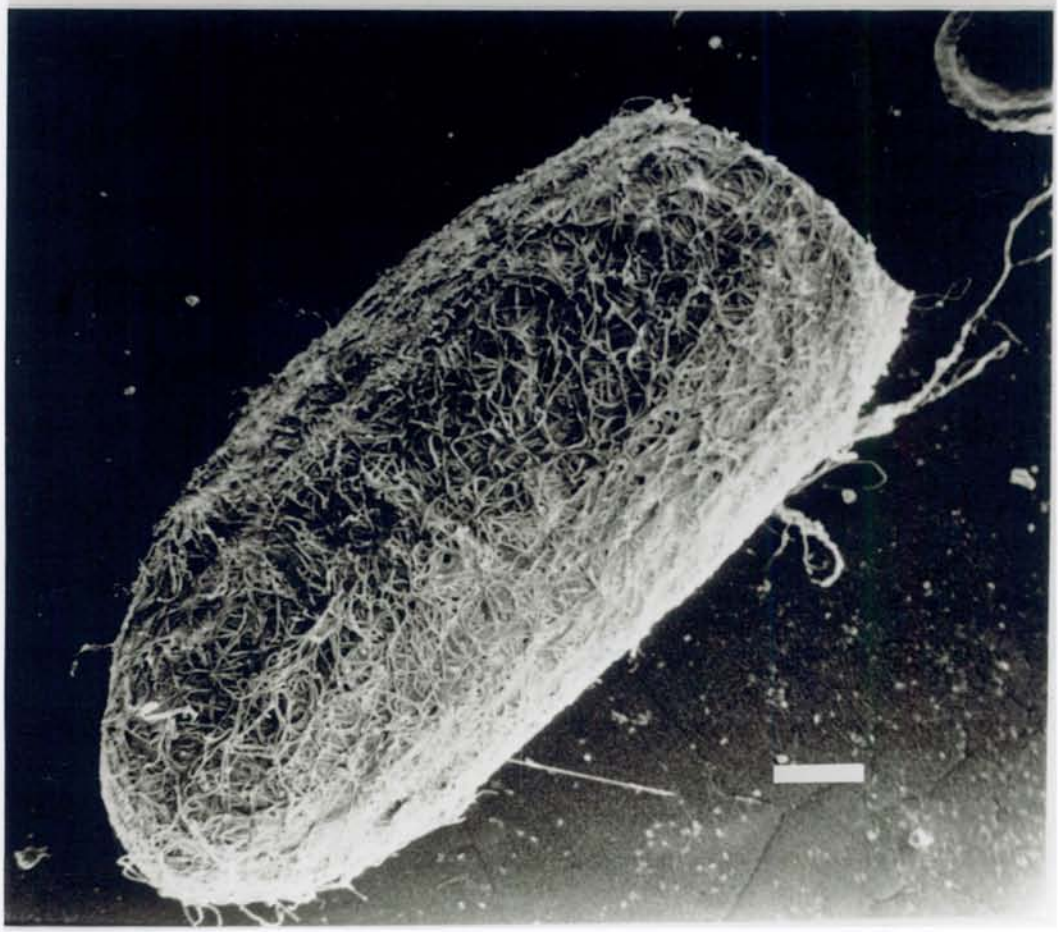
Females = ▨

Plate 3a. Electron micrograph of the cocoon of a diapausing prepupa of *M. demolitor.* Scale bar is 0.5mm long.

Plate 3b. Electron micrograph of the cocoon of a non diapausing prepupa of *M. demolitor.* Scale bar is 0.5mm long.

a

b



7.4 Discussion.

Diapausing prepupae of *Microgaster demolitor* may be dormant for more than 200 days (see chapter 8), compared to normal developmental times of approximately 4 days (chapter 5). The increase in weight shown by the diapausing prepupae of *M. demolitor* may reflect a build up of fat bodies, as it does in many other species (Danks 1987, Madubunyi & Koehler 1977, Bennett & Thomas 1964).

The increased cocoon weights of diapausing individuals are possibly due to the difference in the structure. The ribbing and closer weave of the cocoons of diapausing parasitoids may indicate a denser structure. This may help to reduce desiccation and maintain a humid environment around the diapausing parasitoid.

The heavier cocoons produced by parasitoids reared in *H. punctigera* might relate to the distribution of *Heliocoverpa spp.* in Australia. Zalucki *et al.* (1986) that *H. punctigera* is ubiquitous in Australia, even in areas of very low and unpredictable rainfall. Fitt *et al* (1990) expanded this, suggesting that *H. punctigera* is more abundant in sub tropical and temperate cropping areas. *H. armigera* was thought to be restricted to coastal regions, extending approximately 100 miles inland (Zalucki *et al.* 1986). However, Fitt (1990), found *H. armigera* larvae as far west as the South Australian border, although *H. punctigera* larvae were by far the most dominant, comprising approximately 98% of the total sample (Gregg *et al.* 1990). In general, the highest concentrations of *H. armigera*

are predominantly in the wetter east coast regions, however it is wide spread through out inland Australia, albeit in low densities. In contrast, it appears that *H. punctigera* occurs more frequently in unpredictable and harsh climatic areas, such as inland Australia, even though it is common along the east coast.

Consequently, a parasitoid emerging from *H. punctigera*, is more likely to encounter harsher and more desiccating conditions, requiring a heavier cocoon, than one emerging from *H. armigera*. It may be, therefore, that *M. demolitor* uses its host species as signalling a high probability of adverse condition. (This interpretation is supported by the observation that parasitoids reared from *H. punctigera* are more likely to enter diapause than those reared from *H. armigera* (chapter 6)). This effect may be compounded in inland Australia, where *H. punctigera*, the dominant host, breeds predominantly during winter (Fitt pers. comm. 1991), which is often cold and dry. At this time, average monthly temperatures seldom rise above 20°C (Bureau of Meteorology 1988), which enforces a very slow development rate in the parasitoid. Desiccation over the whole developmental period may be greater if the developmental period is excessively prolonged and possibly the increase in the cocoon weight of the parasitoid is an attempt to decrease water loss.

As males have significantly lighter cocoons than females, they may be more sensitive to environmental changes then females. That is, if males have lighter cocoons, which are thinner and possibly more porous than the females' cocoons, they may not require as great a change in environmental parameters to initiate

development of the diapausing prepupae; certainly males develop more quickly than females in the pupal stage (chapter 5). A greater sensitivity to emergence cues would then allow the males to emerge before the females and hence to increase their chance of reproductive success. A disadvantage to the males under these conditions is that, as diapausing prepupae, they are not as well buffered against the environment and may desiccate more quickly than the females.

In summary, individuals of *M. demolitor* which entered diapause, had heavier prepupae and cocoons than those which developed normally. Female parasitoids produced heavier cocoons than males, whether they entered diapause or not. Diapausing individuals also had a distinctly different cocoon structure. Parasitoids reared in *H. punctigera* had heavier cocoons than those reared in *H. armigera*, possibly reflecting the difference in environmental regimes occupied by the host, that is, *H. armigera* is found predominantly in wetter coastal areas while *H. punctigera* is often found in drier, harsher environments, such as inland Australia.

Chapter 8 Factors affecting diapause duration.

8.1 Introduction

Changes in temperature and/or photoperiod are responsible for diapause termination in many temperate arthropods. Consequently many experiments examining diapause duration have varied photoperiod at constant temperatures, or varied temperatures at constant photoperiods to isolate diapause termination factors (Danks 1987). Only a few cases suggest that moisture directly controls diapause development in arthropods (Danks 1987); for example the orthopteran, *Melanopus differentialis* (Slifer 1946). Of these few cases, even fewer have been substantiated by experimental evidence (Tauber & Tauber 1976). They have, however, shown that, although not necessary to terminate diapause, water is essential in the post diapause developmental phase of some arthropods (Danks 1987, Tauber & Tauber 1976). For example, the European corn borer has a two phase diapause process, whereby changes in photoperiod terminate diapause but water is required to activate the neuroendocrine system to allow post diapause development to be completed (Beck 1967). In some species of braconids, such as *Pholetesor ornigis* and *P. pedias*, postdiapause development is hastened at high humidities (Laing and Heraty 1987).

The significance of the timing of diapause termination by parasitoids is partly determined by the presence of their host/s. That is, a parasitoid breaking diapause, only to find that its host/s are still dormant, is at a distinct disadvantage

compared to one that emerges from diapause at the same time as, or later than, its host. *M. demolitor* has additional problems; emergence must not only be synchronous with, or after its host/s, but must also avoid periods when the host is migrating out of the area. For example, *M. demolitor* terminating diapause when no plants suitable for its host to feed on are present, is liable to find that the population of adult hosts emerging from diapause, as well as established individuals, migrate from the area, leaving the parasitoids without any larval hosts for oviposition.

Diapause has only been shown to occur in *Microgaster demolitor* in a few studies, (see chapter 5), and the mechanism for diapause termination has not previously been isolated. Kay (1982b) reported that *Microgaster demolitor* collected from the Darling Downs region emerged from diapause in August/September in 1977/78. Cobb (1983) found a "diapause like phenomenon" in a lab based colony of *Microgaster demolitor* imported into America from Southern Queensland in 1981, which appeared to be terminated in some individuals when they were moved from 17°C and 21°C to 27°C. Robertson (in Hafez 1951) reported that overwintering in Queensland by *Microgaster demolitor* lasted an average of 5 months, while Hafez (1951) found similar results for *Microgaster demolitor* (originally from S.E. Queensland), in Giza, where the parasitoids overwintered for 4 months.

Diapause termination in the native hosts of *M. demolitor* , *H. armigera* and *H. punctigera*, is controlled primarily by temperature (Wilson *et al.* 1979, Murray pers comm 1990). It is thought that both species of hosts have to be exposed to

temperatures below 12°C to terminate diapause and then temperatures above 16°C to start normal growth (Murray pers comm 1990).

This study aims to determine whether variations in photoperiod, temperature, or humidity stimulate termination of diapause in *M. demolitor*. It also attempts to ascertain whether the sex of the parasitoid, the host species it was reared in or the state of the parasitoid's pupal cocoon have any effect on the time *M. demolitor* spends in diapause. The study also considers how diapause termination cues in the parasitoid may relate to the diapause biology and ecology of its hosts *H. punctigera* and *H. armigera*.

8.2 Methods

8.2.1 General

All parasitoids used in the following experiments originated from Toowoomba and were reared in *H. punctigera* under a photoperiod of 14L:10D unless otherwise stated. Diapausing individuals were identified by the presence of distinct ribbing on their cocoons (see chapter 7). *Heliocoverpa* sp. larvae were parasitised at the 2nd instar by mated female parasitoids. All parasitised larvae were reared individually in single cells of a 12 cell limbro tissue culture tray containing artificial diet (see appendix 2).

All analyses of variance were performed using the General Linear Models option in SAS using type III sums of squares to contend with the unbalanced data sets. Similarly, all regression analysis was performed using the Regression option in SAS.

8.2.2 Sex and host effects

Parasitoids were reared at 26°C and 38 to 58% R.H. as both larvae and pupae. Cocoons were checked daily for emergence of the wasp. At emergence the parasitoids were sexed by the presence or absence of an ovipositor. The effects of sex (n=30 for females, n=15 for males) and host species (n=38 for *H. punctigera*, n=7 for *H. armigera*) on diapause duration of the parasitoid were examined via a two way analysis of variance.

8.2.3 Temperature effects

To determine whether variation in temperature would induce termination of diapause, parasitoids were reared at 26°C, (38 - 58% R.H.) in the larval stages. Individuals that entered diapause were then either transferred to 30°C (41 - 62% R.H) n=10, to 24°C (34 - 53%R.H.) n=11, or left at 26°C, n=5. Photoperiods were maintained at 14L:10D. Only parasitoids that had been in diapause for a period of less than 20 days were used. All parasitoids were then

left under these conditions until emergence occurred. The differences between the mean diapause duration of these three groups of parasitoids were then analysed with a one way analysis of variance.

8.2.4 Photoperiod effects

The effects of increased and decreased photoperiods on diapause duration were established by moving parasitoids reared at 14L:10D photoperiod to either 10L:14D or 16L:8D. Parasitoids were initially reared at 26°C, (38 - 58% R.H.). Parasitoids that entered diapause at this regime were then split into three groups. One group of 13 parasitoids were moved to a photoperiod of 10L:14D, a group of 13 to a photoperiod of 16L:8D and the final group (n=8) was left at the original photoperiod. All three groups of parasitoids were maintained at the original temperature, 26°C.. The effects of these treatments were then analysed via a one way analysis of variance.

8.2.5 Humidity changes

The effect of increased and decreased humidities was investigated by moving diapausing parasitoids from a humidity range of 38 - 58% R.H, (control), to either 72 - 91% R.H. or 5% (±5%) R.H.. Humidity was raised by adding shallow troughs of water into the base of the constant temperature cabinet.

The 5% R.H. regime was produced by placing silica gel within a glass desiccator at a temperature of 26°C. The diapausing parasitoids were then placed within the desiccator in 24 cell plastic Limbro tissue culture plates.

All animals moved to the new humidity regimes had been in diapause no longer than 20 days. 40 parasitoids were left at the original humidity, (38-58%), 37 were moved to the higher humidity while 14 were placed at the lowest humidity . All parasitoids were reared at 26°C. The difference in diapause duration between the control and the treatments was then analysed with a one way analysis of variance.

8.2.6 The effects of cocoon weight and pupal weight

Parasitoids reared in *H. punctigera* and *H. armigera* entering diapause at 26°C and in a humidity regime of 38 - 58% R.H. were used. The combined cocoon and prepupa was weighed on a Cahn electronic microbalance ($\pm$0.05 mg) twelve hours after emergence of the parasitoid from the host. The cocoon was later weighed separately after the wasp had emerged. The prepupal weight was then calculated by subtracting the cocoon weight from the weight of the cocoon and prepupa combined. Any relationship between pupal weight (n=22) or cocoon weight (n=22) and diapause duration was then examined with linear regression analyses.

8.2.7 Effects of cocoon damage

To determine whether cocoon damage induced a release from diapause, part of the surrounding cocoon was totally removed, with a sterile scalpel blade, to expose the diapausing prepupa to the environment. Care was taken not to cause damage to the prepupae and no more than one third of each cocoon was removed. Once the cocoon was modified the parasitoid was left at the environmental regime in which it was reared, a humidity range of 38 - 58% R.H. and 26°C, until emergence occurred. Five diapausing prepupae reared under the same conditions and at the same time were left undamaged as a control. These individuals were siblings of those for which the cocoon was modified.

8.3 Results

8.3.1 Host and sex effects

The host in which *M. demolitor* was reared had no influence on the duration of diapause ($F=0.77$, $df=1,41$, $p=0.38$; table 8.1). Male and female wasps remained in diapause for similar lengths of time ($F=0.37$, $df=1,41$, $p=0.55$; table 8.1) and there was no significant interaction between these two factors ($F=0.64$, $df=1,41$, $p=0.43$).

Table 8.1: Diapause duration, in days, with associated standard errors for *M. demolitor* reared in different hosts as well as differences between sexes.

	Host		Sex	
	H. punctigera	*H. armigera*	male	female
diapause duration	112.8 (9.3)	96.6 (16.7)	108.3 (13.1)	111.2 (10.7)

8.3.2 Temperature and photoperiod effects.

Transfer of diapausing individuals from 26°C to 30°C or from 26°C to 24°C did not affect the time spent in diapause ($F=1.57$, $df=2,23$, $p=0.23$), (table 8.2). Similarly, an increase or decrease in photoperiod did not affect diapause duration ($F=0.18$, $df=2,31$, $p=0.84$), (table 8.2).

Table 8.2: Diapause duration, in days, with associated standard errors for *M. demolitor* reared at different temperatures and photoperiods.

	Temperature			**Photoperiod**		
	24	26	30	10:14	14:10	16:8
diapause duration	64.3 (6.0)	62.7 (10.3)	84.1 (15.8)	51.5 (6.0)	48.4 (2.7)	51.9 (3.2)

8.3.3 Humidity effects

Parasitoids removed from 50 % R.H. and placed at 80% humidity broke diapause an average of 37.9 days later, but those left at 50% R.H. remained in diapause for an average of 91.9 days. Parasitoids moved to 5% R.H. remained in diapause on average 166 days, (figure 8.1). These differences were significant ($F=133.5$, $df=2,89$, $p<0.001$).

8.3.4 Cocoon and prepupal weight effects.

No significant relationship between the duration of diapause and cocoon or prepupal weight was found (table 8.3, figure 8.2). However there appears to be some suggestion that prepupae with very light cocoons have a short diapause duration (figure 8.3).

Table 8.3: Results of a multiple regression analysis on diapause duration versus cocoon and prepupal weight.

Parameter	estimate	D.f.	S.E.	p
Intercept	80.01	1	86.4	0.36
Pupal weight	-11.63	1	13.7	0.40
Cocoon weight	47.80	1	45.8	0.30

8.3.5 Cocoon damage

Damage to the cocoon reduced the time spent in diapause ($F=11.46$, $df=1,9$, $p=0.008$, figure 8.4). The average time to termination of diapause was 23.33 days once part of the cocoon was removed compared to 62.20 days if the cocoon was left undisturbed.

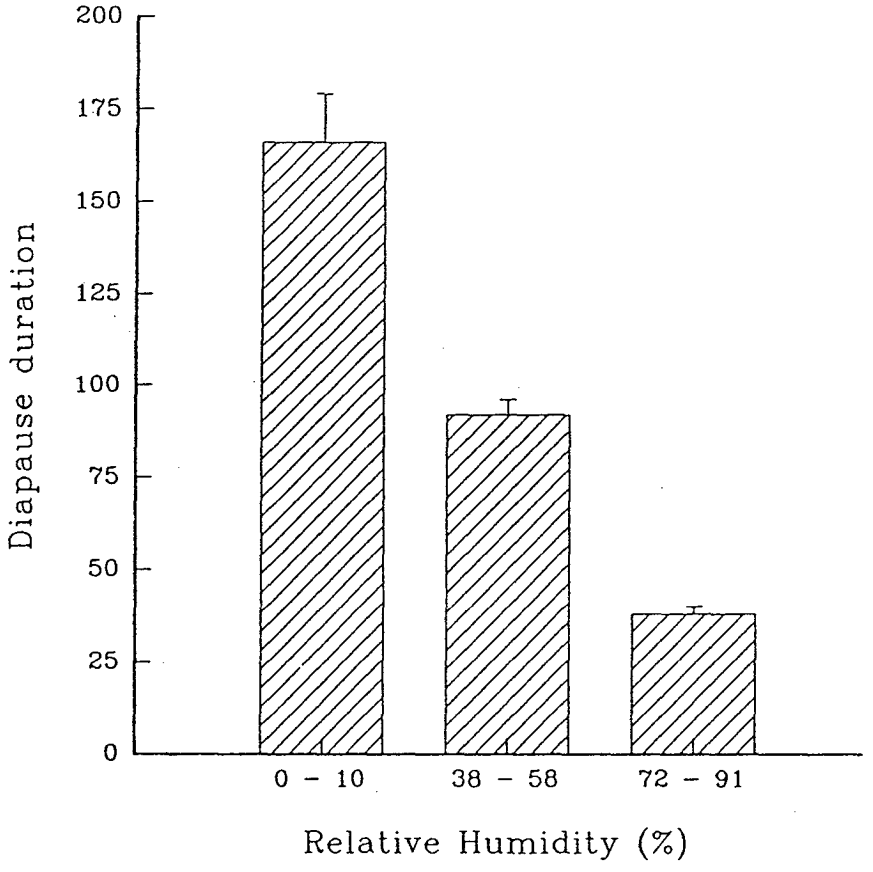


Figure 8:1. Time, in days, to terminate diapause in *M. demolitor* when transferred to high or low humidities. (Vertical bars are 95% confidence limits. 38-58% R.H. is the control).

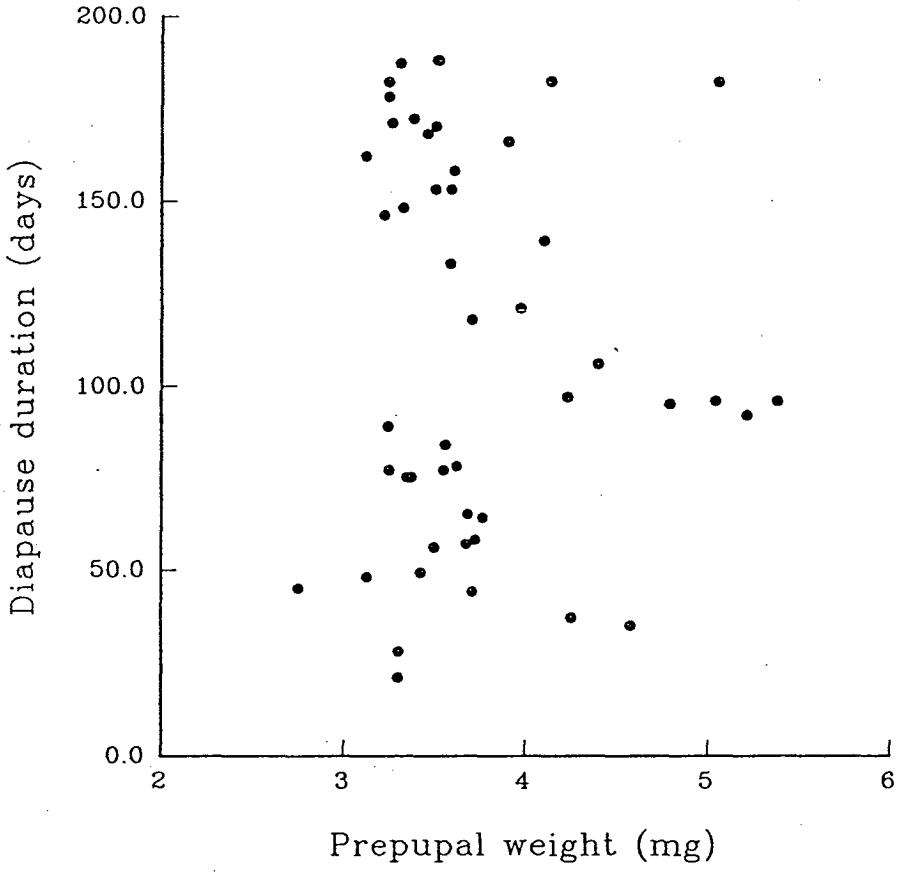


Figure 8:2. Scatter plot of prepupal weight, (in mg), of diapausing *M. demolitor* versus diapause duration (in days).

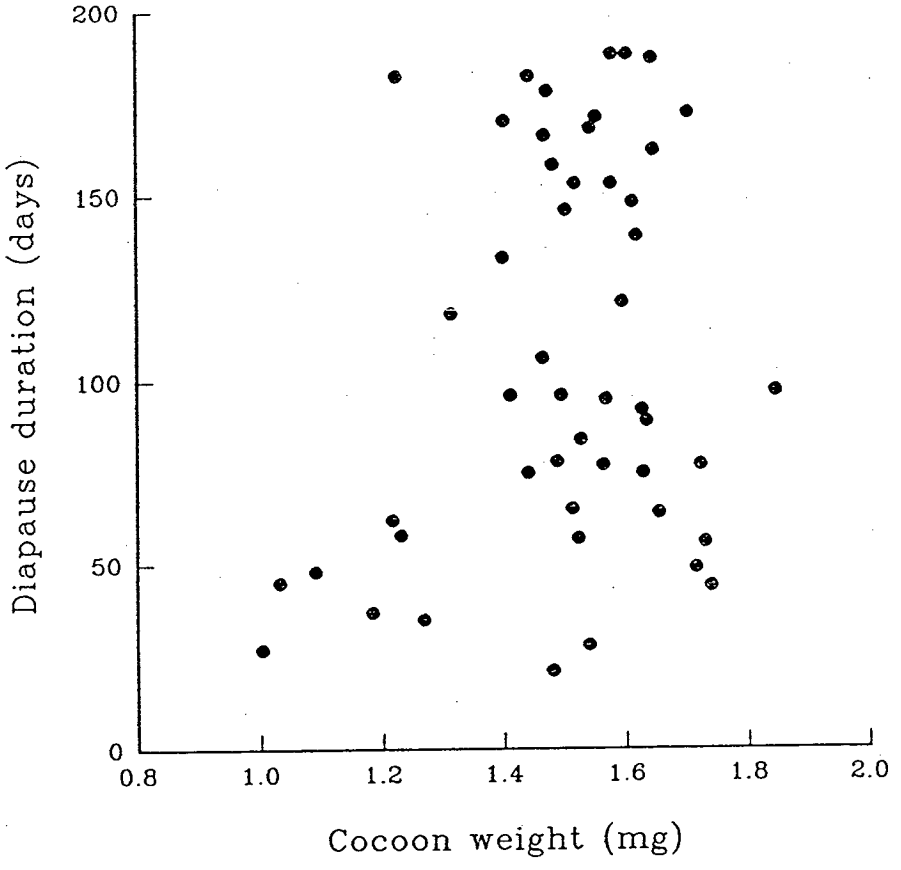


Figure 8:3. Scatter plot of cocoon weight, (in mg), of diapausing *M. demolitor* versus diapause duration (in days).

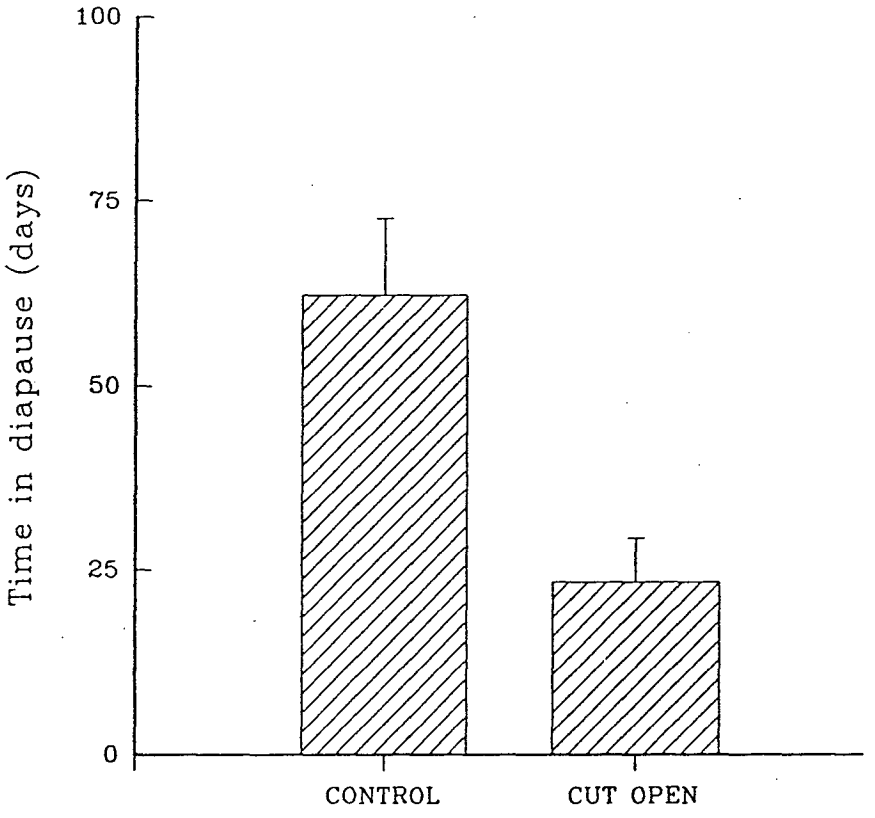


Figure 8:4. Time taken, in days, for *M. demolitor* to terminate diapause after partial removal of the cocoon. (Vertical bars are 95% confidence limits).

8.4 Discussion

In *M. demolitor* neither photoperiod, temperature nor the host species in which the larva was reared, showed any effect on diapause duration. This study shows that the major influence on diapause termination in *M. demolitor* is humidity. That is, as humidity increases, the time in diapause decreases. This is also the same environmental factor which has most influence on diapause initiation in this parasitoid (see chapter 7). However, it appears that the parasitoid is using a different set of cues than that of its hosts, which use temperature and perhaps photoperiod, to terminate diapause.

Captures of *H. punctigera* in Central Australia increased after winter and spring rains (Gregg *et al.* 1989), and there is some suggestion that rainfall may indirectly effect *Heliocoverpa* spp population sizes. In both the Namoi and Darling Downs regions, hot dry weather present in 1988/89 when the first generation of larvae were produced, caused significant decreases in *Heliocoverpa* larval numbers and resulted in decreased numbers for the rest of the season (Fitt pers. comm.). The use of high humidity as a primary cue for diapause termination by *M. demolitor* may therefore serve to synchronise the parasitoid with high abundances of its host/s.

In effect, the use of high humidity may allow the parasitoid to synchronise its diapause termination with the periods in which *Heliocoverpa* spp. are most likely to be abundant, as well as times when there should be a predominance of

plants for the host to feed on, thus reducing the chances of mass migration by the host due to lack of food.

As cocoon weight was not a reliable predictor of diapause duration it is unlikely to be the thickness of the cocoon that is important in the determination of diapause duration. That is, if diapause duration was determined by cocoon thickness, thicker and hence heavier cocoons would be present on parasitoids which spent longer times in diapause. However, the indication that very light cocooned individuals spend short periods in diapause suggests that a minimum cocoon size is necessary for long diapause duration.

Partial removal of the cocoon initiates release from diapause, perhaps suggesting that some property of the intact cocoon may determine when or if the parasitoid is to break diapause. Slifer (1946), found that a wax like layer existed in the hydropyle of the eggs of the grasshopper *Melanopus differentialis*, and is dissolved away with time to allow water to enter the egg. Perhaps a similar system is operating here, with a chemical or wax like layer/substance being present in the cocoons of diapausing parasitoids which is broken down by water, that is, an increase in the surrounding humidity may cause the layer to be degraded faster than under lower humidities. Once this layer is penetrated humidity receptors on the prepupae may then trigger development of the parasitoid.

This proposed mechanism is speculative, but would account for the observed data and should ensure that parasitoid emergence only occurs under environmental conditions which allow the hosts to terminate diapause, as well as decrease the probability of the hosts migrating from the area due to lack of food caused by dry conditions.

Chapter 9 Host Preferences

9.1 Introduction

If the relative frequency of either host types or instars parasitised, differs from the relative frequency of types of hosts or instars available, then a parasitoid can be said to be displaying a preference for a certain host type or instar (Hooper & King 1984). This preference may affect fitness, since the parasitoid's development, longevity or size (and thus possibly fecundity), will be detrimentally affected if an unsuitable host or instar is selected.

Insertion of the ovipositor into the host is not necessarily an index to host suitability (Doutt 1959), as some species will readily oviposit in hosts where development of the parasitoid does not take place. This occurs in *Phaenocarpa persimilis* which parasitises different species of *Drosophila*, only in some of which the parasitoid will develop (Prince 1976).

The existence of preferences for certain host species from a range of suitable species has been experimentally determined in many parasitoid species such as the ichneumonid *Campoletis perdistinctus* (Lingren & Noble 1971), the Torymid *Monodontomerus dentipes* (Drooz & Fedde 1972), the braconid *Monoctonus paulensis* (Calvert 1973) and the Cynipid *Charips victrix* (Gutierrez 1970).

For *Microgaster demolitor*, there has been no experimental work to determine if this parasitoid discriminates between its two closely related native hosts, *H. armigera* and *H. punctigera*, in Australia. However, there is some suggestion that discrimination between these two species of hosts by the parasitoid is occurring in tobacco crops in Northern Queensland. In this region it appears that *M. demolitor* is more often found in *H. armigera* (Titmarsh 1985). If so, this preference displayed by the parasitoid may vary from northern to southern locations, coinciding with the differences in the distribution of its hosts, that is, predominately *H. armigera* in the north and *H. punctigera* in the south. The preference displayed by the parasitoids may also be influenced by differences in seasonal phenology of its hosts. This ability to distinguish between these two hosts would then allow the parasitoid to tailor its strategies to suit the ecology of the different hosts.

Similarly the ability to be able to distinguish between different instars may provide an adaptive advantage, as mortality rates may vary between younger and older larvae. Johnson (1987) indicated that *Microgaster demolitor*, collected from Stanthorpe, Queensland, in 1981, showed a preference for 2nd and 3rd instar larvae of *P. includens*. This type of study has not been completed for the native hosts of this parasitoid.

The objective of this study was to determine if *Microgaster demolitor* can distinguish between *H. armigera* and *H. punctigera*, and whether it discriminates between different instars of these hosts. That is, does this parasitoid have either a preferred host or a preferred instar for oviposition.

9.2 Methods

9.2.1 Host species preference

The preference by parasitoids for different host species was determined by placing 5 second instar larvae of each of the two host species, *H. armigera* and *H. punctigera*, in a sealed petri dish with a small opening in the lid in which a cotton plug was inserted. A mated female wasp from Grafton, Toowoomba, Emerald or Mareeba, which had not previously oviposited, was introduced into the container for approximately 2 hours. The species of host which the parasitoid either encountered and parasitised or encountered without ovipositing was recorded. This procedure was carried out for three parasitoids from each location.

Once a host had been parasitised it was removed and replaced with an unparasitised larva of the same species. These removed hosts were then placed individually into single cells of a twelve cell Limbro® tissue culture tray

containing approximately two cubic centimetres of an artificial diet (see appendix 2). They were then reared at 26°C and 14L:10D to ensure that oviposition had occurred.

The data from each location were then analysed separately by an unweighted logistic regression.

9.2.2 Host instar preference

The preference by the wasp for different host instars was determined in a similar fashion but with 12 larvae of *H. punctigera* in the petri dish at any one time. Within the petri dish there were two specimens from each of the six instars that occur in *Heliocoverpa*. Hosts that were parasitised were replaced and reared on artificial diet at 26°C to establish that oviposition had occurred. Three female unmated parasitoids from the Toowoomba population were used in these trials, each for a total period of 4 hours, in two, 2 hour sessions.

The data were analysed using an unweighted logistic regression.

9.2.3 Source of hosts

All hosts used were reared from a laboratory based culture maintained at the Entomology Branch, Department of Primary Industries, Brisbane. Trials were carried out at room temperature which ranged between 20 and 30°C, and under the illumination of a 75 watt, clear, light globe, suspended 1 metre above the petri dish.

9.3 Results

9.3.1 Host species preference

For all four locations no significant difference was found in the preferences displayed by individual females from within the same location (Grafton, $\Delta dev=0.001$, $\Delta df=1$, $p=0.97$; Toowoomba, $\Delta dev=0.294$, $\Delta df=1$, $p=0.59$; Emerald, $\Delta dev=1.946$, $\Delta df=1$, $p=1.63$; and Mareeba, $\Delta dev=0.039$, $\Delta df=1$, $p=0.85$). However, a significant effect was found in all locations except Toowoomba, for host (Grafton, $\Delta dev=6.437$, $\Delta df=1$, $p=0.01$; Toowoomba, $\Delta dev=1.923$, $\Delta df=1$, $p=0.17$; Emerald, $\Delta dev=14.761$, $\Delta df=1$, $p=<0.001$; and Mareeba, $\Delta dev=14.022$, $\Delta df=1$, $p=<0.001$) . In Mareeba and Emerald, *M. demolitor* females preferred *H. armigera*, the Grafton females preferred *H. punctigera* while the Toowoomba females had no preferred host (figure 9.1). That is, with decreasing latitude the preference by *Microgaster demolitor* for hosts changed from *H. punctigera* in the south to *H. armigera* in the north.

9.3.2 Host instar preference

Analysis of the effect of parent and instar on the frequency of oviposition in *H. punctigera* showed that parent had no significant effect ($\Delta dev=1.07$, $\Delta df=1$, $p>0.05$) but that the instar significantly affected the frequency of oviposition ($\Delta dev=17.3$, $\Delta df=1$, $p<0.05$). That is, *M. demolitor* from Toowoomba, has a distinct preference for 2nd and 3rd instar larvae and more so for 3rd's than 2nd's (figure 9.2).

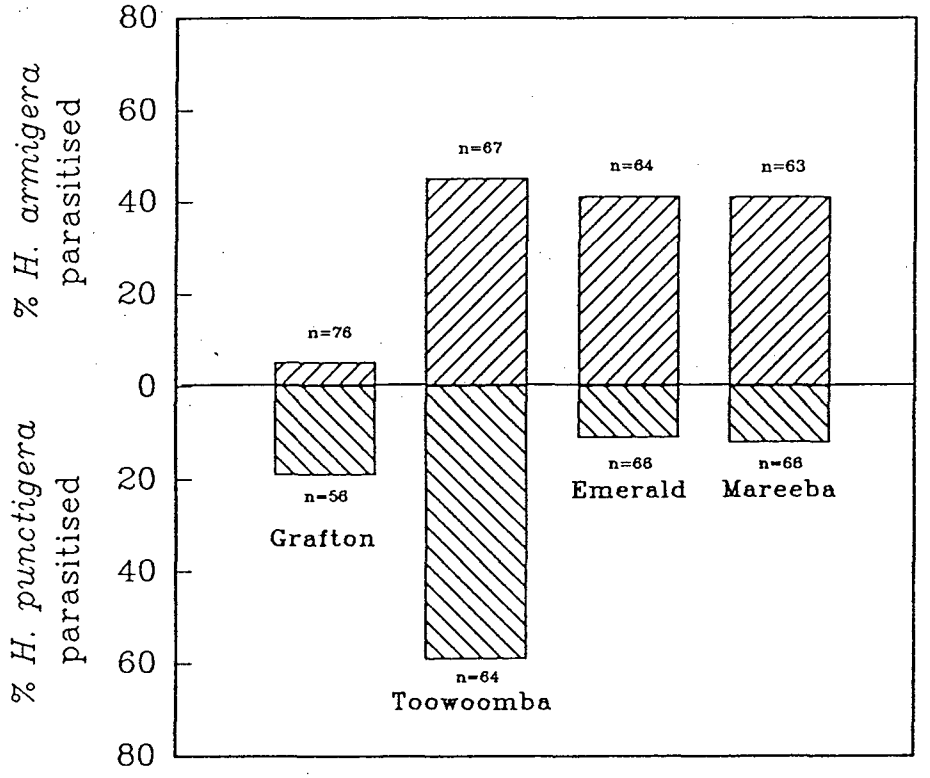


Figure 9:1. Host preference, for either *H. punctigera* or *H. armigera*, shown by four separate populations of *M. demolitor*.

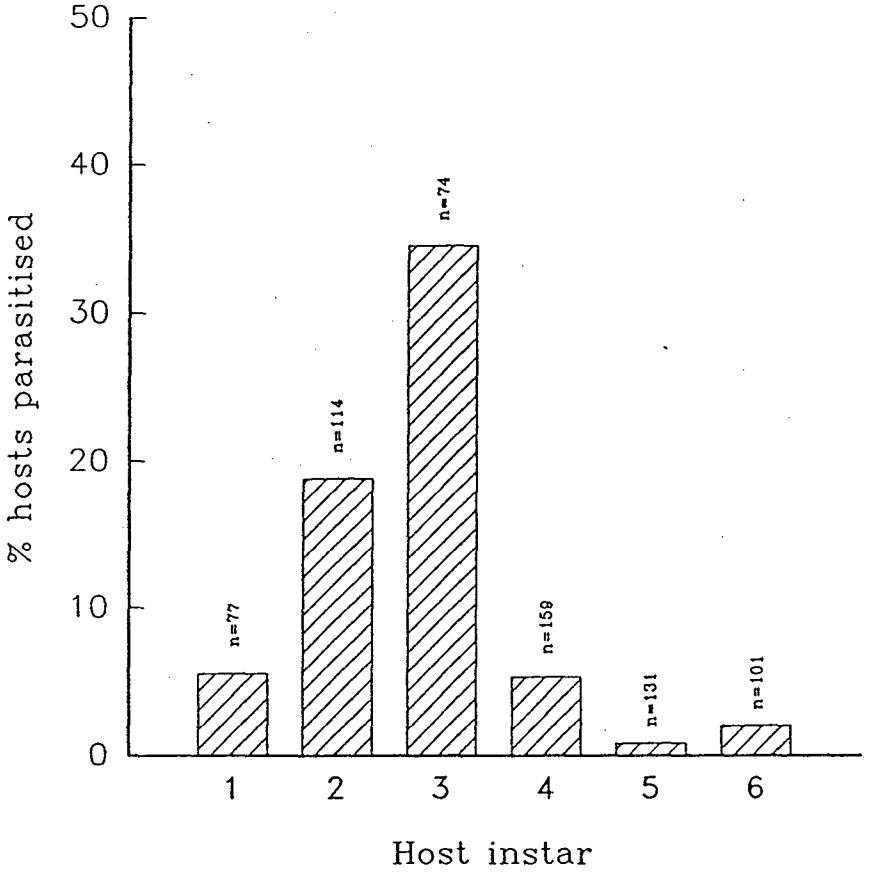


Figure 9:2. Instar preference for *H. punctigera* displayed by *M. demolitor* from Toowoomba.

9.4 Discussion

The preference for different species of *Heliocoverpa* spp in different geographical regions of Australia by *Microgaster demolitor* appears to be related to the general distribution and seasonal phenology differences in the two hosts.

In Mareeba, the dominant, if not the only *Heliocoverpa* spp. present up to October is *H. armigera* (Titmarsh 1985). After this time, the proportion of *H. armigera* larvae present in the field decreases. However, it is not known whether this is caused by a combination of actual decreases in *H. armigera* numbers and large influxes of *H. punctigera* or by the latter factor alone (Titmarsh 1985). Regardless of these changes in population proportions, *H. armigera* is present in the area over a longer period of the year than *H. punctigera.* Thus there would seem to be a selective advantage for parasitoids showing a preference for *H. armigera* in this region, allowing them to utilise hosts earlier in the season and hence produce a greater number of generations during the year.

In Toowoomba, however, the differences in seasonal phenology in the two host species are quite marked. Usually *H. punctigera* and *H. armigera* arrive as migrating populations into the region during mid to late September, with *H. punctigera* being the predominant species (Titmarsh pers comms 1990). Local populations of *H. punctigera* usually terminate diapause in October with *H. armigera* terminating later in the season (Murray pers comms 1990). *H. punctigera* is then the predominant species in this area up until January when it

then disappears leaving *H. armigera* as the dominant *Heliocoverpa* spp. in the area until May, when it to disappears (Titmarsh 1990).

Thus, a parasitoid, in this region, with a preference for one species of host may be disadvantaged in comparison to one that had no preference. That is, a parasitoid, having developed within its preferred host, *H. armigera* late in the season would enter diapause, only to be faced with a population of predominantly *H. punctigera*, its non-preferred host, when it recommenced development at the start of the next season. A similar scenario exists for parasitoids with a preference for *H. punctigera*. Thus there would seem to be a selective advantage for parasitoids without a preference for hosts in this region, as they could exploit whichever host was dominant.

A possible disadvantage related to a non-preference for hosts may be an increase in searching time and hence a decrease in possible oviposition. Host location in parasitoids usually involves several steps, the first being habitat location (Doutt 1959, Glas & Vet 1983, Mueller 1983, Vinson & Iwantsch 1980). Zalucki *et al.* (1986) showed that of 159 species of host plants recorded for *H. punctigera* and *H. armigera*, only 43 were utilised by both species. In general, it appears that *H. punctigera* occurs on dicotyledonous plants while *H. armigera* is found on both di and monocotyledonous plants (Zalucki *et al.* 1986). Thus, a parasitoid with a preferred host may have a tighter primary searching regime and may then spend less time searching unproductively in crops which that particular host does not use.

The preference for *H. punctigera* shown by the population of *M. demolitor* from Grafton is slightly confusing. Although *H. punctigera* is seasonally abundant in subtropical and temperate Australia (Fitt 1989), along the east coast of Australia *H. armigera* in these areas is abundant (Zalucki *et al.* 1986). Based on this information, a non-preference for either host species would appear more advantageous. However no information on seasonal phenology of either of the hosts from this region is available and it may be this which determines the host preference in this area.

The preference for 2nd and 3rd instar larvae of *Heliocoverpa* by *M. demolitor* is similar to that found by Johnson (1987), who showed that *Microgaster demolitor* preferred 2nd and 3rd instar larvae of its host *Pseudoplusia includens*. The preference displayed by *Microgaster demolitor* for certain instars of its native hosts reflects two factors, firstly the high mortality of 1st instar *Heliocoverpa* larvae and secondly the large amount of damage a 4th, 5th or 6th instar larvae can inflict on a parasitoid which is determined to oviposit in these individuals.

Titmarsh (1985) showed that a high degree of mortality occurred in the egg and 1st instar stages of *Heliocoverpa* spp in comparison with later stages. Similar results have been found in other lepidoptera (Courtney & Duggan 1983, Dempster 1971). As a result of this higher mortality, any parasitoid laying eggs in these individuals may be disadvantaged as the probability of mortality of the

hosts at this stage is substantially greater. However, in 2nd and 3rd instar larvae, mortality rates decrease, compared to those for the egg and 1st instar, and thus the survival rate of the parasitoid may also be increased.

Once *Heliocoverpa* reaches its 4th instar, it is substantially larger than the attacking parasitoid and is able to defend itself (see chapter 4). This defence may be so forceful that the parasitoid is only able to effectively oviposit in a small number of host larvae before it is killed, or becomes incapacitated, in contrast to those wasps which preferentially attack 2nd and 3rd instar larvae and are capable of producing 50 to 80 offspring (see chapter 4).

The possibility that *M. demolitor* may use other hosts in the field should not be overlooked. The relationship between *M. demolitor* and *Heliocoverpa* spp. is known, mainly because of the economic importance of these hosts, but other hosts present in the field may play an important role in the ecology of this parasitoid. For example, *Neocleptria punctifera*, a noctuid similar to *Heliocoverpa*, and possibly more abundant than *Heliocoverpa* outside cropping areas, has been recorded as a host for *M. demolitor* (Fitt pers comms 1991). To rectify this, extensive larval collections need to be done, to determine, what, if any other, lepidopteran larvae are native hosts for this parasitoid.

In summary, ovipositing females of *Microgaster demolitor* discriminate between host species and host instars. Instar preferences probably represent a compromise between the improved host survivorship provided by older larvae and

the low risk of injury involved with younger larvae. Species preferences differ between geographic regions and are perhaps determined by differences in distributions and seasonal phenology of the hosts.

Chapter 10 Conclusion.

The use of humidity as a controlling factor of diapause incidence and termination by *M. demolitor* allows it to synchronise its life cycle with its hosts, which use different cues for diapause. By using humidity, *M. demolitor* is able to enter diapause if the hosts population is

i) likely to decrease in number due to high mortality caused by increased temperature and usually decreased rainfall;

ii) if the number of host plants decreases due to decreases in rainfall causing mass migration of the hosts from the region (assuming *M. demolitor* does not undergo similar movements) or

iii) when the hosts themselves enter diapause, usually during winter, when photoperiods and temperatures decrease. However, the winter months in these areas are also dry, with decreased relative humidities stimulating diapause incidence in the parasitoid.

All these result in a decrease in the population of hosts, either directly or indirectly, and as such result in decreased oviposition site for the parasitoid.

This probable non-migratory tendency is further supported by the differences between the populations of *M. demolitor* in regard to their

developmental biology and host preferences. That is, the hosts undergo large migrations and thus there appears to be genetic mixing between different populations, with developmental biology between the populations being similar. However, it seems that the parasitoids are relatively sedentary, with little mixing of genetic material, allowing their developmental biology to be shaped by the different environmental regimes.

There are always some developing *M. demolitor*, (except in Mareeba where *H. armigera* is present all year round), entering diapause, regardless of the conditions, and there is never 100% diapause incidence, this being in contrast to diapause strategies of most temperate insects. This strategy is possibly related to the differences between the migratory tendencies of the hosts in relation to the parasitoid. That is, the hosts often migrate from their old larval host plants to newer plants, which may be in the local area, or very large distances away. However, if the parasitoid does not undergo these large migratory movements, it must have some means of surviving if faced with a departing population of hosts. Thus the strategy of placing a small percentage of offspring into diapause, regardless of the conditions, will help to minimise the impact on the parasitoid population under these conditions. Hosts are also present in some regions, surviving on wild host plants in small numbers, for part or all of the year. So, even under harsh environmental conditions, there is a possibility of *M. demolitor* finding a host, however the probability is very low. The strategy of committing a

small percentage of offspring into normal development under harsh conditions may enable them to exploit the small number of hosts which have not migrated or entered diapause yet, and are surviving on wild host plants.

Thus it appears that *M. demolitor* is gambling on these outcomes, committing some offspring to diapause in ideal conditions, just in case migration of the hosts occur, while, in poor conditions, also keeping some offspring out of diapause on the possibility that small numbers of hosts may be present on wild host plants.

From the studies carried out it is evident that the different populations of *M. demolitor* are behaviourally and developmentally different. Furthermore, the percentage of diapause occurring in the populations is different under similar environmental regimes.

These differences present a number of possibilities for biological control work. If discrete populations of parasitoids exist with a preference for one particular species of host, then the introduction of the parasitoid into an area where its non-preferred host is dominant, may result in a decrease in the parasitisation rate. This could then be interpreted incorrectly, that is, that the parasitoid population was ineffective against *Heliocoverpa* when really it is just ineffective against one particular species of *Heliocoverpa*.

This preference for different hosts shown by the populations of parasitoids may, in fact, increase its effectiveness. *Heliocoverpa* spp populations often show temporal variation in abundance, such as in Toowoomba where *H. punctigera* is the abundant species early in the season while *H. armigera* is dominant later in the season. The release of parasitoids with a preference for *H. punctigera* during the start of the season and those with a preference for *H. armigera* near the end of the season may result in higher yearly parasitisation rates and hence better control of *Heliocoverpa* spp.

There is also the possibility that within the same area, two populations of parasitoids exits with different host preferences. That is, a population of parasitoids with a preference for *H. punctigera* may search predominantly in host crop plants that support this species while those parasitoids with a preference for *H. armigera* may search in host plants which are principly infested by *H. armigera.*

This understanding of the diapause and quiescence of these parasitoids will then allow large numbers to be reared for field release. As *M. demolitor* is a solitary larval braconid, the same number of hosts have to be reared as number of parasitoids to be released. As the longevity of the adult parasitoid is approximately 2 weeks, very large numbers of parasitoids, and thus of hosts, have to be reared in a very short time if sufficient numbers for a field release are to be generated. This then requires large amounts of room and is financially very expensive. However, if the parasitoids can be reared and placed in diapause, then

they can be stored for several months and later brought out of diapause when sufficient numbers have been generated.

If this parasitoid is to be a used as a biological control agent, its effectiveness would be enhanced if used as part of a integrated approach. That is, *M. demolitor* has a preference for 2nd and 3rd instar hosts. Consequently, later instars and pupae are not affected by this parasitoid. An overall approach of egg, larval and pupal parasitoids might then have a greater chance of controlling the population.

It is suggested then, that although these populations of parasitoids are morphologically indistinguishable, they may in fact represent a clinal range of variation, a set of sibling species or a discrete polymorphism. If they are in fact distinct species then electrophoretic studies may be the best method for separating them, and in any event, an examination of their genetics and capacity for interbreeding is an obvious next step in their study.

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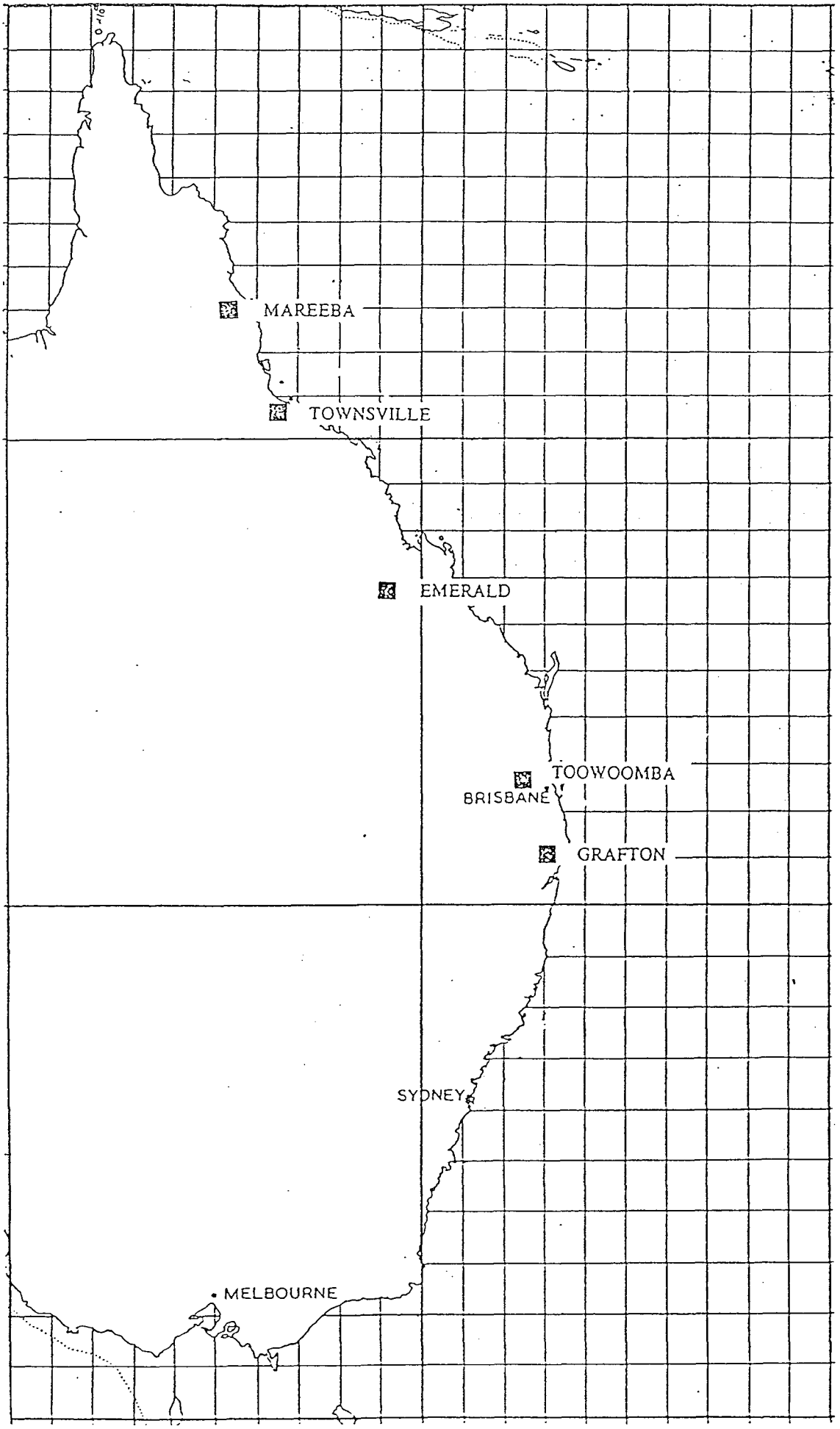
Appendix one.



Appendix two.

A modified Shorey's artificial lepidopteran diet.

(a) 300 grams of lima or soybeans (steamed in a pressure cooker for 20 minutes in 930 ml of water).
(b) 60 grams of yeast
(c) 6 grams of ascorbic acid
(d) 6 grams of nipagen
(e) 2 grams of sorbic acid
(f) 30 grams of wheat germ

Blend the steamed beans into a fine paste and allow to cool to room temperature. Then add ingredients (b) to (f) inclusive and blend once more.

In a separate container dissolve 25 grams of agar in 600mls of water and heat until boiling. Allow the mixture to cool to approximately 70°C.

Add the agar to the paste and blend until mixed.

Pour out the resulting mixture into a flat tray and allow to set. Refrigerate at 5°C until ready to use.

Do not use diet that is any more than 1 week old.