EFFECT OF NUTRITION ON *COTESIA PLUTELLAE* (HYMENOPTERA: BRACONIDAE) AND ITS PARASITISM ON THE DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* (LEPIDOPTERA: PLUTELLIDAE)

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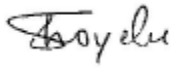
Declaration

I, Olalekan Joseph SOYELU, hereby declare that this work was carried out by me and it has not been previously submitted for any degree at this or any other University. All reference materials contained herein have been duly acknowledged.



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Dedication

I would like to dedicate this work to the Most High God for His grace in my life. To my loving wife, Oluwaseyifunmitan, and my beautiful daughter, Ibukunoluwa. Also, to my parents, thanks for your prayers.

Abstract

Crucifers are important vegetable crops in South Africa. However, they are subjected to attack by lepidopterous pests, especially the diamondback moth *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). The cost of pesticides and the increasing resistance of *P. xylostella* to chemical control make it necessary to explore alternative control methods. Biological control is an alternative method with bright prospect as it does not have any adverse effect on the crop and its human consumers. South Africa has a huge number of parasitoids attacking *P. xylostella* and *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) is the most abundant larval endoparasitoid. At peak performance, percent parasitism of *C. putellae* on *P. xylostella* is greater than 80%. However, the population of *C. plutellae* is usually inadequate during early spring and this is also the period when field infestation by *P. xylostella* increases. This study was therefore conducted to examine the possibility of improving the fitness of *C. plutellae* by incorporating a protein source (beebread) in its diet. The suitability of beebread, as well as its effect on the biology, sensory response and foraging behaviour of *C. plutellae* was studied.

Host-parasitoid colonies were established successfully on honey and honey-beebread, but *C. plutellae* lived longer and produced more offspring on the latter. Also, wasps provided with honey-beebread produced more female offspring than parasitoids fed with honey (t = 2.59; d.f. = 239; P = 0.01). Female-biased offspring were produced by wasps fed with honey or honey-beebread while

wasps that were denied sugar sources produced male-biased offspring. Maternal diet did not affect egg load of newly emerged *C. plutellae* and post-eclosion egg maturation did not depend on type of diet given to the parasitoid. In addition, sizes of egg and body weight of newly emerged *C. plutellae* were independent of maternal diet. Though honey and honey-beebread elicited > 95% feeding responses in *C. plutellae*, the parasitoid showed preference for cues associated with the latter in Y-tube olfactometer bioassays. This is an indication that the parasitoid would likely feed on honey-beebread more than it would feed on honey in an agroecosystem. Obtained electroantennograms further showed that the parasitoid is more responsive to honey-beebread than *P. xylostella* suggesting that the parasitoid would benefit from this high protein diet without competition from its host.

When given host and food experiences before olfactometer trials, *C. plutellae* improved on its innate attraction to the resources. The parasitoid showed preference for food odour when hungry and this preference intensified after it was allowed to either smell the food or have a brief food reward before the experiments. Preference for host-related cues also increased after coming in contact with the host in addition to a brief food reward while satiated females showed stronger preference for hosts. Therefore, *C. plutellae* is able to distinguish between the food and host cues and it made foraging decisions based on its physiological state to balance its food and host needs.

This appears to be the first report of honey-beebread as a diet for *C. plutellae*. Further trials on a relatively bigger scale will be needed to validate its suitability.

Keywords: Beebread, biological control, cabbage, *Cotesia plutellae,* diamondback moth, electroantennogram, foraging cues, olfactometer.

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CHAPTER 1:

General introduction

1.1 Background

Cabbage, *Brassica oleracea* var. *capitata* (L.), is one of the six main vegetables derived from wild *B. oleracea* (of European and Mediterranean origins) through selective breeding for particular characteristics of the plant. The other five vegetables are kale (*B. oleracea* var. *acephala* L.), Brussels sprouts (*B. oleracea* var. *gemmifera* Zenker), kohlrabi (*B. oleracea* var *gongylodes* L.), cauliflower (*B. oleracea* var. *botrytis* L.) and broccoli (*B. oleracea* var. *italica* L.). Cabbage was bred from the wild stock for its enlarged terminal buds (spherical cluster of immature leaves) which is widely consumed raw, cooked, or preserved in a variety of dishes (*Iziko Museums*, 2008). The crop was introduced into South Africa in 1652 (Frere, 1885) and over the years, it has become very popular among the populace. Based on national consumption data, cabbage is the fourth most important vegetable after potato, tomato and onion (More, 2006). It is a good source of many minerals, particularly potassium, and it is relatively high in sulphur, calcium, vitamins A, C, B_1 and B_2 (More, 2006; Tiwari *et al.*, 2003). In 2007, South Africa produced 145,000 tonnes of cabbages and other brassicas (FAOSTAT, 2008).

In South Africa, a wide variety of arthropod pests feed on cabbages and they limit annual production, especially, by the rural resource-poor farmers. The pests listed by Annecke and Moran (1982) include insects of the order Hemiptera (cabbage aphid, bagrada bugs); Lepidoptera (moths, semi-loopers, cutworms, bollworms); Diptera (fly maggots); Coleoptera (flea beetles) and arachnids of the

subclass Acari (mites). Among the listed insect pests, diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), poses the greatest threat to cabbage production (Charleston and Kfir, 2000). Different control measures are implemented annually to curtail the menace of DBM, with the non-chemical control methods being increasingly preferred over the application of chemical pesticides. The established pesticide resistance and negative impacts of pesticides in the environment have increased the interest in alternative control methods. Emphasis has been placed on biological control, plant resistance, cultural control and other non-polluting methods (Lim *et al.*, 1997). Historically, the adverse effects of pesticide use were brought to public attention by the publication of 'Silent Spring' (Carson, 1962). Consequently, 1962 can be seen as marking the start of a transition from the 'chemical era' (a period when chemicals were viewed as panacea for pest problems) to the 'integrated pest management (IPM) era' (Gurr *et al.*, 2000).

Biological control methods were initially seen as a marginal component of integrated pest management but as public pressure to reduce pesticides mounted in the 1980s and 1990s, a dramatic change in perspective occurred. Following large-scale successes with IPM based on the restoration of natural enemies, occasioned by pesticide removal, the role of biological control as the natural baseline of pest management came to the fore (Waage, 2000). This event led to a growing public and governmental interest in renewable, biological

processes for sustainable agriculture, thereby creating new demand for biological control research and implementation in all its forms.

1.2 Motivation for the study

There is well documented evidence showing that control of the DBM with synthetic pesticides has not been as successful as desired. Resistance of DBM to different classes of synthetic insecticides has been widely reported (Talekar and Shelton, 1993; Sereda *et al*., 1997), hence forcing farmers to either apply higher doses or increase the frequency of spraying. Pyrethroid resistance in DBM is conferred by decreased sensitivity at the site of action in the nervous system (Hama *et al*., 1987), while resistance to organophosphorus compounds (parathion and methyl parathion) has been attributed to Glutathione S-transferase degradation (Sun, 1992). The effects of pesticides in insects are usually irreversible, and increased rates of pesticide applications leave behind pesticide residues that are harmful to farmers, consumers and the environment. Unregulated pesticide treatments increase production costs and lead to the exclusion of susceptible non-target insects (Waage and Cherry, 1992), especially the useful natural enemies (Talekar and Yang, 1991; Talekar and Shelton, 1993). To avoid these adverse effects, earlier workers saw an urgent need in adopting environment-friendly control measures, especially the use of natural enemies. Biological control, particularly the use of parasitoids, is seen as one of the most promising means of suppressing insect pest populations, especially where control with insecticides has failed (Charleston, 2004). Parasitoids are, in

general, host-specific and can parasitize several hundred hosts in a density-dependent manner that does not select for resistance (Annecke and Moran, 1982). In this way, the frequency of insecticidal applications can be greatly reduced (Talekar and Yang, 1991). The importance of parasitoids is also based on the assertion that they are usually self-perpetuating (Lim, 1986).

1.3 The system to be studied

1.3.1 *The pest*

The diamondback moth is a well-known destructive insect pest of brassicaceous crops in many parts of the world (Sarfraz *et al.*, 2006) and it was reported in South Africa in 1917 as a pest on cabbage (Charleston and Kfir, 2000). Cabbage is grown all year round in the Eastern Cape. Because of this and the mild climatic conditions, DBM is present all year, but seasonal variation occurs. Infestation levels are highest during the spring (September to November) and autumn (March to May), but low during the winter months (June to August) (Waladde *et al.*, 2001; Smith and Villet, 2003). The larvae are voracious defoliators and they are capable of causing more than 90% crop loss (Verkerk and Wright, 1996). The pest requires over U.S. $1.0 billion in estimated annual global management costs (Talekar and Shelton, 1993) in addition to the crop losses it causes. DBM was believed to have originated in the Mediterranean region (Tsunoda, 1980), the origin of cultivated brassicas, but Kfir (1998) has suggested otherwise. Considering the richness and high diversity of parasitoids associated with a large number of indigenous brassicaceous plants in South Africa, he was of the

opinion that diamondback moth must have originated from this part of the continent.

Adult female moths deposit small, almost round, yellowish-white eggs singly or in small groups on both sides of leaves of host plants. Larvae hatch after a few days and begin feeding just under the surface of the leaf tissue (difficult to see without a microscope). After days of feeding, mid-instar larvae exit the leaf and feed on the leaf surface. Initial damage results in small incomplete holes caused by young larvae and larger complete holes caused by maturing larvae. The entire plant may become riddled with holes under moderate to heavy populations. Larvae also feed in the developing heads of cabbage, causing deformed heads and encouraging soft rots (Hutchison *et al.*, 2004). If disturbed, DBM larvae wriggle away quickly and drop from the leaf on a silk thread. They climb back on the leaf on this thread once the danger has passed. The presence of larvae in florets can result in complete rejection of produce, even if the level of plant tissue removal is insignificant. There are four larval instars before pupation occurs and at 25 ºC, the life cycle from egg to adult emergence takes approximately 24 days (Charleston, 2004).

Its natural host range is limited to cultivated and wild Brassicaceae (Sarfraz *et al.*, 2006), although records of sporadic occurrences on other crops exist (Löhr, 2001). An abrupt host shift to sugar snap- and snowpeas (*Pisum sativum* L.) was reported in Kenya, resulting in heavy damage to these crops. Pupae of cabbage-

fed DBM were observed to be significantly heavier than those that fed on peas (Rossbach *et al*., 2006). Earlier, Löhr and Gathu (2002) did not observe any significant difference in pupal mortality for larvae from both hosts when larvae of equal weight were compared, suggesting acceptable suitability of peas for larval development once the new host is accepted. Obtained results confirmed the existence of a new DBM strain adapted to pea and from observation, DBM started feeding on peas in an outbreak situation when population on the original host became extremely high.

Control of P. xylostella

Cultural control

Cultural control measures are common among subsistence, small-scale farmers in Africa (Abate, 1997) and involve manipulating existing environmental factors, as opposed to adding new technologies such as insecticides and natural enemies (Pedigo, 2002). These control practices could be in the form of trap cropping, intercropping, use of plants with repellent or insecticidal properties, crop rotation, or specific responsive actions to reduce pest attack, such as weeding (Abate *et al*., 2000).

Trap cropping

The trap crop hypothesis put forward by Vandermeer (1992), proposes that the observed reduction of pests on a principal crop is due to the attractiveness of a second crop in its vicinity to such pests. The idea is that the presence of a

second crop attracts a pest which would otherwise normally attack the principal crop. The principle of trap cropping depends on the fact that pests may show a distinct preference for a certain plant species, cultivar, or crop stage (Hokkanen, 1991) either in their oviposition or feeding behaviour. For many plant-feeding insects, the selection of an oviposition site is a critical stage in their choice of host. This is especially true when the newly-hatched offspring are not capable of searching for additional hosts or at least not until they have fed on the plant chosen by their mother (Singer, 1986). In many phytophagous insects, particularly in Lepidoptera, immature stages have limited mobility, so their survival is profoundly influenced by the female decision on what host to choose for oviposition (Renwick and Chew, 1994). If the trap crop were a poor host for the pest, it would serve as a sink rather than a source for subsequent generations. This situation has been termed “dead-end” trap cropping (Badenes-Perez *et al.*, 2004).

A number of trap crops have been proposed for *P. xylostella*, such as the Indian mustard, *Brassica juncea* (L.) Czern (Charleston and Kfir, 2000; Åsman, 2002), white mustard, *Brassica hirta* (Moench) (Talekar and Shelton, 1993), collards, *Brassica oleracea* L. var. *acephala* (Mitchell *et al*., 2000) and yellow rocket, *Barbarea vulgaris* (R. Br.) var. *arcuata* (Badenes-Perez *et al.*, 2004; 2005). Badenes-Perez *et al.* (2004) reported a greater efficacy for glossy yellow rocket over collards and Indian mustard, because it was highly attractive for *P. xylostella* oviposition and larvae did not survive on it. The use of trap crops for controlling

P. xylostella has, however, produced mixed results over the years. The method has either been proclaimed as successful (Mitchell *et al*., 2000; Åsman, 2002), unsuccessful (Shelton and Nault, 2004), or unreliable (Musser *et al*., 2005). These contradictory results indicate that a lack of fundamental knowledge about trap crop management for *P. xylostella* exists.

Intercropping

As Finch and Collier (2003) have summarized, host location by insects is made of three basic steps: (i) chemical stimuli (plant odours) indicate when to land; (ii) visual stimuli (colour and contrast) indicate where to land; and (iii) touch and taste indicate host suitability, and hence whether to stay or fly away. According to Andow (1991), crop diversification (intercropping) may reduce pest colonization and crop damage if the addition of nonhost plants interferes with one or more of these steps. The simplest aspect of a cropping system to alter is the visual stimuli present, so that when insects are stimulated to land (step 1) they have difficulty accurately locating the host plant (step 2) (Broad *et al.*, 2008). For most Lepidoptera, visual perception of plant colour and shape are dominant sensory cues when searching for host plants (Renwick and Chew, 1994). Åsman *et al.* (2001) observed a significant reduction in the number of *P. xylostella* eggs laid when white cabbage, *Sinapis alba* L., was intercropped with tall red clover, *Trifolium pratense* L., compared to a cabbage monoculture. Similarly, in a study comparing Brussels sprout, *B. oleracea gemmifera*, and malting barley, *Hordeum vulgare* L., row intercrop to a Brussels sprout monoculture, Bukovinszky *et al.*

(2004) recorded a lower number of *P. xylostella* larvae, pupae and moths per sprout plant in intercropped plots than in monocultures. A host-deprivation experiment (Åsman and Ekbom, 2006) showed that unlike the leek moth, *Acrolepiopsis assectella* Zeller, *P. xylostella* that waited for 10 days before being provided with a suitable host had a lower total fecundity, as well as a shorter oviposition period. The inability of *P. xylostella* to postpone egg production and oviposition like *A. assectella* did in the absence of a suitable host makes the former a good candidate to be managed by the use of intercropping.

Botanical pesticides

Many secondary metabolites found in plants have a role in defence against herbivores, pests and pathogens through deterrence/antifeedant activity, toxicity, modification of insect development or acting as precursors to physical defence systems (Bennett and Wallsgrove, 1994). Morallo-Rejesus (1986) identified 6 plant species that are active against *P. xylostella* in the Philippines and reported that out of the 1,800 plant species said by Grainge *et al.* (1984) to possess pest control properties, only 82 species were active against the DBM. Plants within the Meliaceae, Asteraceae, Fabaceae and Euphorbiaceae contain most of the insecticidal plant species reported. Most studies on botanical pesticides have, however, centered on plants from the mahogany family, Meliaceae, and in particular on members of the genera *Azadirachta* and *Melia*, which are outstandingly effective against insects (Carpinella *et al*., 2002; Akhtar *et al*., 2008). The neem tree, *Azadirachta indica* A. Juss., and the syringa tree, *Melia*

azedarach L., are the species of these genera that are commonly used for pest control (Nakatani *et al.*, 1995). *Azadirachta indica* does not grow in South Africa but *M. azedarach* (originally imported from India for ornamental and shading purposes) is thriving here (van Wyk *et al*., 2002; Charleston *et al.*, 2005).

The active ingredients in plants within the Meliaceae have been identified as modified triterpenes, which are limonoids derived from the precursor 4,4,8-trimethyl-17-furanylsteroid skeleton (Koul *et al.*, 2002). Azadirachtin, a tetranortriterpenoid occurring in *A. indica*, is a well documented example of a secondary plant compound with deleterious effects on behaviour and physiology of a broad range of insect species (Lucantoni *et al*., 2006; Habluetzel *et al*., 2007). Toosendanin isolated from *M. azedarach* also has strong antifeedant, toxic and growth inhibitory effects on insects (Koul *et al*., 2002). Other potent insecticidal tetranortriterpenoids (azedarachin C, salannal, meliacarpinin A, B, C, D and E, salannin, deacetylsalannin, trichilins, nimbolinin B and nimbolidin B) have been isolated from the root bark of *M. azedarach* (Huang *et al.*, 1995; 1996; Nakatani *et al.*, 1995). Meliartenin, which exists as a mixture of two potent isomers, was isolated from fruits of *M. azedarach* and the isomeric mixture is a strong antifeedant (Carpinella *et al.*, 2002). There is ample information in the literature of potent limonoids being isolated from other parts of *M. azedarach*, such as the stem bark and leaves.

Comparative studies have shown that insecticidal activity of extract from *M. azedarach* varies with the species of insect pest involved. Ventura and Ito (2000) observed a higher antifeedant effect on *Diabrotica speciosa* (Genn.) by aqueous extract obtained from syringa flowers followed by extracts from the fruits, stems and leaves in descending order. Brunherotto and Vendramim (2001), however, observed higher bioactivity effects on tomato pinworm, *Tuta absoluta* (Meyrick), for aqueous extract obtained from leaves followed by those from the raw fruits, branches and ripe fruits in descending order. Defagó *et al*. (2006) observed that methanolic extracts obtained from unripe fruits and green or senescent leaves of *M. azedarach* deterred feeding and contributed to mortality of adult elm leaf beetle, *Xanthogaleruca luteola* Muller, equally.

Botanical insecticides have shown encouraging results and already, there are commercial products of plant origin in the market. Despite the success, the products are not able to replace all synthetic insecticides; rather they are only alternatives that may be used in IPM programs in combination with other available control measures (Silva-Aguayo, 2004). Plant chemicals are biodegradable and selective in their activity, suggesting that their application would be environmentally acceptable and compatible with IPM programs, as well as being effective in countering insect resistance. Studies have shown that they are indeed compatible with biological control of *P. xylostella* as they have little or no deleterious impact on its natural enemies (Charleston *et al.*, 2005; 2006a, 2006b).

Chemical Control

Application of synthetic insecticides has dominated efforts to control *P. xylostella* since the mid 1950s (Talekar and Shelton, 1993). Compounds from virtually all classes of insecticides have been used, including organochlorines, organophosphates, carbamates, pyrethroids and acylureas (Fauziah *et al*., 1992; Khaliq *et al*., 2007). Synthetic pesticides are invaluable in suppressing damage to cabbage, but frequent and indiscriminate uses have led to elimination of important natural enemies. This necessitated a continuous use of the chemicals which eventually caused resistance and control failures. In 1953, Ankersmit observed the first ever insecticide resistance to DDT in the diamondback moth (Talekar and Shelton, 1993) and by 1989, the pest had become resistant to 51 compounds (Vasquez, 2001). In many crucifer-producing regions, it has shown significant resistance to almost every synthetic insecticide applied in the field (Talekar and Shelton, 1993). In addition, diamondback moth has the distinction of being the first insect to develop resistance in the field to the bacterial insecticide *Bacillus thuringiensis* Berliner (Tabashnik, 1994). Its resistance to newer insecticide chemistries, including spinosad, indoxacarb and emamectin benzoate, was also reported recently (Zhao *et al.*, 2006).

The management of insecticide resistance in *P. xylostella* has led to a series of studies (Tabashnik *et al.*, 1994; Roush, 1997) and it has been observed that resistance can disappear when the insect is removed from the selection pressure of the insecticide. One way to manage or delay the development of resistance in

P. xylostella is to use insecticides only when needed, but this requires the setting up of action thresholds (Hines and Hutchison, 2001). Another option for delaying rapid development of resistance is rotational application of insecticides which differ in function and resistance mechanism (Hama, 1992). The use of temporal rotations (i.e. alternations) of insecticides is based on the assumption that the frequency of individuals resistant to one toxin declines when a different toxin is applied (Tabashnik, 1994). When any sign of resistance is detected, it is advised that the affected insecticide is changed immediately for another appropriate insecticide. In making a rotational system effective, the intervals between applications need to be long enough so that initial resistance fully disappears before that insecticide is reused. However, Hama (1992) noted that the intervals between insecticide applications are usually very short. With such application regimes, it is very difficult to repress the rapid development of resistance, even if a rotational application is employed. It was, therefore, suggested that a method of integrated pest management could be developed in order to reduce the number of insecticidal applications, incorporating rotational application with other techniques.

Biological control

Biological control is an environmentally sound and effective means of reducing or mitigating pests and pest effects through the use of natural enemies. It is widely recognized as a major component of *P. xylostella* management strategies. All stages of the diamondback moth are attacked by numerous parasitoids and

predators, but parasitoids are the most widely studied. Although over 130 parasitoid species are known to attack various life stages of *P. xylostella*, most control worldwide is achieved by relatively few hymenopteran species belonging to the ichneumonid genera *Diadegma* and *Diadromus*, the braconid genera *Microplitis* and *Cotesia*, and the eulophid genus *Oomyzus*. Larval parasitoids, belonging to genera *Cotesia*, *Microplitis* and *Diadegma*, are the most predominant and effective, followed by pupal parasitoids (Sarfraz *et al.*, 2005). Egg parasitoids do not exert adequate control, as they require frequent mass releases (Talekar and Shelton, 1993).

Investigations of the pest status of *P. xylostella* in different parts of the world indicate that, despite its wide climatic adaptability, it appears to be held in check by parasitoids in some countries (Lim, 1986). Countries that continue to be plagued by *P. xylostella* appear to share the same problem – the absence or ineffectiveness of indigenous parasitoids. The importance of parasitoids in the management of *P. xylostella* has been witnessed by the reduction of damage after one or more parasitoid species have been introduced into an area where native parasitoids were either ineffective or absent (Kfir and Thomas, 2001). Diamondback moth populations native to different regions differ genetically and biologically, and specific parasitoid strains may be associated with a specific *P. xylostella* strain. Therefore, accurate identification based on genetic studies of both host and parasitoid is crucial to attaining successful control of *P. xylostella* through inoculative or inundative releases.

1.3.2 *The parasitoid*

Cotesia plutellae Kurdjumov (Hymenoptera: Braconidae) is the most common native, solitary endoparasitoid attacking larval stages of the diamondback moth (Mosiane *et al.*, 2003; Nofemela, 2004). The parasitoid is dominant in South Africa both at low (< 500 m) and high elevations (> 1000 m) (Kfir, 1997; Mosiane *et al*., 2003). Waladde *et al.* (2001) observed that widespread use of various insecticides regarded as highly toxic to adults of *C. plutellae* did not eliminate the parasitoid. It was, therefore, considered worthwhile to find out whether *C. plutellae* has strains with the potential of developing some degree of tolerance or resistance to certain chemical pesticides. Such strains, if found, would be very useful biocontrol agents as they would remain effective in IPM systems involving pesticide application. In an instance, contribution of *C. plutellae* to total parasitism on DBM in cypermethrin-treated fields ranged between 80–90% while those of other indigenous parasitoids remained below 25% (Smith and Villet, 2003).

The biology of *C. plutellae* on DBM was recently studied in South Africa by Nofemela (2004). Fecundity study revealed that *C. plutellae* is capable of parasitizing hosts successfully on the day of eclosion. Its fecundity was estimated at 42.13 ± 12.20 eggs (mean ± s.d.) and adult female life span was 5.23 ± 2.70 days. A *no-choice test* showed that the parasitoid has a higher preference for third instar hosts than for second and fourth instars while a *choice test* on the other hand, revealed a higher preference for second instar hosts than third and

fourth instars. Host-instar preference experiments showed that the parasitoid laid more female eggs in second instar hosts than in third and fourth instar hosts. Number of days required by *C. plutellae* eggs to develop to adult wasps was significantly lower in fourth instar DBM larvae (11.5 ± 0.71) than in the second (13.95 ± 0.11) and third instar larvae (14.28 ± 0.19). In a comparative experiment, parasitism of *P. xylostella* due to *C. plutellae* was observed to be significantly higher than that due to *Diadegma mollipla* (Holmgren) (Hymenoptera: Ichneumonidae). The former was said to be a better biocontrol agent of *P. xylostella* because it has a shorter generation time, higher production of female progeny in younger hosts, lower interference among searching females, and a relatively wider thermal tolerance (21–33 ºC).

Various authors (e.g. Vuorinen *et al*., 2004; Roux *et al*., 2007) have considered *C. plutellae* to be host specific to DBM, but a summary of laboratory and field records (Fitton and Walker, 1992) noted that *C. plutellae* had been reared from 20 species of Lepidoptera. Furthermore, host specificity assessments using *no-choice tests* (Cameron and Walker, 1997) demonstrated that *C. plutellae* is capable of developing in several species of Lepidoptera other than *P. xylostella* while a *choice test* (Endersby and Cameron, 2004) demonstrated that *C. plutellae* is capable of parasitizing *Nyctemera amica* (White) (Lepidoptera: Arctiidae) in the presence of *P. xylostella*. Shiojiri *et al.* (2000) found that *C. plutellae* responds to volatiles emitted from cabbage plants damaged by host larvae, and not to those emitted directly from insect hosts, suggesting it may

potentially attack several host species on cabbages. Rate of parasitism by *C. plutellae* on DBM populations and the level of control exerted on the pest have been observed to vary geographically. Mean percentage parasitism reported are 36% in Malaysia (Ooi, 1992), 40% in Taiwan (Talekar *et al.*, 1992), 57% in Cotonou (Goudegnon *et al.*, 2004), 70% in the Philippines (Poelking, 1992), 80% in St. Helena (Kfir, 2003) and 88% in South Africa (Smith and Villet, 2003). A taxonomic study (Rincon *et al.*, 2006) on five populations of *C. plutellae* from South Africa, Benin, Martinique, Reunion, and Taiwan showed the parasitoid to be a single species composed of at least two partially incompatible population aggregates.

Nutritional ecology of parasitoids

The juvenile and adult stages of parasitoids usually differ considerably in their nutritional requirements and food ecology. While the former often feed on the visceral mass of their hosts, the latter feeds primarily or exclusively on plant-provided food supplements. Adult parasitoids depend on sugar-rich and energy-giving food sources such as floral and extra floral nectar, plant sap or leached phloem sugars and homopteran honeydew for survival and egg maturation (Wäckers, 2005). These carbohydrates also serve to satisfy energy requirements for locomotion, longevity, and reproductive physiology of parasitoids (Fuchsberg *et al*., 2007; Onagbola *et al*., 2007). Adult females of some parasitoids obtain essential nutrients directly from hosts through so-called host feeding, but even these species often need non-host food sources as a source of energy (Jervis *et*

al., 1996). The host-feeding parasitoid species may obtain some carbohydrates this way (Jervis and Kidd, 1986) but host haemolymph is probably a relatively poor source of carbohydrates. Not only are haemolymph carbohydrate levels generally low (Kimura *et al*., 1992), the main haemolymph sugar (trehalose) is rather poorly metabolized by parasitoids (Wäckers, 2001).

The sugar composition of nectar and honeydew shows a broad variation both regarding the type of sugars present and the overall sugar concentration. Sucrose and its hexose components glucose and fructose are the most prevalent sugars in nectars and honeydews (Koptur, 1992). However, various other sugars can occur as well, sometimes in significant concentrations. Insect species vary considerably with respect to the spectrum of nectar- and honeydew-sugars they can utilize (Ferreira *et al*., 1998). In addition to sugars, natural food sources such as nectar and honeydew may contain various amino acids, proteins, lipids, antioxidants, organic acids and other non-nutritive substances (Wäckers, 2005), and the role of these individual compounds on the fitness of parasitoids have been reported in literature. McDougall and Mills (1997) showed that 43% fructose and sucrose solutions prolonged longevity of *Trichogramma platneri* Nagarkatti to approximately 20 days; an increase by a factor 13 relative to control insects kept with water only. Leatemia *et al*. (1995) compared the effect of different carbohydrate sources (honey, sucrose, fructose) on longevity, fecundity and progeny sex ratio of *T. minutum* Riley. In terms of longevity, the positive effect of the two carbohydrate solutions was almost as strong as the effect of the honey

solution but in terms of fecundity, honey-fed individuals outperformed parasitoids kept with sucrose or glucose. These results indicate that parasitoids use honey compounds other than carbohydrates (e.g. amino acids or proteins) for egg production.

Feeding does not only increase longevity and fecundity of parasitoids, but also affects flight activity (Wäckers, 1994) and attraction to and/or retention in an area (Stapel *et al*., 1997). Parasitoid females that feed on hosts or their by-products reduce the need to shift from host searching to food foraging, whereas parasitoids that only feed on food sources that are not associated with hosts will frequently have to forage for hosts and food separately (Sirot and Bernstein, 1996; Lewis *et al*., 1998). When food is located at a distance from host sites, this switching between resources may become particularly costly, since travelling to food sites limits the amount of time available for host searching, costs energy and increases the risk of mortality (Jervis *et al*., 1996; Stapel *et al*., 1997). Sugar feeding can also increase host encounter rates as a parasitoid's energy reserves determine the individual's overall flight propensity and searching activity (Forsse *et al*., 1992; Pompanon *et al*., 1999). Food-deprived parasitoids primarily engage in food foraging, rather than seeking out hosts while well-fed parasitoids are usually more active and more focused in seeking out their herbivorous hosts (Wäckers, 1994; Takasu and Lewis, 1995).

The timing of feeding is also important for parasitoids as it enhances survival. Many parasitoid longevity studies suggest that adults need to locate food at least once per day to avoid starvation (Irvin *et al*., 2006; Lee and Heimpel, 2008), stressing the importance of frequent sugar sources to parasitoids. As sugar sources can be highly variable in quantity, space, and time, the chances of finding an amount of sugar sufficient to increase longevity from a single feeding event can be critical for the forager's fitness (Jervis and Kidd, 1999). Therefore, the time spent looking for sugar sources in the field could be crucial for parasitoid survival. Judicious habitat management has been a way in which entomologists have altered habitats to improve availability of the resources required by natural enemies for optimal performance. The goal of habitat management is to create a suitable ecological infrastructure within the agricultural landscape to provide resources such as food for adult natural enemies, alternative prey or hosts, and shelter from adverse environmental conditions (Landis *et al*., 2000). For example, well-timed food sprays have been used to supplement in agricultural systems lacking pollen and nectar resources (Mensah, 1997). Alternatively, the establishment of perennial flowering plants close to crop fields may provide similar resources in a more stable fashion over the entire season and for years to come (Long *et al*., 1998). This way, parasitoids would have a better access to food sources and their performance in regulating insect pests would, invariably, be enhanced. The use of floral resources such as *Coriandrum sativum* L. has been evaluated in cabbage crops in New Zealand for the attraction of hoverflies to reduce aphid infestations in the field (Morris and Li, 2000). When coriander

was present in the field, the number of caterpillars of *Plutella xylostella* and *Pieris rapae* (L.) (Lepidoptera: Pieridae) decreased as compared with the control. In this case hoverfly larvae were responsible for the decrease in larval densities. The presence of buckwheat *Fagopyrum esculentum* Moench in the broccoli-strip doubled the parasitism rates on *P. xylostella* by *Diadegma semiclausum* when compared with the control (Lavandero *et al*., 2005). Similarly adding buckwheat to an apple orchard increased parasitism levels of leafrollers by *Dolichogenidea tasmanica* (Cameron) (Stephens *et al*., 1998). Later laboratory experiments with *D. tasmanica* showed not only that buckwheat can enhance leafroller fitness but that it is a potential host plant for oviposition (Irvin et al., 2006). Another example in vineyards showed that by providing buckwheat flowers, parasitism rate of grape leafhoppers (*Erythroneura* spp.) was increased (English-Loeb *et al*., 2003). In a similar way, proximity to buckwheat floral patches significantly increased rates of parasitism of *Aphidius rhopalosiphi* De Stefani-Peres and *Diaeretiella rapae* (McIntosh) (Hymenoptera: Aphidiidae) in a wheat field (Tylianakis *et al*., 2004). A lack of nonhost food resources for natural enemies has long been recognized as a potentially important contributor to the failure of some biological control programs (Gurr and Wratten, 1999).

Developmental strategies in parasitoids

Parasitoids are insects that lay their eggs in the body, or on the body, of other insects. The term was coined by the German writer O.M. Reuter in 1913 (Whitfield, 2003) to refer to those parasites that invariably kill their hosts as part

of the process of exploiting them. Larval parasitoids consume all or most of the hosts' body and then pupate, either within or outside the body of the hosts. By the time the parasitoid larvae pupate, the hosts would be very weak and die soon after. Adult parasitoids usually emerge from the pupae (Fig. 1.1) and start the next generation anew by actively searching for hosts in or on which to oviposit. Parasitoids occur in several orders of insects (Diptera, Coleoptera, Lepidoptera, Trichoptera, Neuroptera, Strepsiptera), but they are especially common in the Hymenoptera, for which estimates suggest that 10–20% of all Hymenoptera may be parasitoid wasps (Godfray, 1994; Quicke, 1997; Whitfield, 2003).

Endoparasitoid larvae and eggs may die owing to a reaction of the host's immune system: the hosts are capable of mounting a defensive response against foreign bodies. Host defence reactions are of several kinds (Strand and Pech, 1995; Carton and Nappi, 1997) but the most commonly encountered type of reaction is encapsulation. Usually in encapsulation the invader becomes surrounded by a multicellular sheath composed of the host's haemocytes. Successive layers of cells can often be discerned, and on the outer surface of the parasitoid egg or larva there often develops a necrotic layer of melanised cells, representing the remnants of the blood cells that initiated the encapsulation reaction. The melanin deposits on the surfaces of encapsulated parasitoid eggs and larvae often provide the first clue to the occurrence of encapsulation. Parasitoid immatures die probably from asphyxiation, although starvation may be the principal cause of death in some cases. Phagocytosis of parasitoid tissues

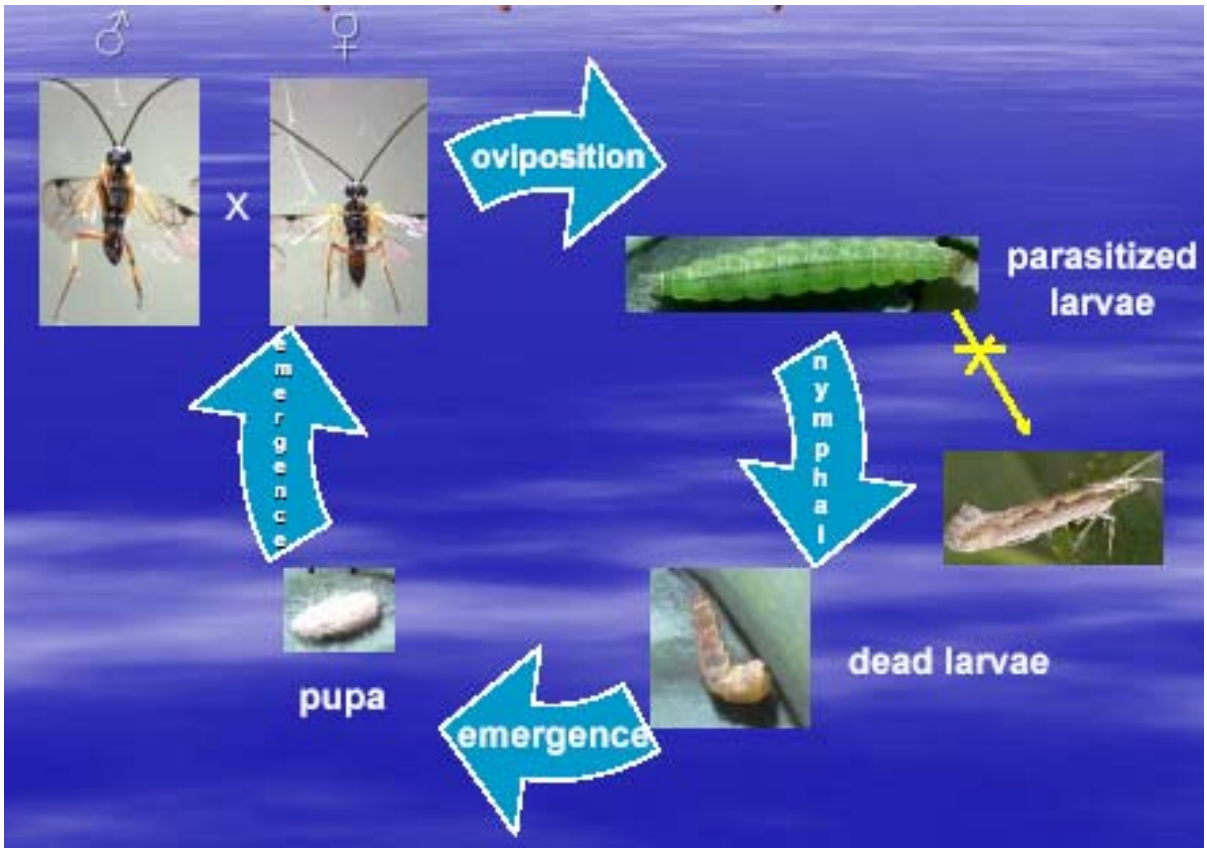


Figure 1.1: Life cycle of *Cotesia plutellae* showing how parasitized larvae are prevented from giving rise to new diamondback moths and young wasps eventually eclose from cocoons. Eight to ten days after oviposition, the nymph eats its way out of the dying larva and builds a cocoon where it pupates. The adult wasp emerges from the cocoon 5–7 days later.

gradually occurs, at least during the initial stages of encapsulation (Jervis *et al*., 2007).

Parasitoids have evolved various mechanisms by which they resist, i.e. evade and or suppress the immune responses of their hosts, thereby ensuring successful development of their progeny.

Evasion of immune responses

Larval endoparasitoids take advantage of some passive means of evading encapsulation by their hosts. They achieve this by either ovipositing or developing in host tissues that are inaccessible to host haemocytes. Many platygastrids, for example, oviposit in the host's gut or ganglia (Strand and Pech, 1995), while some braconids lay eggs in the host's fat body (Carton and Nappi, 1997). Other endoparasitoids oviposit in the host's hemocoel, but passively evade encapsulation because their progeny have surface features that are not recognized as foreign or to which the hemocytes are unable to bind (Schmidt *et al.*, 2001). Immunoevasive ovarial proteins coat the eggs of several braconids in the genera *Cotesia* and *Toxoneuron* (Tanaka *et al.*, 2002; Hu *et al*., 2003). In contrast, eggs of *Cotesia congregata* (Say) are fully susceptible to encapsulation and they depend on *C. congregata* bracovirus (*Cc*BV) infection for protection, but larvae have surface features that protect them from encapsulation, independent of virus infection (Lavine and Beckage, 1996). Other parasitoids, such as the polyembryonic encyrtid *Copidosoma floridanum* (Ashmead), remain enveloped

by their extraembryonic membrane throughout development, for both nutrient absorption and protection from the host's immune system (Corley and Strand, 2003; Giron *et al.*, 2004).

Suppression of immune responses

During oviposition, female parasitoids co-inject a cocktail of maternal secretions made of calyx fluid, viruses and venom into the host hemocoel. The components of each type of secretion may influence host physiology and development independently or in a synergistic fashion.

Calyx fluid and viruses

The family Polydnaviridae consists of segmented, double-stranded DNA viruses that are associated specifically with certain types of parasitoid wasps. Polydnaviruses (PDVs) are divided into two types, bracoviruses (BVs) and ichnoviruses that coexist with wasps in the families Braconidae and Ichneumonidae, respectively (Fleming, 1992). All PDVs persist as stably integrated proviruses in the genomes of wasps and replicate asymptomatically in ovarial cells that form a region of the female reproductive tract called the calyx. Virions accumulate in the lumens of the oviducts, and the resulting suspension of virus and protein is called calyx fluid (Beck and Strand, 2005). This fluid is deposited onto the egg as it is laid. PDVs do not replicate in the wasp's host, but the expression of PDV-carried genes causes physiological alterations in the host that are essential for the survival of the parasitoid's progeny. Thus, a true

mutualism exists between PDVs and wasps, as viral transmission depends on parasitoid survival and parasitoid survival depends on infection of the wasp's host by the virus.

PDVs suppress host's immune system by preventing encapsulation of the parasitoid egg and by modifying the host's growth, development, morphology and behaviour. Studies with several parasitoid-host systems have indicated that PDV-infected hosts do not mount an encapsulation response, which in turn allows the parasitoid's offspring to develop successfully (Lavine and Beckage, 1996; Webb and Luckhart, 1996). Hosts parasitized by the braconid *Microplitis demolitor* Wilkinson are unable to encapsulate parasitoid eggs or other foreign targets because haemocytes infected by *M. demolitor* bracovirus (MdBV) lose the capacity to adhere to foreign surfaces 4 to 6 h postinfection (Strand *et al*., 1999). Haemocytes in primary culture and the hemocyte-like cell line BTI-TN-5B1-4 (High Five cells) from *Trichoplusia ni* (Hübner) also lose the ability to adhere to foreign surfaces after infection by MdBV (Beck and Strand, 2003).

Other parasitoids use virus-like particles (VLPs, e.g. *Leptopilina boulardi* Barbotin) to counteract the host immune reaction. VLPs have structures that superficially resemble viruses but that lack nucleic acids (Quicke, 1997). VLPs are used in several ways by the parasitoid. They can form a shield around the parasitoid egg in order to make it invisible for the host immune system. VLPs can also be used to attack the haemocytes directly.

Venom

Venom is produced in specialized glands that are associated with the reproductive system of female hymenopteran wasps. The venom fluid is chemically described as consisting of alkaloids, terpenes, polysaccharides, biogenic amines (such as histamine), organic acids (formic acid), and amino acids, but the majority are peptides and proteins (Asgari, 2006). The venoms of parasitic Hymenoptera serve very different functions in ectoparasitoids and endoparasitoids. Venoms from many ectoparasitoids that have been studied act on their hosts through various physiological effects including permanent paralysis or killing the hosts, host development arrest and host metabolic regulation (Nakamatsu and Tanaka, 2003), as well as inhibition of host immunity (Rivers *et al*., 2002). In contrast, the role of endoparasitoid venom in suppressing host immune defence and regulating host development has not been clearly determined in most cases (Zhang *et al*., 2005). Its presence, in some instances, is not essential for successful parasitism to occur (Webb and Luckhart, 1994). In some braconid species, however, venom may act synergistically with calyx fluid or polydnavirus to inhibit host-encapsulation response or regulate host development (Beckage and Gelman, 2004). In addition, a limited number of studies suggest that endoparasitoid venom alone can upset host immune defence or delay host development. For example, venoms from *Pimpla hypochondriaca* Retzius (Hymenoptera: Ichneumonidae) and *Pteromalus puparum* (L.) (Hymenoptera: Pteromalidae) suppress immune cellular response in their respective hosts, *Lacanobia oleracea* L. (Lepidoptera: Noctuidae)

(Parkinson *et al*., 2002) and *Pieris rapae* L. (Lepidoptera: Pieridae) (Cai *et al*., 2004), and the venom from *Aphidius ervi* Haliday (Hymenoptera: Braconidae) alone acts in inducing developmental arrest and death of its host, *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae) (Digilio *et al*., 1998).

Concept of fitness in parasitoids

Parasitoid fitness (measured by life history traits, such as body size, fecundity, longevity, mate location ability, foraging efficiency, developmental rate, and survival of immature stages) varies with host species, as well as size and/or age of the host (Harvey and Vet, 1997; Harvey, 2000). Parasitoids use a variety of olfactory, visual, mechanosensory, and gustatory cues to recognize suitable host species and to distinguish between different sizes and/or developmental stages of the same host. A female parasitoid completes the host-selection process by inserting her ovipositor into the host to further evaluate its suitability and nutritive quality (van Alphen and Jervis, 1996). After selecting a victim, a host-feeding parasitoid decides whether to feed from the host, to oviposit, or to do both. If it chooses to oviposit, then it decides on sex allocation and offspring number. Haplodiploidy (the production of haploid males from unfertilized eggs and diploid females from fertilized eggs) allows female wasps to determine the sex of their offspring (Fellowes *et al.*, 2007). The size of parasitoids is often determined by host size and as there is a positive correlation between parasitoid body size and fitness, larger hosts should be preferred. However, this relationship is much stronger for female offspring, such that an ovipositing wasp maximizes her

fitness by placing female eggs in better quality hosts. Female-biased sex ratios are clearly the preferred outcome of parasitoid mass-rearing for biocontrol interventions.

The majority of adult parasitoids depend entirely or primarily on carbohydrate-rich food as their main source of energy (Jervis *et al.*, 1993) which is stored as glycogen in the fat body or as either trehalose or glucose in the hemolymph (Rivero and Casas, 1999; Steppuhn and Wäckers, 2004). The derived energy is channeled towards metabolic activities such as growth, flight, food- and host-searching, reproduction and survival (longevity). In the presence of limited resources, the ability of a parasitoid to make appropriate 'choices' in allocating energy to different functions becomes a good measure of its fitness. When energy supply becomes limited, a parasitoid is not able to maximize all traits associated with fitness and, as a result, energy allocation towards different functions trades off (Pelosse *et al.*, 2007). For instance, fecundity and host-searching efficiency, which are crucial to female reproductive success, are both energy-demanding thus necessitating a trade-off in energy allocation (Ellers *et al.*, 2000; Jervis *et al.*, 2005). High reproductive success would be attained by female parasitoids if they approach a perfect match between the number of eggs they carry and the number of hosts encountered during adult life (Rosenheim, 1996; Sevenster *et al.*, 1998). Consequently, the availability and spatial distribution of the hosts may determine how the resources should be invested towards survival and reproduction (Ellers and van Alphen, 1997).

Apart from feeding and mating, parasitoid fitness is also affected by a wide range of environmental variables such as temperature (Colinet *et al.*, 2007), photoperiod (Sagarra *et al.*, 2000) and season (Ellers *et al.*, 2001). Temperature (as a fundamental component of the microclimate) becomes more important in the hot, humid summer conditions. In general, longevity of adult insects is inversely related to temperature (McDougall and Mills, 1997), probably due to an increased use of energy reserves at higher temperatures. However, the degree to which temperature affects longevity varies with species and gender, and some species are more tolerant of temperature extremes than others (Nofemela, 2004). Availability of host patches and developmental history of individuals or generations are two season-dependent factors whose changes may influence parasitoid fitness. Ozkan (2007) observed an increase in egg load of *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae) that was reared in continuous darkness. He also noted that the parasitoid often rested in darkness. Earlier, Scott and Barlow (1984) observed that the parasitoid rarely attempted flight in continuous darkness, and they reported that decreased longevity was associated with increased flight activity. It would, therefore, be logical to infer that reduced flight activity saved energy, which the parasitoid channeled toward achieving increased egg load (Hoferer *et al.*, 1999).

1.4 The research problem

The scarcity of *C. plutellae* in the field during early spring, when the number of DBM start increasing, has been a subject of concern. As the weather warms up

in spring, the population of DBM increases suddenly, reaching its peak, while that of *C. plutellae* remains low, and by late spring when the parasitoid population increases to an appreciable level (Fig. 1.2), a measurable damage to the cabbage crop is already done (Waladde *et al.*, 2001). Efforts have been directed toward ensuring a continuous existence of effective *C. plutellae* populations in cabbage fields throughout the growing season, especially during the early phase. Attempts at introducing *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae) into South Africa remains unfruitful. This ichneumonid effectively complemented the activity of *C. plutellae* in controlling DBM in Malaysia (Ooi, 1979) and Taiwan (Talekar *et al.,* 1992; Talekar and Yang, 1993). Its initial introduction from England in 1936 (Evans, 1939) failed, because apart from recoveries made in 1937, the wasps were never noticed again (Greathead, 1971). In addition, subsequent attempts at establishing *D. semiclausum* in South Africa have been without success (Nofemela, S.R., pers. comm., ARC-PPRI, Pretoria, South Africa, 2008).

Planting nectar-producing floral vegetation within or near crops is often recommended to provide *C. plutellae* and other parasitoids with nutrients (sugar and pollen) and shelter (Ellis *et al.*, 2005; Berndt *et al.*, 2006; Winkler *et al*., 2006). The presence of supplementary nutritional resources in close vicinity of hosts can improve the fitness of the natural enemy and thus, its rate of parasitism. Supplementary nutritional resources could be used to rear *C. plutellae* colony in a facility, which would in turn serve to augment field

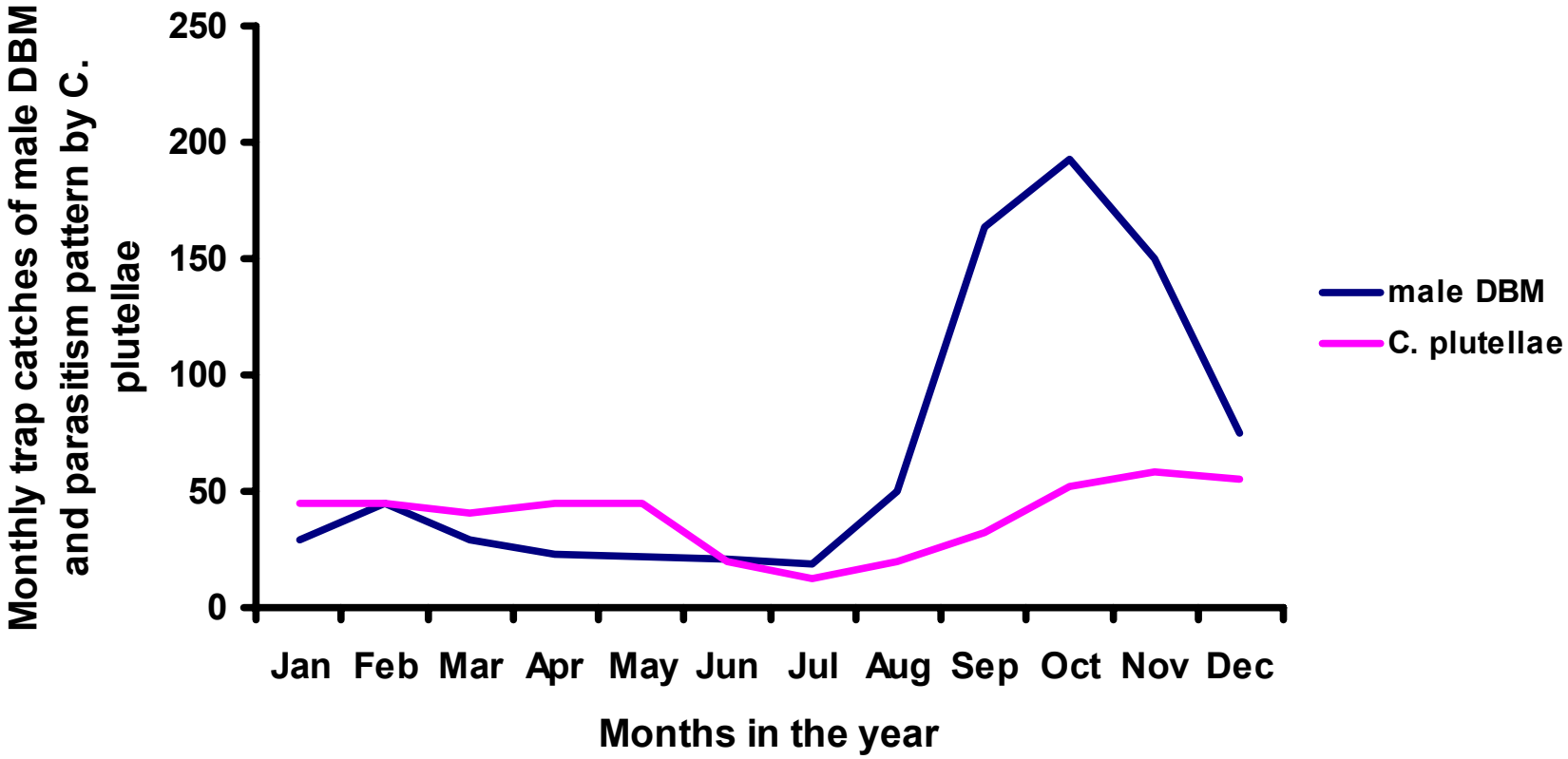


Figure 1.2: Population dynamics of the diamondback moth and pattern of *Cotesia plutellae* parasitism at Alice and Zanyonkwe in 1998 (Deduced from Waladde *et al*., 2001).

populations. Augmenting of *C. plutellae* field population is necessary in early spring.

1.5 Objectives of the study

The main objective of the study was to determine if a novel diet would be able to improve the fitness of *C. plutellae* significantly and sustain a considerable parasitoid population in the laboratory. Such a diet could be incorporated into a large-scale parasitoid multiplication program and resulting *C. plutellae* population would be used to augment in the field during early spring, when the population of this parasitoid is usually low.

A honey-based diet (honey-beebread) and pure honey were investigated for their impact on *C. plutellae*. In this thesis, I addressed the following aspects: (i) performance of *C. plutellae* and *P. xylostella* on the two food sources in the laboratory; (ii) effects of diet on biology (body size, egg load, fecundity and longevity) of *C. plutellae*; (iii) response of *C. plutellae* to diet cues; and (iv) ability of *C. plutellae* to associate food and host with their respective odour.

1.5.1 Thesis outline

The present thesis addresses four main questions:

Chapter 2: What is the effect of honey and honey-beebread on the performance of *C. plutellae* population under laboratory conditions?

A number of studies have been conducted on the impact of supplementary food sources on parasitoid fitness in the laboratory but focus has often been on carbohydrate sources such as pure honey and specific sugars. Synovigenic parasitoids like *C. plutellae* require host hemolymph, which is proteineous, for adequate reproductive performance. In an effort to reduce the dependence of the parasitoid on its host for protein, a dietary protein source, the beebread, was added to honey. A comparison was then made between host-parastoid colonies supplied with either honey or honey-beebread. In contrast to previous studies which often determined short-term effects of supplementary food sources on parasitoids, I monitored the performance of *C. plutellae* and *P. xylostella* populations on the two diets for 10 months. The diets sustained the pest-parasitoid colonies showing that they are good sources of energy for the insects. There were periodic fluctuations in the number of parasitoid/pest which could be due to diet effect. Possible reasons for these fluctuations were further investigated in the second question.

Chapter 3: How does each food source affect the biology of *C. plutellae*?

As performance of a parasitoid is a product of its body size, fecundity, egg load and longevity, I investigated diet effect on the biology of *C. plutellae.* Parasitoids provided with honey-beebread lived longer and produced more offspring than wasps that were fed honey. However, oviposition period did not differ between the two treatments. Maternal diet did not have significant effects on body size and egg load of newly emerged wasps. Likewise, commencement of

posteclosion egg maturation in *C. plutellae* did not depend on diet but on maternal age, reaching peak egg load within 48 h of eclosion.

Chapter 4: Do cues from honey and honey-beebread elicit similar behavioural responses in *C. plutellae*?

Perception of cues is very crucial to utilization of available resources. Having determined that the two food sources can sustain *C. plutellae* population, I attempted to assess both phagostimulatory and olfactory responses of the parasitoid to honey and honey-beebread. The two diets were highly stimulatory, eliciting a feeding response of > 90%. This result was consistent with parasitoid responses to specific sugars that are common in honey. However, in a *choice test* using Y-tube olfactometer, I observed that the wasps preferred odour emanating from honey-beebread to that from honey. This preference was further confirmed using the EAD technique, where higher antennal responses to honey-beebread were recorded.

Chapter 5: Can *C. plutellae* associate food and host with their respective odour?

As honey and honey-beebread were shown to support *C. plutellae* and aroused remarkable responses from the wasps, ability to associate odours with resources might be adaptive. Such ability should decrease the time and energy spent when switching from host to food searching. Female *C. plutellae* with different experiences and feeding status were given a choice to respond to food odour

versus clean air in the first experiment, while food odour was tested against volatiles produced by DBM-infested cabbage seedlings in the second experiment. It was observed that *C. plutellae* has the capacity to associate the two resources with their specific odour.

A summary and conclusion of the research is provided in **Chapter 6**.

CHAPTER 2:

Effects of honey and honey-beebread on the performance of *Cotesia plutellae* in the laboratory

2.1 Introduction

In the wild, adult insects utilize a broad range of food sources, such as floral nectar, extra-floral nectar, pollen, (rotting) fruits, and homopteran honeydew (Wäckers, 2005; Begum *et al.*, 2006). The large number of parasitoid species that do not engage in host feeding are entirely dependent on these nutritional sources. Honeydew and floral nectar commonly contain the monosaccharides glucose and fructose, and the disaccharide sucrose (Wäckers, 2001). However, honeydew also contains oligosaccharides, such as maltose and melibiose (disaccharides), melezitose, raffinose, and erlose (trisaccharides), which are very rare in floral nectar (Baker and Baker, 1983). These oligosaccharides reduce the suitability of honeydew as a food source for parasitoids because (1) the sugars do not elicit a feeding response as readily as do common nectar sugars (Wäckers, 1999), (2) honeydew sugars crystallize quickly compared to sucrose, making it difficult for parasitoids to feed (Wäckers, 2000), and (3) oligosaccharides increase the lifespan of parasitoids to a lesser extent than simpler sugars (Wäckers, 2001; Lee *et al.*, 2004).

Nectar and other sugar supplements are being increasingly recommended as a tool to enhance parasitoid performance (Jacob and Evans, 1998), but identification of selected diets is required in order to avoid inadvertently exacerbating pest damage. Wanner *et al*. (2006) observed a significant influence of floral nectar on flight capacity of *Cotesia glomerata* (L.) as defined by the number of flights, the longest single flight and the total distance flown during a

given period of time. Ellis *et al.* (2005) and Lavandero *et al.* (2005) also observed that parasitoids were more active in habitats in which flowers were in bloom than in nearby habitats without flowers. Studies have shown that distinct differences exist among insect species in their ability to utilize sugars (Ferreira *et al.*, 1998). The ability of some parasitoids to accept a number of sugars that fail to elicit a feeding response in their respective herbivorous hosts have been reported, thereby making the use of (selective) food supplements relevant in biological control programs (Romeis and Wäckers, 2000; Winkler *et al.*, 2005).

Parasitoid performance is closely associated with the quality and quantity of food available to it. Also, the relationship between a parasitoid and its host may be influenced both by the nutrient composition of the diet and the ability of the parasitoid to utilize the diet. This Chapter examines the responses of a multitrophic system [an insect herbivore (*P. xylostella*) and its parasitoid (*C. plutellae*)] to two supplementary food sources (pure honey and honey-beebread). Previous studies (e.g., Berndt and Wratten, 2005; Irvin and Hoddle, 2007) showed that supplementary food sources are able to impact positively on parasitoid body mass, reproduction, developmental rate, longevity and searching efficiency, while some other studies (e.g., Lee *et al.*, 2004) have elucidated that different diets are able to effect different fitness levels in natural enemies. Although nutritional composition of honey depends on the floral source, honey is mostly sugars and it is not a significant source of vitamins or minerals. Beebread on the other hand is rich in essential amino acids, carbohydrates, fatty acids,

vitamins, minerals, trace elements, enzymes and hormone precursors (FAO, 1996). Therefore, the null hypothesis that a diet with higher nutritional value would produce natural enemies with greater performance level was tested in this Chapter.

2.2 Materials and Methods

Diets used in the study

The honey used for this study is produced by *Apis mellifera* L. (Hymenoptera: Apidae) from Karoo wildflowers and packaged by Speelmanskop Honey, Cradock, South Africa. Beebread was obtained from the honeycombs donated by Makana Meadery, Grahamstown, South Africa (www.iqhilika.co.za). One part of the cake-like beebread was crushed in three parts of honey to give a paste referred to in this thesis as honey-beebread. The insects were able to utilize beebread in this state.

Standardized *P. xylostella* and *C. plutellae* colonies

The parent insects were obtained from DBM-infested cabbage plant materials collected from farms in the vicinity of Alice (32°46' S, 26°50' E, altitude 540 m), East Cape Province of South Africa. The DBM and *C. plutellae* which emerged from the field materials were used to establish standardized pest-parasitoid cultures supplied with either honey or honey-beebread. Replicated cages (five per diet) of *P. xylostella–C. plutellae* colonies were established in December 2008. Each cage was started with 40 2nd instar DBM larvae, 6 *C. plutellae*

(2♀:4♂) and 24 cabbage seedlings in 6 containers. The parasitoids were removed from the cages after four days and each cage was supplied with 10 2nd instar DBM larvae 13, 14, 19, and 20 days after cage establishment (DAE) to serve as oviposition substrates for emerging parasitoids. Addition of DBM larvae was stopped at 20 DAE because the emerging parasitoids had enough oviposition substrates to keep the colonies going. The ventilated insect rearing cages used in this work were adopted from Stemele (2005) and they were made of Perspex and nylon mesh (Fig. 2.1). The insect colonies were maintained in the laboratory at 21.09 ± 0.77 ºC (mean ± s.d.), 49.30 ± 8.35% r.h. and 15L:9D photoperiod. This temperature was chosen because apart from the fact that *C. plutellae* has a relatively wider thermal tolerance (21–33 ºC), percent parasitism and percent emergence of the parasitoid were highest at 21 ºC (Nofemela, 2004). Diet, honey or honey-beebread, was streaked thinly on the interior top surface of the cages, while water was provided through a wick of cotton wool in water-filled glass vials, hung on the interior top surface of the cages. Fresh cabbage seedlings grown in a compost medium were regularly supplied in the rearing cages to serve as host plant food for the DBM.

2.3 Data collection and analysis

Weekly assessment of the performance of *C. plutellae* under each treatment started four weeks after cage establishment. The number of diamondback moth and *C. plutellae* present in each cage was counted every Monday between 1000

Figure 2.1: Ventilated insect rearing cages (53 × 53 × 53 cm) with four sides made of clear Perspex, while two opposite sides were entirely covered with nylon mesh to allow free flow of air through the cages. The rearing cages were directly illuminated by a number of FLUORA L58W/77 fluorescent tubes (OSRAM, Germany) which provided adequate light to support the photosynthetic process in the cabbage seedlings.

and 1100h, starting January 12 to September 28, 2009. Changes in the number of pest-parasitoid system were presented in graphical form. The weekly data were subjected to one-way analysis of variance (Program GLM, SAS Institute, 1999) to determine diet effect on the number of emerged DBM and *C. plutellae*. Sample means were separated between treatments using Least Significant Difference procedure. Correlation analysis was carried out to determine relationships between the number of DBM and *C. plutellae* in the cages.

2.4 Results

The first set of *C. plutellae* emerged in established cages 13 days after the larvae were exposed for parasitism. Emerging wasps suppressed DBM population and this became evident in honey-beebread cages three weeks (February 2) before such was recorded in honey cages (February 23) (Fig. 2.2). Negative correlation was recorded between the number of DBM and *C. plutellae*, but the relationship was statistically significant in honey-beebread cages (c = −0.38; d.f. = 37; P = 0.019) and insignificant in honey cages (c = −0.15; d.f. = 37; P = 0.376). Peak population of *C. plutellae* was often followed by a sharp decline after which the number rises again. Average number of DBM that emerged in honey cages was higher than that from honey-beebread cages (F = 4.78, d.f. = 1, P = 0.035). On the other hand, there was no significant difference in the number of *C. plutellae* that emerged in the two treatments (F = 2.80, d.f. = 1, P = 0.103).

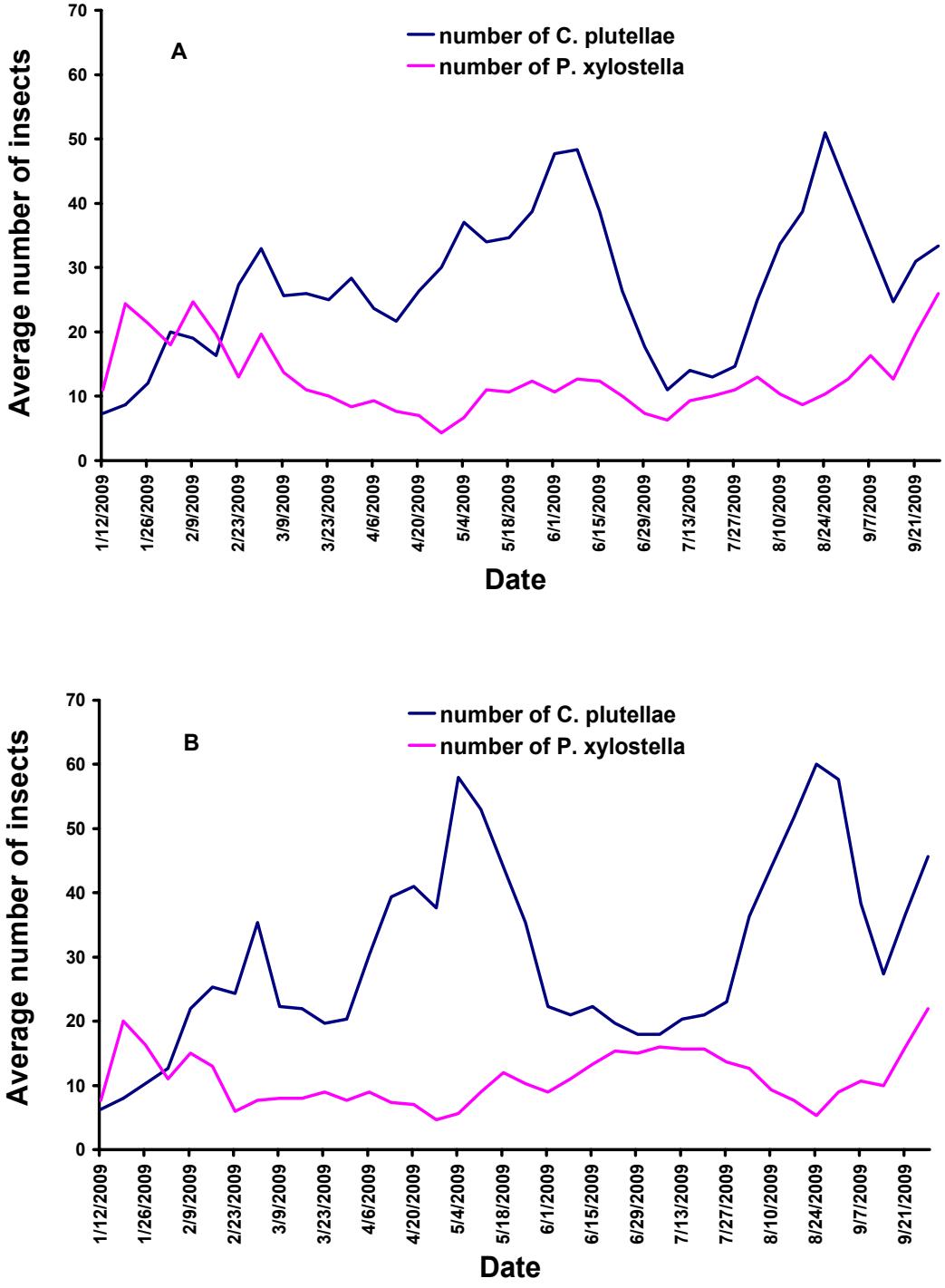


Figure 2.2: Relative abundance of *C. plutellae* and *P. xylostella* in cages provided with (A) honey and (B) honey-beebread between January 12 and September 28, 2009.

2.5 Discussion

Honey-beebread, just like pure honey, showed potential as a good food source for maintaining *P. xylostella–C. plutellae* colonies in the laboratory. However, it appeared that wasps in honey-beebread cages attained adequate fitness faster than those in honey cages as DBM was suppressed much earlier in the former. This disparity could be due to the quality of diet, honey-beebread being more nutritious than honey. It is noteworthy that pest population remained perpetually low right from the date it was subdued in each treatment. This showed that the parasitoid was successfully established in the two treatments and the negative correlation between the number of DBM and that of *C. plutellae* in the cages confirmed that reduction in DBM population was due to parasitism.

The sharp decline in the number of *C. plutellae* after each peak population and subsequent increase in parasitoid number could be attributed to the phenomenon of density-dependent parasitism. Mortality factors, such as parasitism, acting on an insect population can cause three possible dynamic changes. They can: (i) affect the average population density, (ii) induce fluctuations in numbers, and (iii) contribute to the regulation of population numbers (Kidd and Jervis, 2007). For a factor to regulate, the strength of its action must be dependent on the density of the population affected. Thus, its proportional effect has to be greater at high population densities and smaller at low densities. It is logical to assume that since the parasitoid needs its host to multiply, it would rather not eradicate the host in its entirety but maintain the number within certain limits. In the present

study, *C. plutellae* attacked more larvae at high larval densities and when the larval population was depleted, the parasitoid reduced the rate of parasitism. This reduction allowed DBM larvae to increase in number, accompanied by an increase in rate of parasitism till peak attack was attained and the whole process of number regulation was repeated. Emergence of wasps from eggs laid at high larval densities gave rise to the peaks in Fig. 2.2, followed by the sharp descent recorded for fewer wasps that emerged from reduced number of eggs laid at low larval densities. It could, therefore, be said that the regulatory activity of the parasitoid kept the colonies alive in the laboratory.

CHAPTER 3:

Effects of diet on fecundity, egg load, body size and longevity of *Cotesia plutellae*

3.1 General introduction

It is generally appreciated among entomologists that adult parasitoids require food in order to maintain aspects of fitness, such as longevity, fecundity, and offspring sex ratio (Jervis *et al*., 1992). Adult parasitoids are known to survive on food items like nectar, pollen, homopteran honeydew, honey and sucrose solution. In addition, parasitoid species which emerge with a small fraction of their potential egg complement but continue to mature eggs for variable periods throughout the course of adult life (termed 'synovigenic') need to feed on host hemolymph to derive proteins necessary for reproduction (Chan and Godfray, 1993).

Nutritional factors are very crucial to the success of parasitoids. Dietary carbohydrates are important to both the somatic maintenance and reproductive capacity of parasitoid hymenopterans (Thompson, 1999; Casas *et al.,* 2005). Lipids are of key importance to insect reproduction as major constituents of the oöcyte dry mass and as the main source of energy for the developing embryo (Ziegler and Van Antwerpen, 2006). Also, experiments using the larval ectoparasitoid *Exeristes roborator* (F.) have shown that essential amino acids are crucial to survival and growth of the natural enemies (Thompson, 1986).

Energy derived from feeding is used for vital activities such as flight and handling of hosts during parasitism. The capacity of female parasitoids to fly is very important in agroecosystems as the two vital resources for parasitoids, i.e. host

and food, are often not found in the same place. This necessitates frequent movement by flight between host- and food-containing areas for the parasitoids to reproduce successfully (Lewis *et al*., 1998). Adequate strength is, therefore, needed to sustain the highly energy-demanding locomotory behaviour. Metabolic rates during flight may increase 50–100-fold compared to metabolism at rest (Nation, 2008). However, Hoferer *et al*. (1999) reported a 10-fold increase in flying *C. glomerata* compared to resting individuals.

Nutrient limitation or stress has adverse effects on fitness traits of parasitoids. For some parasitoid species whose adults do not obtain proteins via 'host-feeding' behaviour, all of the resources (e.g. fat body) necessary for egg production are obtained during larval feeding and are carried over to the adult stage. In the absence of adequate food source, fat body reserve is depleted rapidly and metabolized for maintenance and repair (Ellers *et al*., 1998). Food stress might also indirectly affect other fitness traits such as host-finding and dispersal efficiency, the ability to overcome behavioural host defences, and even the production of paralyzing venom, the latter being an important prerequisite for parasitoids attacking non-sessile hosts (Taylor, 1988; Nakamatsu and Tanaka, 2003).

Nofemela (2004) studied the biology of South African *C. plutellae* on pure honey. In addition to his results, the present work attempted to provide information on its fecundity, egg load, egg size, body size, and longevity when supplied *ad libitum*

with (i) honey, (ii) honey-beebread, (iii) distilled water, (iv) physiological solution or (v) when starved.

3.2 Fecundity

3.2.1 Introduction

The term fecundity refers to a parasitoid's reproductive output, in terms of the total number of eggs produced or laid over a specified period. This is different from fertility which refers to the number of viable progeny that ensue. From the standpoint of population dynamics, fertility is the more important parameter, as it is the number of progeny entering the next generation. However, because fertility can be relatively difficult to measure, fecundity measurements are often used instead (Jervis *et al*., 2007).

Previous workers (e.g., Wang and Messing, 2003; Bokonon-Ganta *et al.*, 2007) have observed the presence of mature eggs in parasitoid ovaries at or shortly before the time of death giving an indication that parasitoids do not lay all the eggs in their ovaries in a lifetime. A distinction is, therefore, drawn between potential fecundity and realized fecundity. A species' potential fecundity is usually taken to be the maximum number of eggs that can potentially be laid by females. For parasitoid species that eclose with their lifetime complement of eggs (termed 'pro-ovigenic'), potential fecundity is equal to the number of mature eggs contained in the ovaries and oviducts, while for synovigenic parasitoids, it is the total number of mature and immature eggs possessed at eclosion. Realized

fecundity, on the other hand, is the number of eggs that are actually laid over the parasitoid lifespan when excess hosts are available. The value for realized fecundity is often less than the estimate for potential fecundity. Fecundity (potential or realized) is used as a measure of individual fitness in parasitoids (Ellers *et al.*, 1998; Roitberg *et al.*, 2001) and it is influenced by a range of biotic (host density, host quality, food consumption, body size, mating) and physical factors (temperature, photoperiod, humidity, weather).

An important aspect of parasitoid fecundity is sex allocation; which is usually determined by the population's mating structure and environmental conditions. Sex ratios (usually expressed as the proportion of the progeny that are male) can vary from highly female-biased to equality, or much more rarely, become male-biased (Winkler *et al*., 2006; Fellowes *et al*., 2007). A female Hymenoptera is able to determine the sex of her offspring by producing haploid males from unfertilized eggs and diploid females from fertilized eggs (Haploidiploidy). Parasitoids are also selective when ovipositing in their hosts by laying female eggs in better quality hosts and laying male eggs in less suitable hosts. This was explained by the theory of conditional sex allocation (Charnov *et al.*, 1981). An ovipositing female will maximize her fitness by placing female eggs in better quality hosts (Morris and Fellowes, 2002). The theory of local mate competition predicts that ovipositing females would lay an increasingly female-biased offspring sex ratio as the likelihood of sib-mating increases (Hamilton, 1967). Other factors such as parasitoid density can determine sex allocation. It is

predicted, for instance, that the proportion of male offspring produced per female will be higher at high wasp densities than at low densities (Hamilton, 1967).

Winkler *et al*. (2006) and Jervis *et al*. (2007) stated that female parasitoids that are either deprived of food or experienced a reduced intake lay fewer or no eggs at all. In addition, food sources differ in the extent to which they support fecundity in parasitoids. Based on this assertion, effect of nutritional resources on fecundity of *C. plutellae* is investigated in this chapter and the following questions were addressed:

i. to what extent does individual diet affect fecundity of *C. plutellae*, and
ii. is the performance of any diet comparable to that of honey, a supplementary resource that is commonly used to raise insects in the laboratory?.

3.2.2 Materials and methods

Newly emerged parasitoids were confined in 24.5 × 14.5 cm glass jars for about 5 h to ensure mating and fertilization. After this period, a pair (male × female) of wasps was introduced into a similar glass jar containing 2–3 cabbage seedlings infested with a batch of thirty second instar DBM larvae (Fig. 3.1). The DBM larvae served as oviposition substrate for female parasitoid and food (one of honey, honey-beebread, physiological salt, water, or no food) was provided to serve as source of energy. After 24 h, the wasps were transferred into another

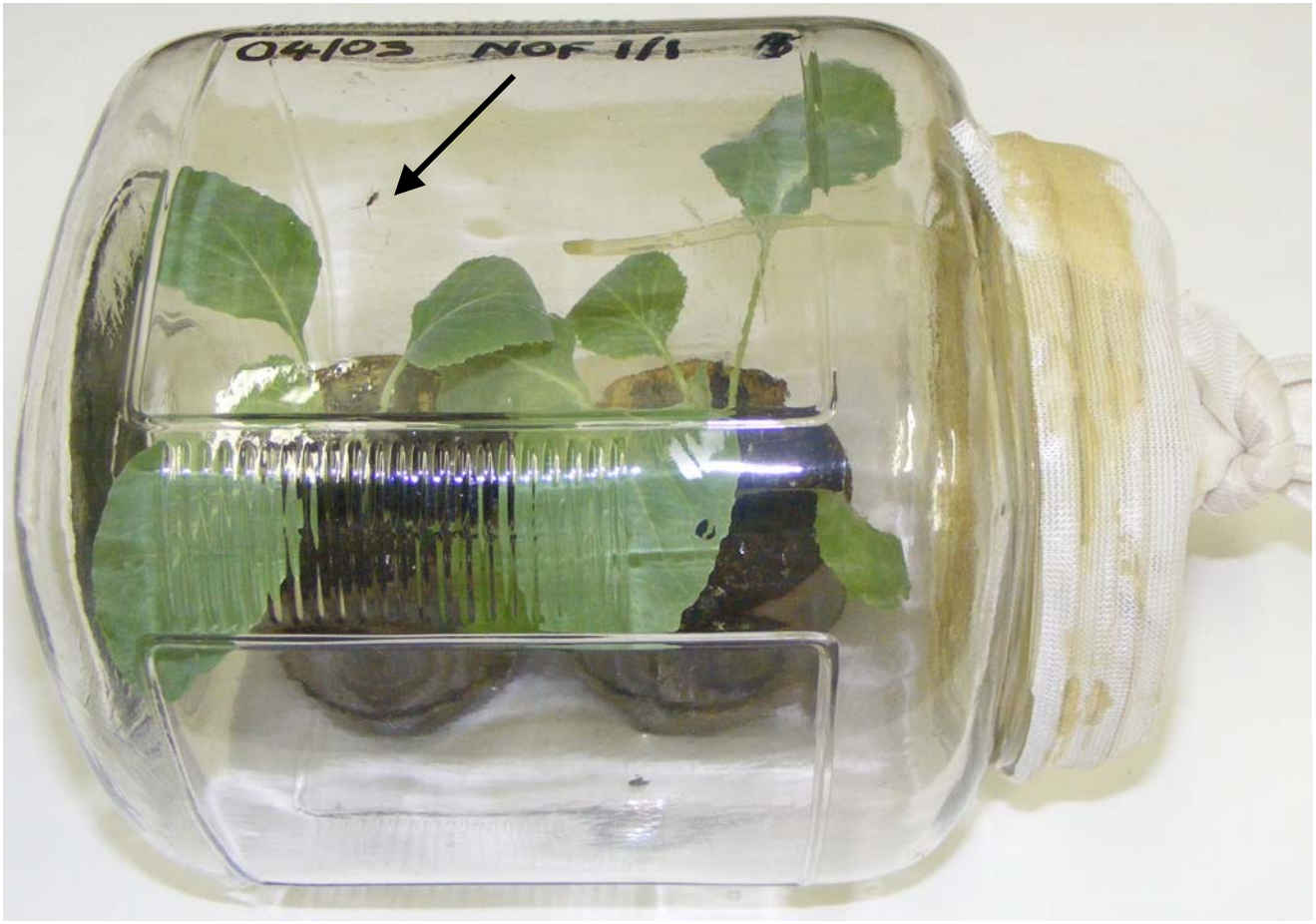


Figure 3.1: A typical set-up for fecundity study showing a glass jar containing cabbage seedlings infested with 30 second instar *Plutella xylostella* larvae which are exposed to a female *Cotesia plutellae* (arrowed) for oviposition.

glass jar containing a new batch of thirty DBM larvae on cabbage seedlings and served a corresponding food. This exercise was repeated every 24 h till the female *C. plutellae* died, marking the completion of a replicate. The process was carried out 30 times for each treatment and each batch of parasitized larvae was reared through until parasitoid eclosion. The total number of offspring per female and offspring sex ratio were recorded.

3.2.3 Data analysis

Obtained data were tested for normality using Shapiro-Wilk method. After this, two separate analyses of variance (Program GLM, SAS Institute, 1999) were carried out to determine effects of diet and maternal age on:

i. fecundity of *C. plutellae* in the first three days of eclosion, and

ii. lifetime fecundity of *C. plutellae* as influenced by honey and honey-beebread.

This was necessary because wasps provided with physiological salt, water, or those that were starved stopped laying eggs two days after eclosion. On the other hand, wasps that were given honey or honey-beebread laid eggs for eight days. Contribution of each source of variation to offspring production was calculated as:

$$\% \text{ contribution} = \frac{\text{Sum of squares (SS) value for } i^{th} \text{ source of variation}}{\text{Total SS value}} \times 100\%$$

Average lifetime fecundity was separated among treatments and oviposition days using Duncan's MRT procedure. Unpaired-sample *t*-test was used to test the null

hypothesis that wasps provided with honey produced the same number of female offspring as those that fed on honey-beebread (GenStat for Windows, 2007).

3.2.4 Results

Maternal diet accounted for most of the variation in offspring production during the first three days of eclosion (Table 3.1). However, as the age of parent wasp increased, diet effect became less significant and age of wasp determined most of the variation in offspring production (Table 3.2). Diet by day of oviposition interaction also became insignificant as the maternal age increased. *Cotesia plutellae* had successful parasitism on the day of eclosion irrespective of the treatment, but oviposition lasted three days among sugar-deprived wasps while it extended to eight days among wasps fed honey or honey-beebread (Table 3.3). Most of the wasps provided with water, physiological salt or those that had no food parasitized DBM larvae in the first two days of eclosion, while less than 50% of the female population parasitized larvae on the third day. Also during the first two days after eclosion, there was a significant reduction in oviposition by parasitoids lacking access to honey or honey-beebread. Generally, fecundity decreased with age of wasp. Average lifetime fecundity was higher in *C. plutellae* provided with honey-beebread than in wasps that were fed honey. However, parasitoids that were given distilled water, physiological salt or those that were starved produced the same number of offspring.

Table 3.1: Analysis of variance testing the effects of five nutritional treatments, age of wasp and diet × age of wasp interaction on fecundity of *Cotesia plutellae* in the first three days of eclosion.

Source of Variation	df	Sum of Squares	Mean Square	F value	% contribution to fecundity
Diet	4	8555.48	2138.87	1072.76***	87.02
Age of wasp	2	234.45	117.23	58.80***	2.38
Replicate	29	106.82	3.68	1.85**	1.09
Diet × Age of wasp	8	125.79	15.72	7.89***	1.28
Error	406	809.48	1.99		8.23
Total	449	9832.02			

, * Significant F-test at P ≤ 0.01 and P ≤ 0.001, respectively.

Table 3.2: Analysis of variance testing the effects of honey and honey-beebread, age of wasp and diet × age of wasp interaction on lifetime fecundity of *Cotesia plutellae*.

Source of Variation	df	Sum of Squares	Mean Square	F value	% contribution to fecundity
Diet	1	15.41	15.41	5.08*	0.14
Age of wasp	7	9189.17	1312.74	432.56***	86.24
Replicate	29	115.47	3.98	1.31	1.08
Diet × Age of wasp	7	15.29	2.18	0.72	0.14
Error	435	1320.13	3.03		12.39
Total	479	10655.47			

*, *** Significant F-test at $P \leq 0.05$ and $P \leq 0.001$, respectively.

Table 3.3: Fecundity of *Cotesia plutellae* as influenced by five nutritional treatments and age of wasp.

Diet	Average fecundity per day[1]								Total fecundity[2] (mean ± s.d.)	% female in progeny	Sex ratio (m : f)
	1	2	3	4	5	6	7	8			
Honey	10.47^{a}	10.97^{a}	8.43^{b}	8.17^{b}	1.00^{c}	1.23^{c}	0.77^{c}	0.47^{c}	41.50 ± 5.46^{b}	57.38	1 : 1.35
Honey-beebread	10.60^{a}	10.80^{a}	9.03^{b}	8.93^{b}	1.80^{c}	1.10^{cd}	1.30^{cd}	0.80^{d}	44.37 ± 5.96^{a}	59.73	1 : 1.48
Salt solution	1.33^{a}	1.33^{a}	0.13^{b}	_	_	_	_	_	2.80 ± 1.77^{c}	39.29	1.55 : 1
Distilled water	3.27^{a}	0.67^{b}	0.27^{b}	_	_	_	_	_	4.20 ± 1.52^{c}	46.03	1.17 : 1
No food	1.50^{a}	1.13^{ab}	0.77^{b}	_	_	_	_	_	3.40 ± 1.48^{c}	36.27	1.76 : 1

Values in the same row [1] or column [2] followed by the same letter are not significantly different at $P \leq 0.05$.

There was a significant diet effect in the number of female progeny produced by *C. plutellae*. Wasps fed honey-beebread produced more female offspring than parasitoids raised on honey (t = 2.59; d.f. = 239; P = 0.01) while those provided with physiological salt, water or starved produced few female offspring. Parasitoids fed honey or honey-beebread had female-biased progeny, while male-biased offspring were produced under the other three feeding regimes.

3.3 Egg load and egg size

3.3.1 Introduction

Egg load can be defined as the number of mature oöcytes (i.e. fully-chorionated eggs) found within the ovaries and oviducts of an insect at any given moment in its lifetime (Riddick, 2006). Emergence with a high percentage of mature rather than immature eggs would seem advantageous to short-lived species that generally expend much of their egg load early-on in adult life; whereas, emergence with a low percentage of mature eggs would seem advantageous to species that oviposit a limited number of eggs over a longer period of time (Ellers and Jervis, 2003). Egg load has been identified, especially among synovigenic parasitoids, as a significant measure of fitness (Jervis and Ferns, 2004). This fitness trait has also been related positively to body size of many pterygote insects (Ellers and Jervis, 2003; Jervis *et al*., 2003), although the relationship may not always hold true for very small-sized, short-lived parasitoids (Ellers and Jervis, 2004) or lab-cultured parasitoids (Riddick, 2005).

Egg size is considered to be a reliable predictor of offspring fitness (Giron and Casas, 2003). A minimal egg size below which offspring can not survive, and above which their fitness increases as size increases was observed in *Eupelmus vuilletti* (Crowford) (Hymenoptera: Eupelmidae). Parasitoids hatching from small eggs suffered higher mortality than neonates emerging from larger eggs. Smaller eggs might result in offspring that suffer a reduction in fitness, in terms of reduced survival, smaller adult size, and/or longer development time. Egg size in *Mastrus ridibundus* (Gravenhorst) (Hymenoptera: Ichneumonidae) increased with adult female size, but fed wasps consistently produced larger eggs than their starved counterparts (Bezemer *et al*., 2005). Egg size is also known to decline with female age (Giron and Casas, 2003), indicating that egg size can change depending on life expectancy and resource availability. Information about effect of diet on egg load and egg size in *C. plutellae* is lacking in literature.

Effects of food on egg maturation vary depending on the species of parasitoid. Riddick (2007) observed a significant increase in egg load of host-deprived *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae) when wasps were given food (honey). On the contrary, rate of egg maturation in another host-deprived braconid, *Macrocentrus grandii* (Goidanich) was slightly less in sugar-fed than in starved females (Olson *et al*., 2000). Egg maturation in host-deprived *M. ridibundus* remained static when females were deprived of honey (Bezemer *et al*., 2005), whereas egg maturation in *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae) occurred regardless of food supply (Wang and Messing, 2003).

There is no record of how food availability affects egg maturation in *C. plutellae*. The present experiment was, therefore, designed to assess:

i. effect of maternal diet on egg load and egg size of newly emerged female offspring, and

ii. effect of diet on egg maturation in mated but host-deprived *C. plutellae*.

3.3.2 Materials and methods

Female offspring from each diet treatment in the fecundity study (Section 3.2) were used in this section and the experimental set-up was two-fold:

Experiment I: Egg load of newly emerged wasps

Body weight of each wasp was determined using an analytical balance (SCALTEC SBA 32**,** d = 0.0001 g, Germany) before it was dissected for mature eggs. Dissections were made under a Kyowa Optical Model SDZ-PL binocular microscope (magnification range 7× to 45×) to determine egg load according to the method of Tylianakis *et al.* (2004). Each female wasp was immobilized by placing the dorsal surface on molten wax inside a dissecting dish. The wasp was then submerged in insect saline solution (Yeager's physiological salt; Appendix 1). The cuticle around the posterior end of the abdomen was cut using fine needles to expose the ovaries and a pair of forceps was used to pull the ovipositor. This brought out the ovaries and the rest of the reproductive system. Ovaries and lateral oviducts were placed on a glass slide, stained in 0.01% solution of methylene blue and cut up to bring out the mature eggs, which were counted. This exercise was repeated with at least 30 wasps per treatment.

Lengths of at least 30 eggs were also measured in each case. Photomicrographs of the female reproductive system and mature eggs were taken using a LEICA EZ4D digital stereomicroscope (knob zoom range 8×–35×) installed with LAS EZ 1.5.0 processing software.

Experiment II: Egg load of developing wasps

Newly emerged *C. plutellae* females were confined with male wasps in 24.5 × 14.5 cm glass jars to ensure mating and fertilization. Each group was given a diet corresponding to that given to the parent stock, but DBM larvae were not provided for oviposition. Members of each cohort were dissected, as described above, on each subsequent day to monitor changes in egg load.

3.4. Body size

3.4.1 Introduction

The relationship between adult body size and fitness has generated a lot of interest in the field of evolutionary biology and ecology (Mayhew and Glaizot 2001; Jervis *et al*. 2003). The size of parasitoids is a major determinant for their fitness, as large body size is often correlated with an increase in the amount of resources carried over from the larval to the adult stage (Hoňek, 1993; Ellers *et al.*, 1998). Large size enables individual parasitoid both to store more energy reserves (which influences longevity) and to produce more eggs (which influences reproductive success) than smaller conspecifics. Also larger parasitoids will be more successful in attacking defending hosts. Numerous laboratory studies (e.g., Zaviezo and Mills, 2000; Harvey *et al*., 2001) have

reported that longevity and fecundity in parasitoids are positively correlated with body size. A number of field studies have likewise demonstrated a positive relationship between body size and fitness variables such as patch finding ability and lifetime reproduction (Ellers *et al*., 1998; 2001). Smaller parasitoids may thus be less successful in biological control.

The study reported here investigated the effect of maternal diet on body size of newly emerged *C. plutellae*. Such report does not exist in literature, and the experiment was designed to test the hypothesis that wasps raised on a more nutritious diet would produce bigger offspring.

3.4.2 Materials and methods

Each newly emerged female wasp that was dissected for egg load was placed on another glass slide, in saline solution, and its hind tibia was used to determine its size. The length of the hind tibia is a standard estimation of size in parasitoids (Godfray, 1994) and this character has been used previously for estimating body size of other parasitoids (Mills and Kuhlmann, 2000). Measurement was done using a 20 mm diameter eyepiece graticule that was fitted into the Kyowa Optical Model SDZ-PL binocular microscope. Hind tibia length of 30 female wasps per treatment was recorded. Hind tibiae of 30 male wasps per treatment were also measured to estimate body size.

3.4.3 Data analysis

Obtained data were tested for normality (Shapiro-Wilk test), after which one-way analysis of variance (Program GLM, SAS Institute, 1999) was carried out to determine diet effect on egg load, egg size and body size of *C. plutellae* offspring. Sample means were separated among treatments using Duncan's MRT procedure. Correlation analysis was used to determine relationships between parasitoid size and weight, and between parasitoid size and egg load. Parasitoid size and weight were compared between male and female offspring to test the null hypothesis that compared means are equal (unpaired-sample *t*-test, GenStat for Windows, 2007).

3.4.4 Results

Female reproductive system

The female reproductive system of *C. plutellae* consisted of a pair of ovaries, a pair of ovarioles (ov) per ovary, two lateral oviducts (lo), a median oviduct (mo), a pair of accessory glands (spg), spermatheca (spt) and ovipositor (op) (Fig. 3.2). The germarium (ge), inside which trophocytes are differentiated into young oöcytes, was connected to the ovariole by a long narrow germ tube (gt). The ovarioles repeatedly folded on each other anteriorly but united posteriorly before the calyx (ca). Each ovary emptied into a lateral oviduct via the distinctly bluish calyx. The two lateral oviducts united to form the median oviduct which opened into the genital chamber. The glandular portion of the spermatheca (spg) was relatively longer but thinner than the storage portion (spt) which was roughly

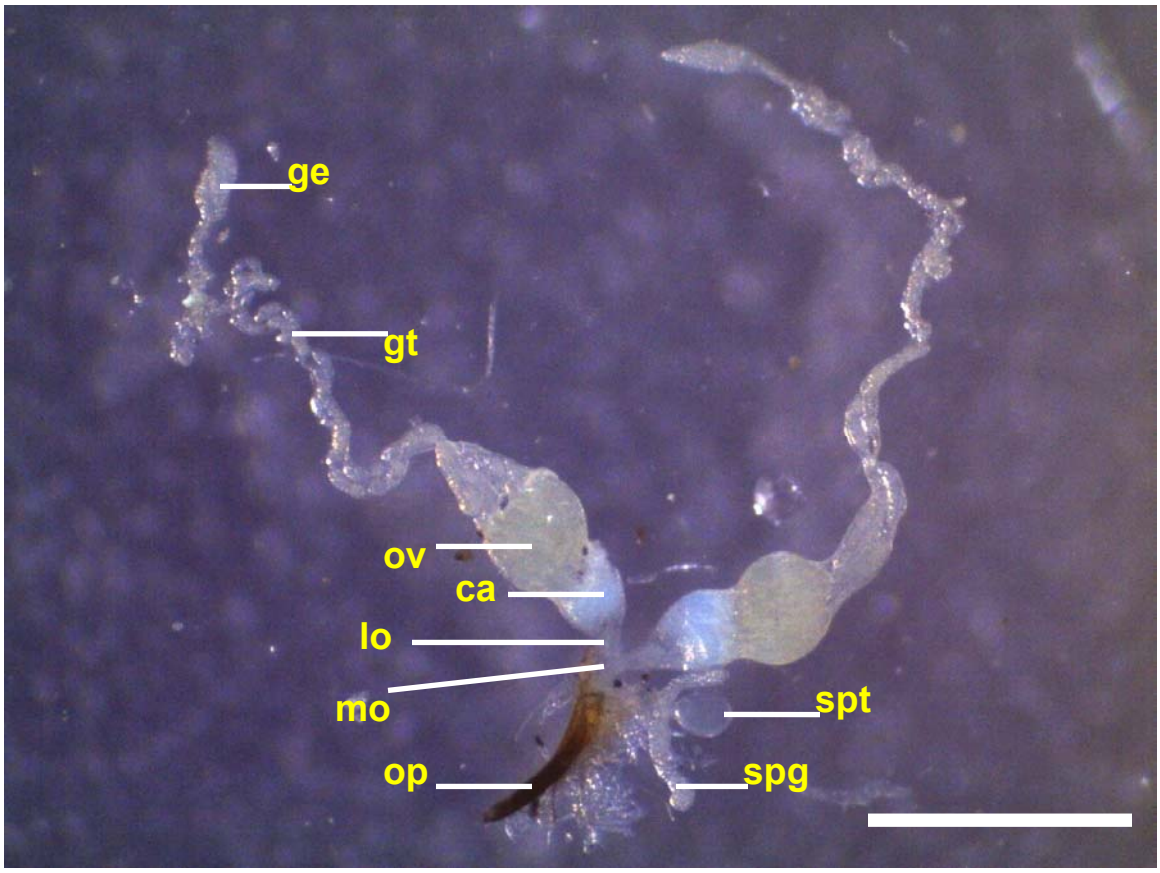


Figure 3.2: Photomicrograph showing the female reproductive system of *Cotesia plutellae*. ge, germarium; gt, germ tube; ov, ovariole; ca, calyx; lo, lateral oviduct; mo, median oviduct; op, ovipositor; spt, spermatheca; spg, spermathecal gland. Scale bar = 500 µm.

spherical in shape. These two structures united and opened into the genital chamber. The rest of the reproductive apparatus was housed within the ovipositor which the parasitoids use in laying eggs when parasitizing DBM larvae.

The ovariole accommodated most of the mature eggs (Fig. 3.3), while a small number was found in the calyx and lateral oviduct. The number of mature eggs found in the calyx and lateral oviduct increased with age of parasitoid: a combined average of 2 eggs at eclosion increased to an average of 13 eggs 24 h posteclosion. The ovaries did not contain equal numbers of mature eggs. Immature eggs were found at the distal portion of the ovarioles and each of them had a nutritive attachment of trophocytes. Mature eggs were chorionated and lacked the attachment of trophocytes. When the ovarioles were dissected, mature eggs were recognized as being turgid and elongated under a microscope.

Egg load of newly emerged wasps

Maternal diet did not have any significant effect on egg load, egg size, body size and weight of newly emerged *C. plutellae* (Table 3.4). However, female offspring were bigger (t = 6.35; d.f. = 149; P = 0.001) and heavier (t = 2.39; d.f. = 149; P = 0.018) than the males. Generally, parasitoid weight was strongly related to body size, while there was no significant correlation between body size and egg load. The only exception was among offspring produced by parasitoids provided with honey-beebread, where a significantly positive correlation was recorded between body size and egg load (Table 3.5).

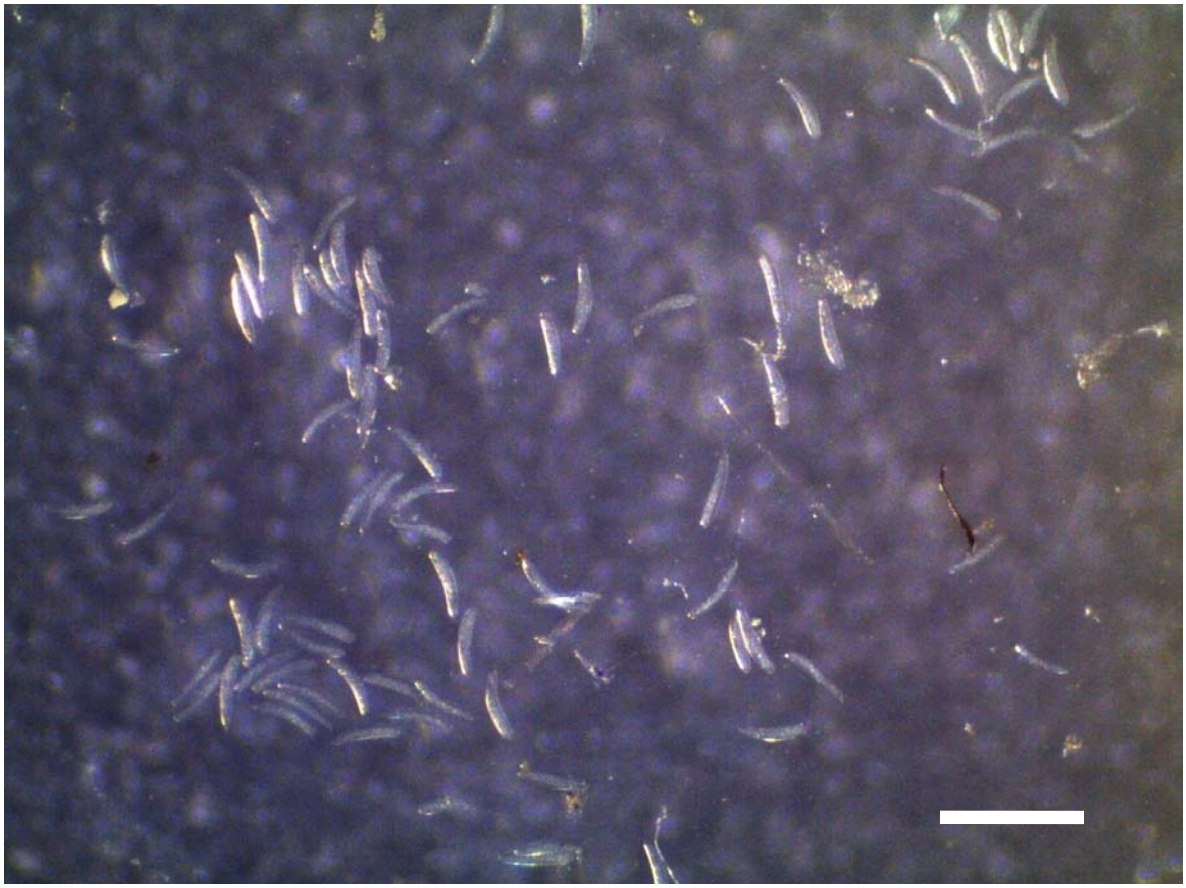


Figure 3.3: Photomicrograph showing eggs dissected out of the reproductive system of *Cotesia plutellae*. Scale bar = 300 µm.

Table 3.4: Mean values of egg load, egg size, body weight and size of newly emerged *Cotesia plutellae* as affected by maternal diet.

Treatment	Egg		Parasitoid			
			Female		Male	
	Egg load (range in parentheses)	Egg length (mm)	Body size (i.e. hind tibia length, mm)	Weight (mg)	Body size (i.e. hind tibia length, mm)	Weight (mg)
Honey (n = 30)	67.83 ± 17.79 (26–103)	0.16 ± 0.02	0.71 ± 0.06	0.51 ± 0.21	0.67 ± 0.06	0.46 ± 0.18
Honey-beebread (n = 30)	68.07 ± 21.75 (26–108)	0.15 ± 0.02	0.71 ± 0.05	0.53 ± 0.17	0.68 ± 0.05	0.50 ± 0.16
Salt solution (n = 30)	63.63 ± 21.60 (28–94)	0.15 ± 0.02	0.70 ± 0.07	0.50 ± 0.16	0.67 ± 0.06	0.45 ± 0.19
Distilled water (n = 30)	64.77 ± 19.44 (20–100)	0.15 ± 0.02	0.71 ± 0.06	0.51 ± 0.20	0.67 ± 0.05	0.45 ± 0.17
No food (n = 30)	64.60 ± 14.37 (26–88)	0.15 ± 0.02	0.70 ± 0.07	0.50 ± 0.15	0.66 ± 0.06	0.45 ± 0.18

Values are expressed as mean ± standard deviation. There is no treatment effect on the variables.

Table 3.5: Correlations between the size and weight of *Cotesia plutellae* and between size and egg load with respect to five nutritional treatments.

	Parasitoid body size (i.e. hind tibia length)											
	General trend across the five treatments		Honey		Honey-beebread		Salt solution		Distilled water		No food	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Body weight	0.52***	0.57***	0.59***	0.70***	0.50**	0.63***	0.53**	0.36	0.49**	0.51**	0.48**	0.70***
Egg load	_	0.15	_	0.90	_	0.51**	_	0.27	_	0.00	_	−0.18

, * Significant F-test at $P \leq 0.01$ and $P \leq 0.001$, respectively.

Egg load of developing wasps

Analysis of variance (ANOVA) showed that diet did not affect posteclosion egg maturation in *C. plutellae* (F = 0.11; d.f. = 19; P = 0.9772), but age of wasp did (F = 198.38; d.f. = 19; P < 0.0001). Egg maturation started within the first few hours of adult emergence and maximum egg load was attained within 48 h (Table 3.6). Invariably, sugar-deprived wasps that were short-lived died with maximum egg load.

Table 3.6: Posteclosion egg maturation in *Cotesia plutellae* as affected by five nutritional treatments.

Treatment	Average egg load per period (n = 20)			
	24 h > t > 0 h	48 h > t ≥ 24 h	72 h > t ≥ 48 h	*96 h > t ≥ 72 h
Honey	65.25 ± 16.43[b]	101.15 ± 16.83[a]	100.45 ± 12.75[a]	100.20 ± 12.66
Honey-beebread	67.95 ± 20.27[b]	100.95 ± 13.88[a]	102.30 ± 15.92[a]	101.45 ± 12.64
Salt solution	64.65 ± 16.36[b]	101.70 ± 12.26[a]	100.70 ± 9.84[a]	–
Distilled water	66.30 ± 18.87[b]	101.00 ± 13.92[a]	100.90 ± 11.16[a]	–
No food	64.10 ± 12.70[b]	101.80 ± 11.14[a]	100.65 ± 11.44[a]	–

Values in the same row followed by the same letter are not significantly different at P ≤ 0.05.

* Values were not included in the ANOVA because most of the wasps placed on treatments other than honey and honey-beebread died within 72 h of eclosion.

3.5 Longevity

3.5.1 Introduction

The life-span of an individual insect can be divided into two phases: (a) the development from hatching of the egg until adult eclosion, and (b) the period of adult life, usually referred to as longevity (Blackburn, 1991). The subject of longevity is crucial to the success of biological control, the assumption being that: (a) the longer a male can live, the more females he can inseminate, and therefore the more eggs he can fertilize; and (b) the longer a female can live, the more eggs she will lay (Roitberg *et al.*, 2001; Rivero and West, 2002).

Longevity is a highly variable species characteristic, influenced by a range of biotic (body size, mating, host density, etc.) and abiotic (diet, temperature, humidity, photoperiod, pesticides or other toxins) factors (Jervis *et al*., 2007). A positive correlation between body size and longevity has been shown for adults of several parasitoid species (Noda and Nakamura, 2004, Eliopoulos *et al*., 2005). The size effect is attributable to the smaller fat reserves that small insects possess at eclosion and *vice versa* (Rivero and West 2002). Frequent mating may reduce an individual's longevity (Sagarra *et al*., 2002; Onagbola *et al*., 2007), indicating that there is a cost, in terms of survival to reproduction. The effect of host density depends on the nature of parasitoid females. In non-predaceous species, host-deprived females are able to live longer than host-satiated ones (Ellers *et al*., 2000; Tran and Takasu, 2000). Presumably, the former live longer because they do not incur the costs of oviposition. However, in

predaceous (host-feeding) species, longevity is found to be shortest in host-deprived females, as they are not able to satisfy their metabolic requirements for maintenance. There is an optimum range of temperatures and humidity outside of which survival is severely reduced. Generally, longevity decreases with increasing temperature within the optimum range (Liu and Tsai, 2002; Seal *et al*., 2002), and with decreasing humidity, especially, in small-bodied insects as they are more prone to desiccation (Jervis *et al*., 2003). Several studies have also revealed that longevity varies with the quality of food consumed. Many parasitoids given carbohydrate-rich foods, e.g., diluted honey or sucrose solutions, live significantly longer than insects that are either starved or given water only (Fadamiro and Heimpel, 2001; Wanner *et al*., 2006).

The present study was conducted to assess the longevity of *C. plutellae* on different diets, as there is no previous record of such in literature. A significant difference in parasitoid longevity on the diets could have an implication on its biological control potential. It is generally assumed that the diet giving longer lifespan would enhance the efficacy of a parasitoid in insect pest reduction.

3.5.2 Materials and methods

Adult male and female parasitoids that were enclosed with DBM larvae for the fecundity study in Section 3.2 were monitored till they died. Longevity was then determined as the number of days from eclosion to death.

3.5.3 Data analysis

Obtained data were tested for normality (Shapiro-Wilk test), and then analyzed using one-way analysis of variance (Program GLM, SAS Institute, 1999) to determine diet effect on longevity. Sample means were compared using Duncan's MRT procedure. The null hypothesis that mated males do not outlive mated females was tested using *t*-test procedure (GenStat for Windows, 2007). Survival probability of *C. plutellae* on each treatment was also presented using Kaplan-Meier curves (StatsDirect, 2009).

3.5.4 Results

Male and female *C. plutellae* lived longer on honey-beebread than they did on honey, whereas there was no significant difference between the lifespan of starved wasps and those that were provided with either water or physiological salt. Median longevity (day at which 50% of the initial number of wasps are still alive) was highest in wasps provided with honey-beebread followed by those that were given honey. In addition, risk of starving to death was lowest among wasps provisioned with honey-beebread followed by honey-fed wasps, and was highest among wasps given physiological salt. There was no sexual effect on longevity except among wasps that were given salt solution (t = 2.69; P = 0.010) or distilled water (t = 2.51; P = 0.015), where females lived slightly longer than the males (Table 3.7). It is evident from Kaplan-Meier curves (Fig. 3.4) that wasps provided with honey-beebread had higher chances of surviving each day and they also lived longer than those fed honey. However, *C. plutellae* did not thrive under

Table 3.7: Indices of adult *Cotesia plutellae* longevity on five nutritional treatments.

	Adult longevity						
	mean ± standard deviation (days)		*t*-test for male vs. female longevity (n = 58)	median longevity (days)		relative death rate (log-rank test)	
Treatment	Male	Female		Male	Female	Male	Female
Honey	13.37 ± 5.18[b]	12.4 ± 5.06[b]	$t = 0.73$, $P = 0.468$	13	13	0.72	0.66
Honey-beebread	19.40 ± 9.11[a]	16.07 ± 8.38[a]	$t = 1.47$, $P = 0.146$	18	14	0.47	0.50
Salt solution	2.13 ± 0.35[c]	2.43 ± 0.50[c]	$t = 2.69$, $P = 0.010$	2	2	2.42	2.48
Distilled water	2.63 ± 0.81[c]	3.13 ± 0.73[c]	$t = 2.51$, $P = 0.015$	2	3	1.92	1.66
No food	2.57 ± 0.50[c]	2.60 ± 0.50[c]	$t = 0.26$, $P = 0.798$	3	3	1.84	2.14

Values in the same column followed by the same letter are not significantly different at $P \leq 0.05$.

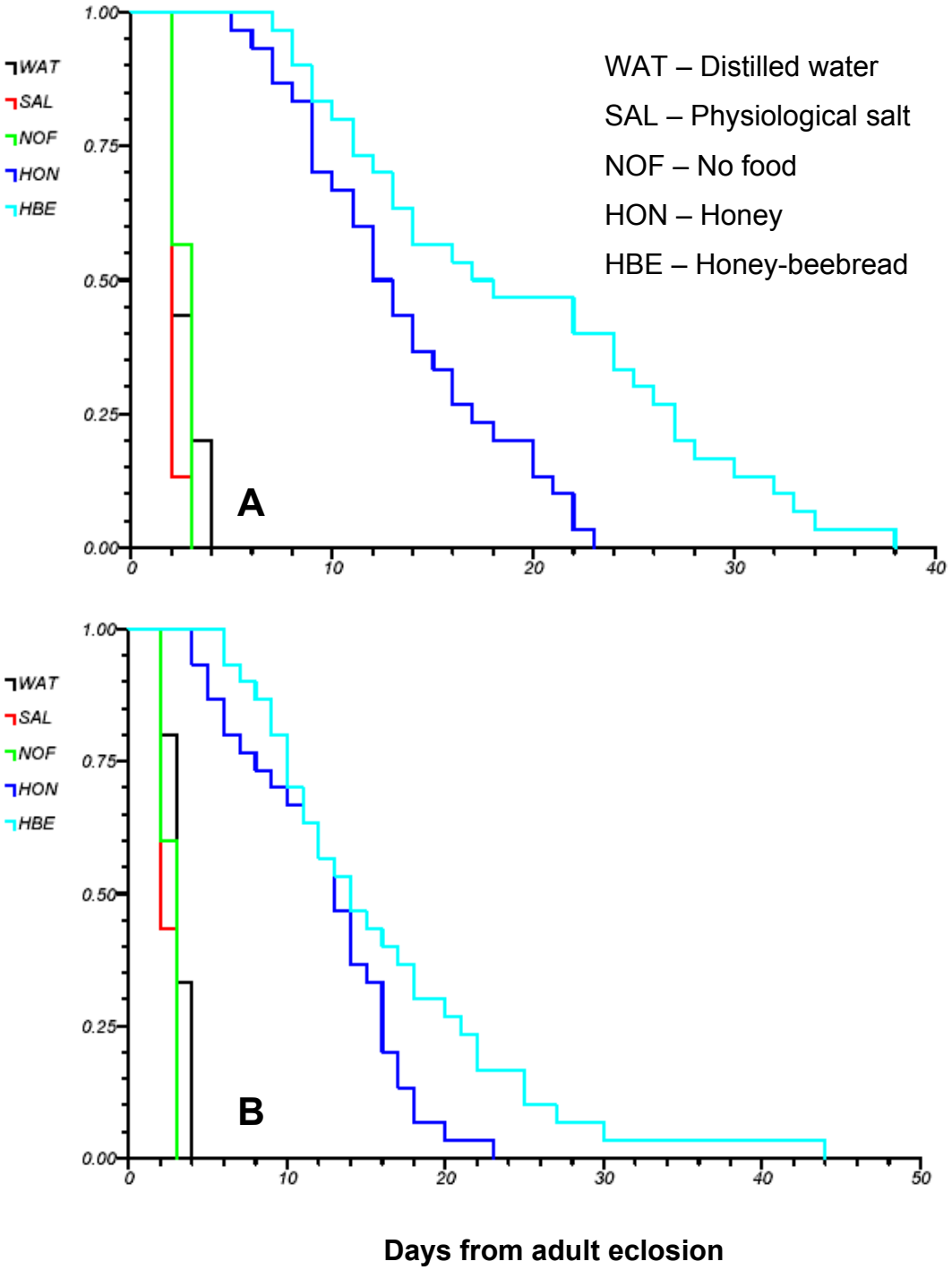


Figure 3.4: Survival probabilities of male (A) and female (B) *Cotesia plutellae* on five nutritional treatments. Data are presented as Kaplan-Meier curves.

water, physiological salt and starved conditions. As expected, chances of survival reduced with age of wasps.

3.6 Discussion

Maternal diet accounted for 87% of the variability in reproductive outcome of *C. plutellae* within the first three days of eclosion, followed by a sharp decline as the wasps aged. This is an indication that availability of a good energy source within the first few days of eclosion is very crucial to the biological success of *C. plutellae* and it is advisable to limit tests for diet effect to such period. The significant Diet × Age of wasp interaction within the first three days of eclosion also suggested that the parasitoid utilized its food mainly for reproductive purposes. There is a possibility that as the female wasps aged, energy from food sources was diverted mostly to body maintenance rather than reproduction and fecundity relied less on quality of food.

Wasps fed honey or honey-beebread presented a fecundity about 13-fold higher than the one of wasps provided with distilled water, physiological salt or those that were not fed at all. This is due to the fact that (i) wasps were short-lived on unsuitable food sources and (ii) wasps facing nutritional stress lacked adequate energy to optimize parasitism, even on the day of eclosion. Efficacy of < 24 h old nutritionally stressed wasps was 10–30% of those that received honey or honey-beebread. Adult parasitoids provided with honey-beebread had higher lifetime fecundity than those that were raised on honey. The high protein content in

beebread was suspected to be responsible for this difference. Previous studies (e.g., Ueno, 1999; 2000) have highlighted the importance of protein sources to female parasitoids. In an attempt to acquire enough protein, females of synovigenic parasitoid wasps may feed on a considerable proportion of hosts and such hosts are not suitable for offspring development. Provision of a protein supplement is thus ideal in the laboratory to reduce the proportion of hosts used for feeding purposes and increase the number parasitized for multiplication. In this study, beebread must have been utilized as a protein supplement, thus reducing the dependence of *C. plutellae* on DBM larvae as protein sources and invariably making more hosts available for parasitism.

Progeny sex ratio of nutritionally-challenged wasps was male-biased, while that of wasps fed honey or honey-beebread was female-biased. This indicates a potential benefit of sugar feeding in the production of female-biased progeny, an important consideration in the utilization of biological control agents. Sex allocation in *C. plutellae* is known to be influenced by host quality (Nofemela, 2004) and results of the present study suggest that diet quality may also be a determinant. Onagbola *et al*. (2007) observed a female-biased sex ratio in the offspring of *Pteromalus cerealellae* (Ashmead) (Hymenoptera: Pteromalidae) when provided with a sugar source as opposed to a male-biased sex ratio when they were denied sugar. The results showing a positive impact of sugar feeding on sex allocation by female parasitoids may have an important ramification for their use in biological control programs. Presence of a protein source was also

suspected to be an added factor determining sex allocation in *C. plutellae* as females provisioned with honey-beebread produced a significantly higher number of female offspring than those that were fed honey. Relationship between dietary protein and female progeny was earlier observed in *Bracon thurberiphagae* Muesebeck (Hymenoptera: Braconidae) (Rojas *et al*., 1995), where a previously male-biased sex ratio was restored to a 1:1 situation after the addition of several amino acids to the diet of adult wasps.

Four ovarioles (two per ovary) seems to be the standard phenotype for braconids. The presence of such in *C. plutellae* is in agreement with the number of ovarioles found in other braconids, such as *Diachasmimorpha longicaudata* (Ashmead), *Doryctobracon areolatus* (Szepligeti), *Doryctobracon crawfordi* (Viereck), *Opius hirtus* (Fisher) and *Utetes anastrephae* (Viereck) (Sivinski *et al*., 2001), *Apanteles galleriae* (Wilkinson) (Uçkan *et al*., 2003), *Fopius arisanus* (Sonan) (Wang and Messing, 2003) and *Cotesia marginiventris* (Cresson) (Riddick, 2007). Some authors (e.g., Albrecht *et al*., 1994; Webb *et al*., 2006) have reported that the ovarian fluid of polydnavirus-carrying parasitic wasps sometimes has a bluish tinge. In this study, the calyx region of fresh *C. plutellae* was consistently bluish in appearance and the presence of this colouration could, therefore, be regarded as a preliminary means of detecting polydnavirus-like particles in this braconid. However, in agreement with Ketseoglou and Bouwer (2008), this method is only reliable when viewing fresh specimens, as the bluish tinge fades with time.

A wide range of egg loads in newly emerged wasps was recorded in the present study, the smallest being 62 (i.e., 26–88) among the progeny of starved wasps. This suggests that considerable variation in egg production can occur between females reared under identical conditions. However, this variation did not reflect differences in body size in this study, indicating that body size does not necessarily determine egg load in *C. plutellae*. A similar observation by Riddick (2005) showed that egg load was not related to body size of *Anaphes iole* Girault (Hymenoptera: Mymaridae).

The presence of an average of 65 mature eggs in the ovaries and lateral oviducts of newly-emerged *C. plutellae* females highlights the storage capacity of this species. All other things being equal, the parasitoid is able to oviposit a large number of eggs into hosts soon after eclosion. Mitsunaga *et al*. (2004) gave 1-day-old inseminated female *C. plutellae* access to 30 second instar DBM larvae for 24 h and the experiment yielded an average of 20 offspring per female. However, a number of days rather than one day of exposure to new (i.e., un-parasitized) hosts would exploit the fecundity of *C. plutellae*. The present study showed that at least eight days of exposure to fresh second instar DBM larvae are needed to assess the realized fecundity of *C. plutellae*.

The fact that some developing eggs were present in the distal portions of the ovarioles suggests that further egg maturation can occur post-emergence. Maternal age and food availability might stimulate further egg maturation and

Quicke (1997) stated that newly-emerged synovigenic parasitoids must begin ovipositing into suitable hosts before egg maturation can recommence. However, the present study has shown that neither food nor oviposition experience is a prerequisite for posteclosion egg maturation in *C. plutellae*. Instead, it depended on maternal age. The egg maturation commenced and progressed in both fed and starved host-deprived *C. plutellae*, reaching its peak within 48 h posteclosion. Although parasitoid egg load was independent of diet supplied, wasps supplied with honey or honey-beebread produced more offspring than wasps given either physiological salt or water alone. This indicates that whereas egg load is a function of an intrinsic factor in *C. plutellae*, parasitoid nutrition among other factors determine fecundity.

The highest average lifetime fecundity recorded for *C. plutellae* in this study was 44.37 ± 5.96 (mean ± s.d.), which is significantly less than the average number of mature eggs at emergence. Also, Nofemela (2004) recorded a maximum of 42.13 ± 12.20 offspring for *C. plutellae* on honey. These suggest that the parasitoid does not have the capacity to lay all the matured eggs in its lifetime. In this study, an average of 19 (n = 10) matured eggs were found in dead *C. plutellae* females that had access to excess second instar DBM larvae throughout their lifetime. Jervis *et al*. (2003) recorded an ovigeny index (OI) of 0.32 for *C. plutellae*. Since OI is the ratio of mature egg load at emergence to the lifetime potential fecundity, it follows that the total number of mature and immature eggs per female could be as high as 200. Obviously, the number of

offspring produced per female wasp together with the number of eggs remaining in the reproductive system at death did not add up to this value, suggesting that (i) the parasitoid did not mature all its immature oöcytes (a maximum average of 102 matured eggs was recorded in this study), and or (ii) egg resorption occurred.

Laboratory studies have shown that in the absence of food, the longevity of both sexes and the fecundity of females are in general significantly reduced (Onagbola *et al*., 2007; Kapranas and Luck, 2008). This trend was evident in the present study as sugar-deprived wasps were significantly short lived than sugar-satiated cohorts. When eclosed female wasps were deprived sugar, their life expectancy was limited to an average of 2 days. This suggests that the resources acquired during immature development and stored in their fat body (lipids and proteins) are limited and rapidly exhausted for somatic maintenance. On average, food availability increased parasitoid longevity by a factor of 10 compared to when wasps were not fed at all. However, *C. plutellae* lived longer on honey-beebread than it did on honey, while physiological salt appeared to be the most unsuitable 'food' source for the parasitoid. Since beebread is very rich in essential amino acids, as opposed to honey (FAO, 1996), it is reasonable to assume that their presence in honey-beebread must have led to the longer lifespan. Rojas *et al*. (1995) observed an increase in the longevity of *B. thurberiphage* on *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae) when the adult diet (30% solution of 50:50 glucose:fructose) was fortified with several

amino acids. Provision of protein sources as supplemental food could be the key to improving mass rearing efficiency of *C. plutellae*. In the field, where hosts are presumably more difficult to find and the rate of parasitoid oviposition may, therefore, be lower, it seems likely that longevity would have a stronger influence on parasitoid fitness.

CHAPTER 4:

Feeding and orientation responses of *Cotesia plutellae* to honey and honey-beebread cues

4.1 General introduction

All creatures rely on physical and chemical cues to locate food and mates, and to avoid predators and danger (Wäckers, 1994; Chesler and Firestein, 2008). Parasitic wasps can accurately locate hosts or food by using olfactory cues, visual signals, contact stimuli and perceived sound. These cues can be used either singly or in an interactive manner to enhance precision in target location (Fischer *et al*., 2001).

Some parasitoid species use visual cues for the recognition and evaluation of their food resources (Fischer *et al*., 2004). Parasitoids can quickly evaluate visual cues such as host shape, size, colour, and movement without the risks associated with host handling. Colour learning in response to food and host rewards has been demonstrated in some hymenopterans. For example, females of *Nasonia vitripennis* (Walker) (Pteromalidae) learned to associate colour with hosts and/or honey (Oliai and King, 2000); *Exeristes roborator* (Fabricius) (Ichneumonidae) learned to associate hosts with colour (Wardle, 1990); *Polybia occidentalis* Olivier (Vespidae) learned to associate sugar with colour (Shafir, 1996), and the Sahara desert ant, *Cataglyphis bicolor* Fabricius (Formicidae), learned to associate a piece of biscuit with colour (Kretz, 1979).

Interaction between vibrational sounding and visual cues was reported in a polyphagous endoparasitoid of lepidopteran pupae, *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae) (Fischer *et al*., 2001). Single-cue experiments

showed that the parasitoid achieved a similar precision of host location when each cue was used singly. The combination of the two cues, however, increased precision of host location by a factor of approximately two to three (additive accuracy). Cases of additive accuracy have also been recorded in other parasitoids. A larval parasitoid of *Helicoverpa* / *Heliothis* spp., *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae), a solitary larval endoparasitoid of frugivorous tephritid fruit flies, *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae), and the generalist tachinid, *Exorista mella* Walker (Diptera: Tachinidae), combined olfactory and visual cues for enhanced host location efficiency (Wäckers and Lewis, 1994; Jang *et al*., 2000; Stireman, 2002).

The present study was conducted to assess the feeding response of *C. plutellae* to honey and honey-beebread in comparison to distilled water, physiological salt and five specific sugars. Also, reliability of odour emanating from honey and honey-beebread as olfactory cues for foraging *C. plutellae* was investigated.

4.2 Phagostimulatory response

4.2.1 Introduction

Many adult parasitic Hymenoptera require food to satisfy metabolic energy and reproductive needs (Quicke, 1997). Energy and maintenance requirements are generally met by sugar-foraging, while host-feeding provides wasps with protein nutrients necessary for successful reproduction (Beach *et al.*, 2003). After coming in contact with a food source, the parasitoid has to decide whether the

food is suitable or not and this function is performed by the gustatory system, which coordinates the sense of taste.

Taste receptors have a characteristic pore near or at the tip of the cuticular structure and they respond to stimulus molecules in solution. These receptors are numerous on the mouthparts, as well as on the tarsi and ovipositor of some insects (Nation, 2008). The sensillae generally contain numerous chemosensitive neurons such as the sugar cell, water cell, and the (deterrent) salt cell (Wäckers, 1999). It is generally assumed that during taste perception, sugars bind to gustatory cells possessing pyranose and furanose sites (Boevé and Wäckers, 2003). The pyranose site binds sugars such as glucose, maltose, sucrose and trehalose, while the furanose site binds fructose, galactose and mannose (Wäckers, 1999). The generated chemical energy is then transformed into electrical energy in the neurons which carry taste sensations to the insect brain for interpretation.

The capability of hymenopteran parasitoids to detect sugars varies greatly (Winkler *et al*., 2005; Williams and Roane, 2007), and it is sometimes positively correlated with longevity (Irvin *et al*., 2007) and metabolic utilization (Chen and Fadamiro, 2006). Increased longevity would allow the wasps more time to search for and parasitize hosts, thus leading to a possible increase in realized fecundity. An increase in longevity afforded by sugar foraging is especially important when host densities are low and parasitoids must spend considerable time searching.

In order to successfully exploit a food source, the insect must show a positive phagostimulatory response to its component sugars. Most parasitoids readily accept sucrose, fructose, and glucose (Jervis *et al*., 1993; 1996), the most common components of honey, nectar and honeydew. The study reported here was, therefore, conducted to (1) describe the response of *C. plutellae* females to honey, honey-beebread, physiological salt, specific sugars and distilled water (control), and (2) to assess relative acceptance of the test solutions based on feeding durations.

4.2.2 Materials and Methods

The method described by Winkler *et al.* (2005) was followed to test the feeding behaviour of *C. plutellae* to honey, honey-beebread, physiological salt, distilled water and 1 M sugar solutions (fructose, glucose, sucrose, maltose, trehalose). Two-day-old mated, unfed but water-satiated females were tested. To ensure water satiation at the time of the experiment, the wasps were provided with a soaked cotton wool for a period of 30 min prior to the trials. Subsequently, each wasp was transferred to separate 6.0 × 2.5 cm (height × diameter) glass vials which were then inverted. The bottom of each glass vial had a tiny drop of food and as soon as the wasp's tendency to walk upwards had brought it in contact with the test material, its responses were recorded. The reaction was scored as acceptance (if feeding lasted more than 5 s) or rejection (feeding for less than 5 s). Observations were made for a maximum of 10 min for each wasp, beginning at the time the parasitoid first came in contact with the droplet. Durations of

successful feeding bouts within the period were summed up to assess relative acceptance time to each treatment. A minimum of 30 females were tested for each treatment and each individual was tested once.

4.2.3 Data analysis

Phagostimulatory outcome was expressed as percent positive feeding response to each treatment. Replicated feeding duration was subjected to one-way analysis of variance (Program GLM, SAS Institute, 1999) and sample means were separated among treatments using Duncan's MRT procedure.

4.2.4 Results

Honey, honey-beebread, the disaccharide sucrose and its constituent sugars, fructose and glucose, evoked > 90% acceptance while maltose and trehalose led to < 50% phagostimulatory response in *C. plutellae*. Response to distilled water was < 10% and physiological salt was the least stimulatory of the treatments evoking an acceptance of < 5% (Table 4.1). In general, feeding occurred in discontinuous bouts and the total time spent feeding by each wasp within the 10 min of observation was summed up. The wasp fed for < 10 s on physiological salt while duration of < 50 s was recorded on distilled water. Feeding duration on specific sugars ranged between 70 and 130 s while much higher values of up to 330 s were recorded on honey and honey-beebread (Fig. 4.1). Time spent feeding on honey and honey-beebread was significantly higher than that spent on the sugars. With the exception of maltose, all sugars tested led to longer

Table 4.1: Feeding response of water-satiated *Cotesia plutellae* to honey, honey-beebread, physiological salt and specific sugars compared to distilled water during 10 min observation periods.

Treatment	*Sample size	Percent positive response
Honey	33 (32)	96.97
Honey-beebread	41 (39)	95.12
Physiological salt	32 (01)	3.13
Fructose	31 (30)	96.77
Glucose	30 (29)	96.67
Sucrose	31 (29)	93.55
Maltose	30 (14)	46.67
Trehalose	30 (11)	36.67
Distilled water	32 (03)	9.38

* Values outside parentheses represent the number of wasps tested per treatment while values within parentheses represent the number of wasps that showed positive phagostimulatory responses.

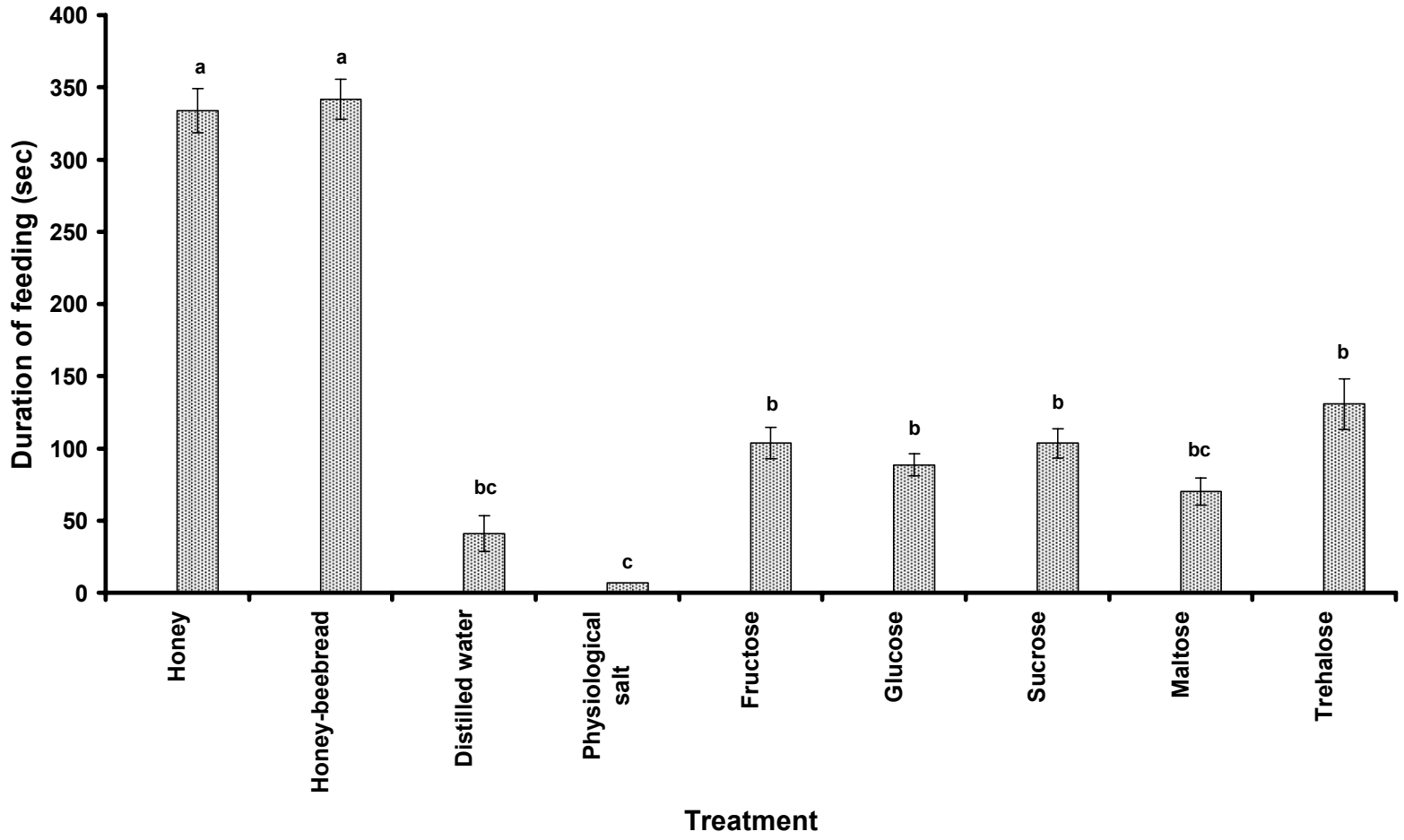


Figure 4.1: Duration of feeding (± s.e.) by water-satiated female *Cotesia plutellae* on honey, honey-beebread, specific sugars, physiological salt and distilled water. Bars having the same letter are not significantly different at $P \leq 0.05$.

duration of feeding than did the distilled water control.

4.2.5 Discussion

A feeding response can be elicited by stimulating gustatory chemoreceptors. Sensilla involved in taste perception contain gustatory receptor neurons (GRNs) that are specialized for either sugar, water, or concentrations of salt (Klowden, 2007; Nation, 2008). In the present experiments, acceptance or rejection of a treatment was typically preceded by contact with the mouthparts. These observations were also reported for *C. glomerata* (Wäckers, 1999), and suggest a role of mouthparts for sugar perception in parasitic Hymenoptera. However, the involvement of taste receptors in other parts of the insect body cannot be ruled out.

Honey, honey-beebread, fructose, glucose and sucrose could be categorized as 'highly stimulatory' food sources of *C. plutellae* based on the > 90% acceptance, maltose and trehalose as 'moderately stimulatory' sugars and the physiological salt as an 'antifeedant'. The poor acceptance of distilled water was due to the fact that the wasps were water-satiated prior to the trials. A good fit between phagostimulatory response and longevity has been shown for a number of insects (Wäckers, 2001; Irvin *et al*., 2007; Williams and Roane, 2007). The results of longevity study (Section 3.5 of this thesis) and observations made in the present experiments complement one another in establishing a direct

relationship between acceptance and longevity of *C. plutellae* on honey, honey-beebread, physiological salt and distilled water.

Feeding durations on pure honey and honey-beebread were higher than time spent feeding on sugar solutions suggesting that similarly high sugar concentrations in the field (i.e. concentrated honeydew) might have a strong impact on daily time allocation. A wasp spending a long time on a highly concentrated sugar source makes her an easy target for predators (Morse, 1986) and delays her return to host searching and egg laying activities. Application of sugar spray (e.g., diluted honey-beebread) becomes pertinent in this respect as a high amount of sugar can be consumed in a relatively short feeding time when diluted solutions are applied in the field (Siekmann *et al*., 2001). The long feeding durations on honey and honey-beebread suggest a reduced rate of fluid intake due to high viscosity (Wyckhuys *et al*., 2008). An exponential increase in viscosity is a major characteristic of increasing sugar concentration (Borrell, 2006), a factor that affects the handling of food in insects (Heyneman, 1983).

Sugars account for 95–99% of honey dry matter. The majority of these are the simple sugars fructose and glucose which represent 85–95% of total sugars. Small quantities of other sugars are also present, such as disaccharides (sucrose, maltose and isomaltose) and a few trisaccharides and oligosaccharides (FAO, 1996; Beach *et al*., 2003). Parasitoids vary considerably with respect to the spectrum of sugars that they can utilize. Wäckers (1999) reported that *C.*

glomerata showed ~100% acceptance to 2 M solutions of fructose, glucose, sucrose, erlose and maltose while melezitose elicited < 55% feeding response. On the contrary, Beach *et al*. (2003) observed > 90% acceptance of 2 M melezitose, fructose, glucose, sucrose, erlose and maltose by *Anaphes iole* Girault (Hymenoptera: Mymaridae). In addition, Winkler *et al*. (2005) recorded a high response in *D. semiclausum* to fructose, glucose, sucrose and maltose. The present study, together with the cited literature, shows that parasitoids from different hymenopterous families readily accept sugars that are dominant in nectar or honey.

4.3 Orientation response

4.3.1 Introduction

Movement towards or away from objects in the environment is mediated by the senses of smell and sight. However, olfaction is the primary sense used by insects to detect and locate food resources (Pernal and Currie, 2002). This is crucial to foraging success as odour identification guides them to food location without wasting time and energy unnecessarily. In insects, information about food odour is encoded by olfactory receptor cells with characteristic response spectra, located in several types of cuticular sensilla. The insect antenna is a very special biological organ with numerous sensillae, containing over a thousand olfactory receptor neurons (ORNs), which have the ability to sense and code odour cues with remarkable sensitivity and specificity (Elmore *et al*., 2003; Keller and Vosshall, 2003). Roux *et al.* (2005) suggested that *C. plutellae* uses *Sensilla*

coeloconica Type I located on the ventral side of antennomeres 6–15 (one per antennomere) and the numerous *S. placodea* (on all the 16 antennomeres) in odour detection.

Olfactory sensilla walls possess many pores through which odor molecules diffuse to reach the dendrites of sensory cells after passing through the intermediate pore tubules. Acceptors (odorant-binding proteins) for the stimulus molecules are thought to be present in the dendritic membranes and they transport the molecules across the sensillum liquor (an aqueous medium) to the dendritic endings (Nation, 2008). When odor molecules bind with the acceptors, the membrane conductance is changed, generating a decrease in the resting potential. Nerve impulses are subsequently generated and conducted to the central nervous system. The time interval from stimulus onset to the generation of nerve impulses ranges up to about 500 ms at very low odor concentrations. The antennal receptor cells send their axons to the antennal lobe where the axons terminate in glomeruli. The information running through the antennal nerve is processed and evaluated in the brain at several levels. The output neurons send their axons via the tractus olfactorio-globularis towards other brain centers, and terminate in the calyces of the mushroom bodies (corpora pedunculata) as well as in the lobus lateralis protocerebralis (Visser, 1986).

The attraction or deterrence of parasitoids to chemical cues is usually assessed using different types of olfactometer (e.g., Vuorinen *et al.*, 2004; Tamò *et al.*,

2006). In this section, response of *C. plutellae* to odours of honey and honey-beebread was assessed using a Y-tube olfactometer. The objective of the study was to test if the two diets would elicit similar response in the wasp.

4.3.2 Materials and methods

Olfactometer bioassays

Preference of water-satiated freshly emerged but mated *C. plutellae* females for odours emanating from honey and honey-beebread was tested in a Y-tube olfactometer as described by Rossbach *et al.* (2005). The glass olfactometer had the following dimensions: main arm 29.5 cm, side arms 15.5 cm, inner diameter 2.3 cm, angle between two side arms ~45º. Each side arm had a detachable bulbous component which served as a container for odour sources (Fig. 4.2). Prior to the trials, wasps were kept in glass receptacles around the test area for them to acclimatize to the environment. Tests were conducted in a bioassay room at 22 ± 2 ºC and four fluorescent tubes (FLUORA L58W/77) provided a steady illumination for the test area.

In two-choice bioassays, female parasitoids were allowed to respond to various combinations of the following stimuli:

(a) Pure honey: approximately 2 g in a suspended glass vial cover

(b) Honey-beebread: approximately 2 g in a suspended glass vial cover

(c) Clean air control: compressed air, from a cylinder, which passed through a water-containing jar before entering the odour chambers.

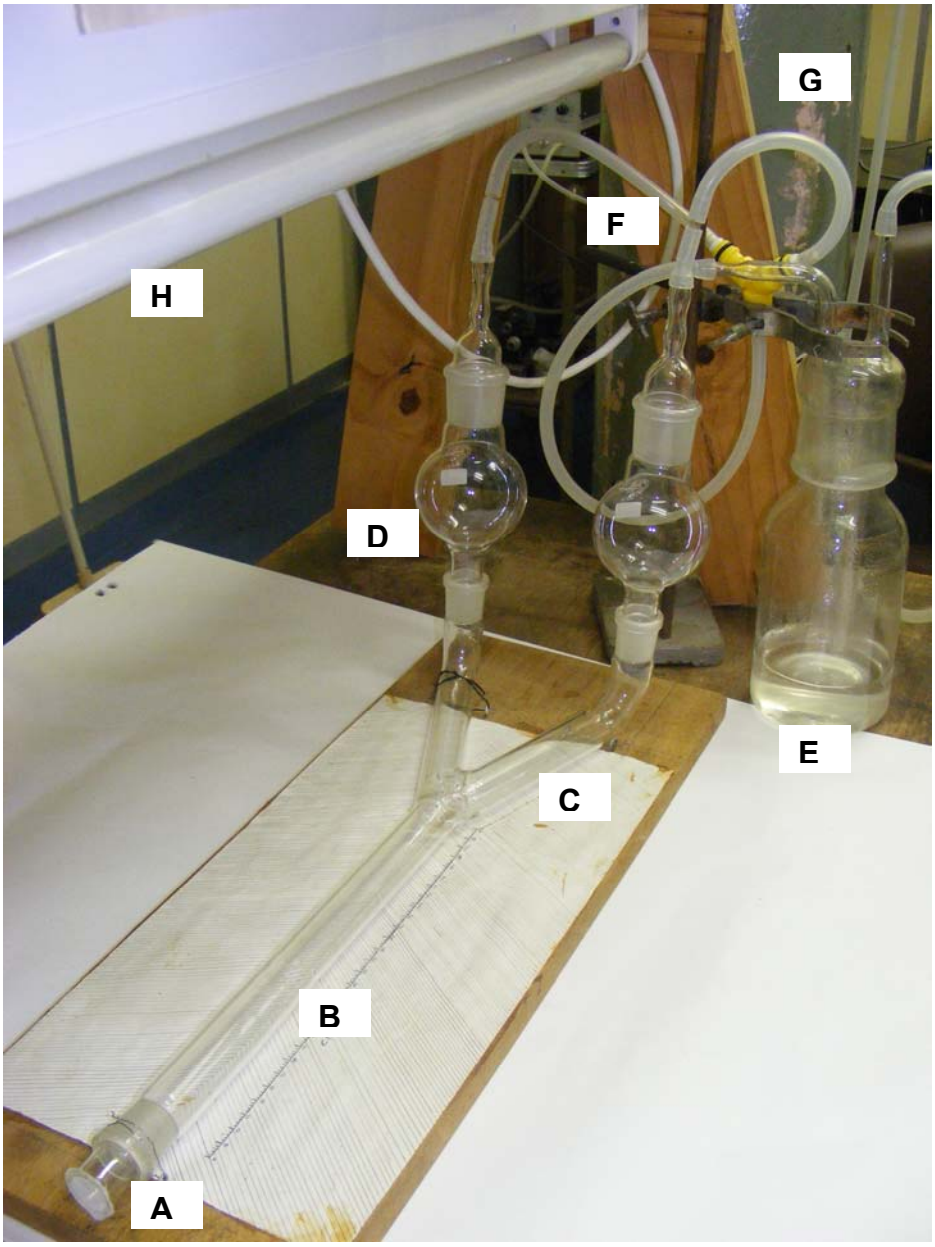


Figure 4.2: A typical Y-tube olfactometer set-up showing the receptacle for releasing insects (A), main arm (B), side arm (C), odour chamber (D), water container (E), Teflon tube (F), air cylinder (G) and fluorescent tube (H).

The odours were introduced into the apparatus by pushing humidified clean air into odour chambers at 0.09–0.10 m/s to carry the volatiles via the side arms into the main arm of the device. After this, a naïve female (i.e. mated but without oviposition experience) was introduced at the base of the main arm and its movement in the apparatus was observed for 10 min.

Parasitoids that went beyond the intersection and remained in one of the side arms for at least 20 s were recorded as having made a first choice. Females that remained in the main arm or spent less than 20 s in one of the side arms were recorded as showing no response. Those that switched between the two arms without initially spending more than 20 s in one of the arms were recorded as not having made a definite choice. To avoid bias, odour sources were switched between the left and the right arms of the Y-tube after every 5 insect and a new set of glass wares was used each time. After testing a given odour combination, the olfactometer was washed, rinsed with acetone and dried in an oven at 70 ºC for at least 24 h.

Behavioural sequence

Activities of introduced wasps were monitored throughout the 10 min mark using a stopwatch for good documentation. Time taken by the wasps to choose a given side arm and time taken to make a homing behaviour on each treatment was recorded.

4.3.3 Data analysis

Data from olfactometer bioassays were subjected to Chi-Square analysis (SAS Institute, 1999) to determine significant differences between the number of insects choosing either test odour. Time taken by the wasps to decide for each treatment and time taken to make homing behaviour was subjected to one-way ANOVA (Program GLM, SAS Institute, 1999), followed by Duncan's multiple range tests for comparison of treatment means. Actions associated with parasitoids that made a choice and those that did not make any choice were outlined separately.

4.3.4 Results

Olfactometer bioassays

Freshly emerged water-satiated *C. plutellae* preferred odour emanating from food to that of clean air in separate experiments (honey vs. clean air: $\chi^2 = 4.50$, $n = 32$, $P = 0.0339$; honey-beebread vs. clean air: $\chi^2 = 8.91$, $n = 22$, $P = 0.0028$). However, in *choice* experiment involving the two food sources, the parasitoid showed a weak preference for odour emanating from honey-beebread (honey vs. honey-beebread: $\chi^2 = 3.95$, $n = 57$, $P = 0.0469$) (Fig. 4.3).

Behavioural sequence

The behavioural patterns, used in the analysis, are described in Table 4.2 and typical sequence was divided into two based on observation: (1) activities that led to a decision, and (2) activities that did not lead to a decision.

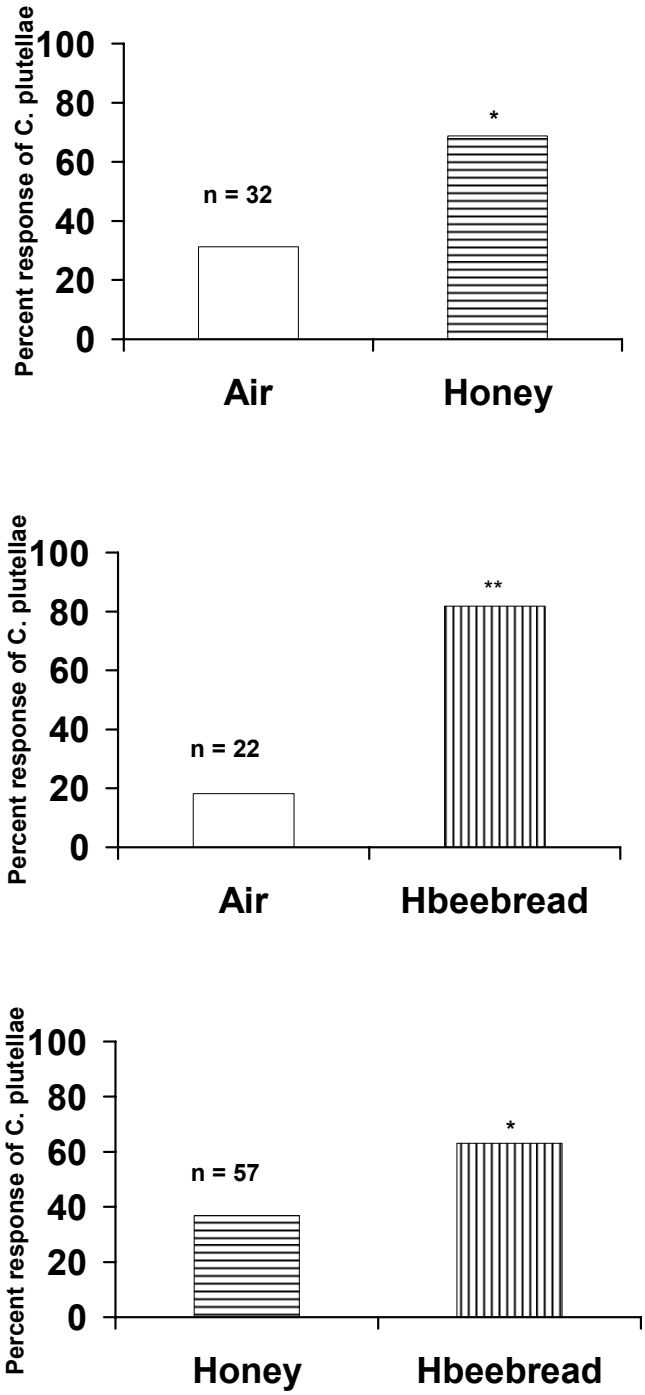


Figure 4.3: Responses of water-satiated *Cotesia plutellae* females to honey vs. clean air, honey-beebread vs. clean air, and honey vs. honey-beebread.

*, ** indicate significant χ^2-test at 0.05 and 0.01 levels of probability, respectively.

Table 4.2: Patterns of behavioural sequence of *Cotesia plutellae* and abbreviations used in text.

Pattern	Definition
walk	The female walks continuously with antennal movements (right / left, up / down, U-shape position with dorsal side or tip in contact with the glass)
groo	Grooming (wasp rubs its antennae with forelegs and abdomen with hindlegs)
pree	Preening (wasp rubs its wings with hindlegs then rubs the tarsi of the legs together)
hom	Homing behaviour (wasp goes beyond the side arm into the odour chamber)
ante	Antennation (wasp moves the antennae vigorously in different directions and may touch the glass sometimes)
rem	Wasps either remain static or move round the circumference of the receptacle without entering the main arm of the olfactometer

Decision

Female wasps either spent 15 s in the receptacle (with antennae in an elevated position) or started walking (*walk*) rapidly along the main arm soon after it was introduced into the olfactometer. Most of the time, the antennae were U-shaped with the dorsal side of apical antennomeres (or sometimes only the tips) in contact with the glass. When it had gone around midway of the main arm, it may stop for about 3 s to groom (*groo*) and at about ⅔ of the length of the main arm, it may hesitate for about 2 s to preen (*pree*) its wings. Some of the parasitoids completed their decision-making process before they got to the intersection. These ones entered the chosen arm without hesitating at the intersection and time taken from introduction to first choice was 35 s. A large number of these early-deciding wasps showed homing (*hom*) behaviour. For others, there was a brief stop at the intersection with the antennae moving in all directions before they decided to enter one of the side arms. Some wasps may hesitate midway in the side arm, antennate (*ante*) for a few seconds before deciding to go further for the decision whereas for some, they may decide to leave for the second arm after < 20 s. Very few of these late-deciding wasps showed homing behaviour and time taken from introduction to first choice was mostly 3–5 min.

Parasitoids took less time in making positive decisions for honey-beebread compared to honey while they took longer time in deciding for clean air. Approximately, the wasps needed 3, 3.5 and 5 min to choose honey-beebread, honey and clean air respectively. Time taken for wasps to make homing

behaviour on honey was about twice the time needed to do the same on honey-beebread (Fig. 4.4). None of the wasps homed on clean air.

No decision

Some of the wasps remained (*rem*) inside the receptacle for up to 5 min and they were, therefore, eliminated from the experiment. Others left the receptacle after about 2 min and walked slowly along the main arm. They often moved to and fro the ⅓ and ⅔ regions of the main arm and some wasps returned to the receptacle, remaining there till the expiration of the 10 min. Some wasps also went as far as the intersection only for them to remain there and antennate intermittently whereas others returned to the main arm. A few wasps entered a side arm, hesitated for some seconds to antennate and left the arm under 20 s. Such wasps either returned to the main arm to repeat the same sequence all over again or kept on interchanging between the two side arms without spending at least 20 s in any of them.

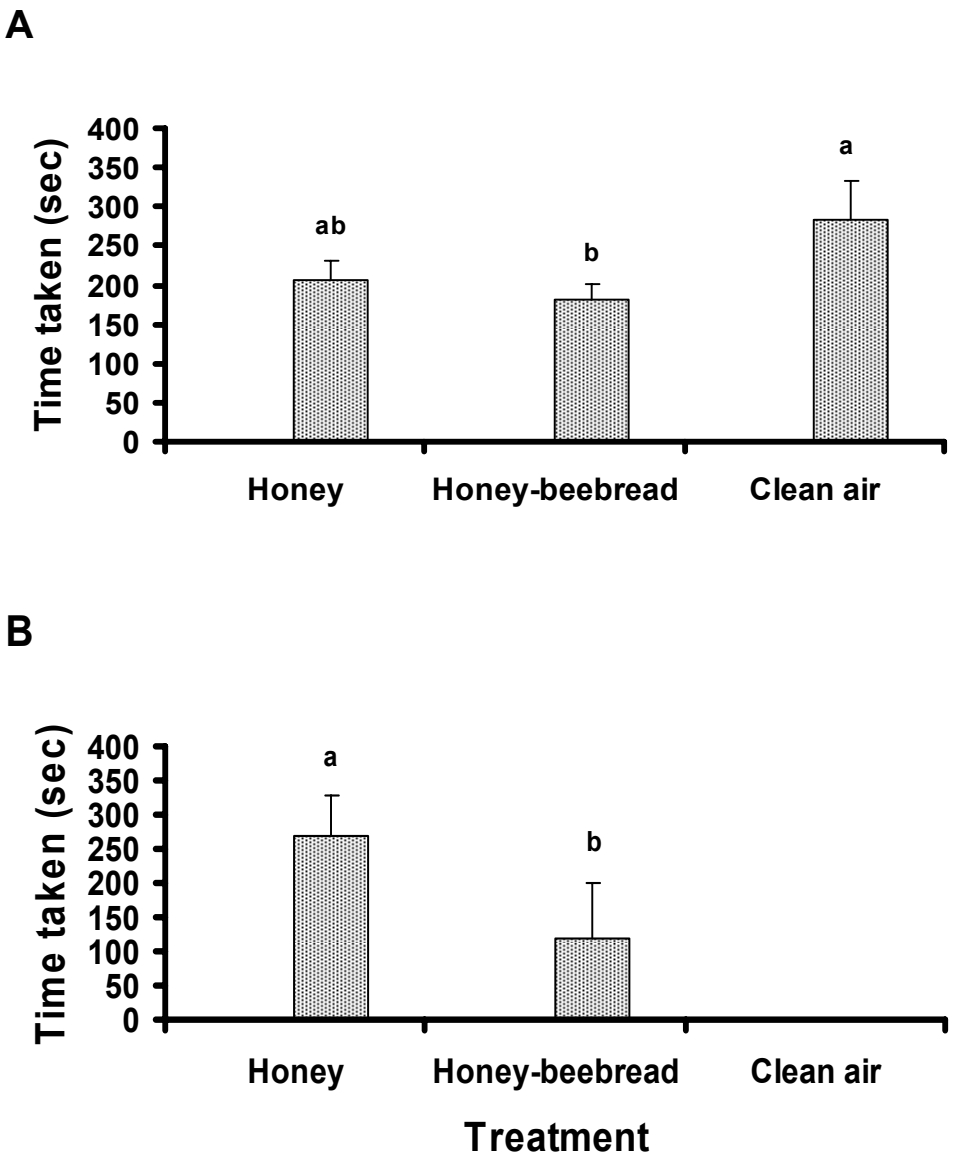


Figure 4.4: A. The means (+ s.e.) of the total time spent in the olfactometer before *Cotesia plutellae* made positive choice for honey, honey-beebread or clean air. B. Average time spent in the olfactometer before the wasps entered odour chamber (homing) containing honey or honey-beebread. Bars having different alphabets are significantly different at $P \leq 0.05$.

4.4 Antennal response

4.4.1 Introduction

The investigation of insect chemical communication has been greatly facilitated by the use of electrophysiological methods. In insect olfaction, these techniques were pioneered by Schneider in 1957 (cited in Schiestl and Marion-Poll, 2002) who, for the first time, recorded an electroantennogram (EAG) detected by an excised antenna of male *Bombyx mori* (L.) (Lepidoptera: Bombycidae) that was stimulated by the female sex pheromone. EAG is essentially the sum of many olfactory receptor potentials recorded more or less simultaneously by an electrode located in the sensory epithelium (Klowden, 2007). The principle of EAG is to record voltage changes between the tip and the base of an antenna during stimulation by a volatile (Fig. 4.5).

Electroantennogram recording of insect olfactory responses has been an asset for the bioassay for chemical components of pheromones and for the development of synthetic attractants. The EAG technique gives a good account of responses to volatiles and previous studies established direct relationships between EAG response and field behaviour of insects (Karg *et al*., 1997; Brindis *et al*., 2008). The purpose of this study was to undertake an analysis of the olfactory sensitivities of *C. plutellae* and that of its host, *P. xylostella,* to odours emanating from honey and honey-beebread. The outcome of this study would determine if the insects are able to distinguish the two food sources.

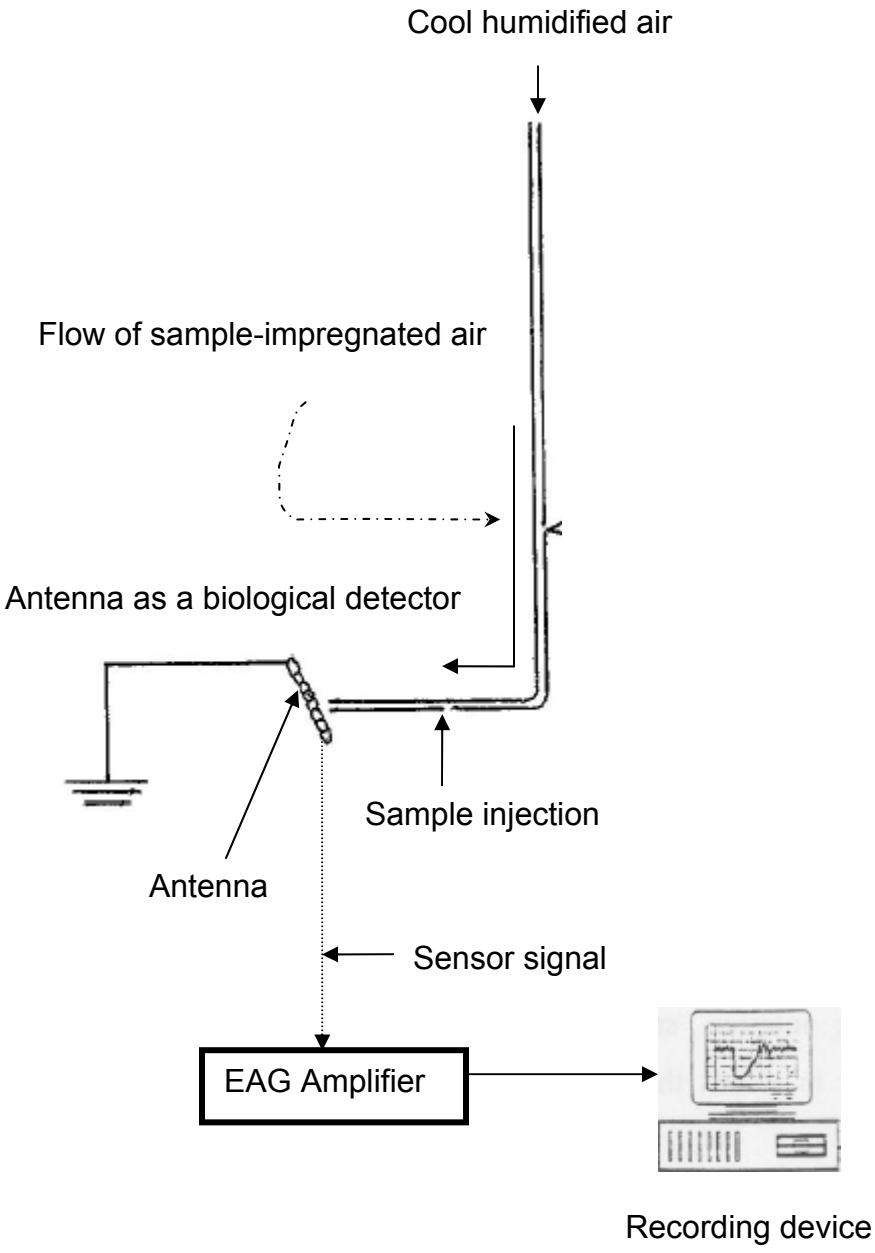


Figure 4.5: Schematic presentation of an EAD setup. The receptors on the whole excised antenna respond to the stimulus blown across it. The responses of all the sensory cells are amplified and displayed.

4.4.2 Materials and methods

Sample preparation

For the stock solutions, 3.2805 g of each diet was dissolved separately in 3 ml distilled water to give an equivalent of 1093.5 µg diet/µl dH_2O. The glass vials containing the solutions were then shaken in an ultrasonic bath DECON FS 100 for 20 min to break diet particles and make the solution finer. This concentration was further diluted in distilled water to obtain the following serial solutions: 364.5, 121.5, 40.5, 13.5, 4.5 and 1.5 µg diet/µl dH_2O.

Odour stimuli

Five microlitres (thus yielding a dose of 5467.5, 1822.5, 607.5, 202.5, 67.5, 22.5, and 7.5 µg diet respectively) of each serial solution and distilled water (control) were pipetted onto filter paper strips. Another strip of filter paper was treated in a similar manner with 15 µl of the stock solution, giving a dose of 16402.5 µg diet. The strips were air-dried for some minutes and later inserted into separate Pasteur pipettes. This experiment was carried out to investigate whether the EAG response profile changed with increasing concentrations.

Antennal preparation

The antennae used in the tests were obtained from one- to two-day old males and females. The insects (*C. plutellae* and *P. xylostella*) were individually anesthetized with CO_2 after which the head was cut off carefully under a Kyowa Optical Model SDZ-PL binocular microscope. Using a pair of forceps,

entomological pins and a modified blade, the head was carefully dissected and an antenna was removed. The base of the antenna was put on the positive terminal of a gold-plated electrode while the tip was placed on the negative end. The antenna was held fast to the terminals with an electrogel. The terminal portion of the antenna was cut off to allow haemolymph come in contact with the gel for electrical conduction.

Electroantennogram recordings

Antennal responses of the insects to the two diets were investigated using an electroantennogram (EAG) method (Das *et al*., 2007). The tip of the loaded pipette was inserted into a side hole (2.5 mm in diameter, 80 mm upstream from the outlet) on the main airflow tube (6 mm inner diameter) in which a continuous moistened airflow was blown onto the prepared antenna. Using an electronic stimulus controller (CS-05, Syntech), a 0.3 s puff of air was injected through the large end of the Pasteur pipette, transporting the volatiles to the antenna for stimulation. Each antenna was tested with a series of stimuli in the order: solvent, 7.5, 22.5, 67.5, 202.5, 607.5, 1822.5, 5467.5, 16402.5 µg diet, and solvent. At least 1 min was allowed between two stimuli to provide time for recovery of antennal responsiveness. The signals generated by the antenna were passed through a high-impedance amplifier (UN-06, Syntech) and processed with a PC-based signal processing system (EAG Version 2.7 Syntech®, The Netherlands).

4.4.3 Data analysis

To evaluate comparative antennal sensitivity to the different stimuli tested, the data were standardized by expressing the EAG values (mV) as a percentage of the standard stimulus. The corresponding normalized values were generated by the signal processing software and they were obtained by clicking on 'Lists' button under the 'Options' tab. This action led to a pop-up 'Value list' page and under 'List type', 'Norm.' option was chosen. A typical set of mV and corresponding normalized values are presented in Appendix 2. Dependent variable data were not transformed because assumption of normality was not violated according to Shapiro-Wilk test (SAS Institute, 1999).

Analysis of variance of EAG responses for the two sexes of each insect species was performed and sample means were compared using DMRT to indicate significant differences at $P \leq 0.05$ (Program GLM, SAS Institute, 1999). Subsequently, series of *t*-tests were used to compare percent EAG between insect species, between sexes and between diets (GenStat for Windows, 2007). Electroantennogram data for male and female insects were tested separately because of the occurrence of sexual dimorphism with regards to odour detection. Dose-response data were also subjected to linear regression in which dose served as the independent variable and amplitude of response served as the dependent variable. This was used to describe the relationships between substrate dose and EAG response, with separate analyses for each test substrate and each sex.

4.4.4 Results

An example of electrophysiological graphs obtained during this study is shown in Fig. 4.6. The EAG responses of each insect species at each dose are also presented in Fig. 4.7. Both sexes of the diamondback moth and *C. plutellae* distinguished between clean air and all the doses of honey and honey-beebread tested. The diamondback moth and *C. plutellae* showed equal responses to honey at 22.5 and 1822.5 µg while a dose of 202.5 µg elicited higher responses in the DBM than in the parasitoid. On the other hand, a *C. plutellae*-biased trend was noticed in tests involving honey-beebread. The diet elicited higher responses in *C. plutellae* at 67.5, 607.5, 1822.5 and 5467.5 µg and there was no dose at which the EAG responses of DBM surpassed that of the parasitoid. Generally, EAG response increased with increasing dose.

Honey and honey-beebread elicited equal EAG responses among the males as well as females of the diamondback moth. On the other hand, EAG values recorded for tests involving honey-beebread were higher than those for honey in both male and female *C. plutellae* (Table 4.3). Males of the DBM and *C. plutellae* did not differ in their responses to honey whereas, males of the latter showed higher responses when honey-beebread was tested. Females of the DBM were more sensitive to odour emanating from honey while on the contrary, females of *C. plutellae* recorded higher EAGs than the DBM when honey-beebread was tested (Table 4.4). Comparisons of means within each insect species (Table 4.5)

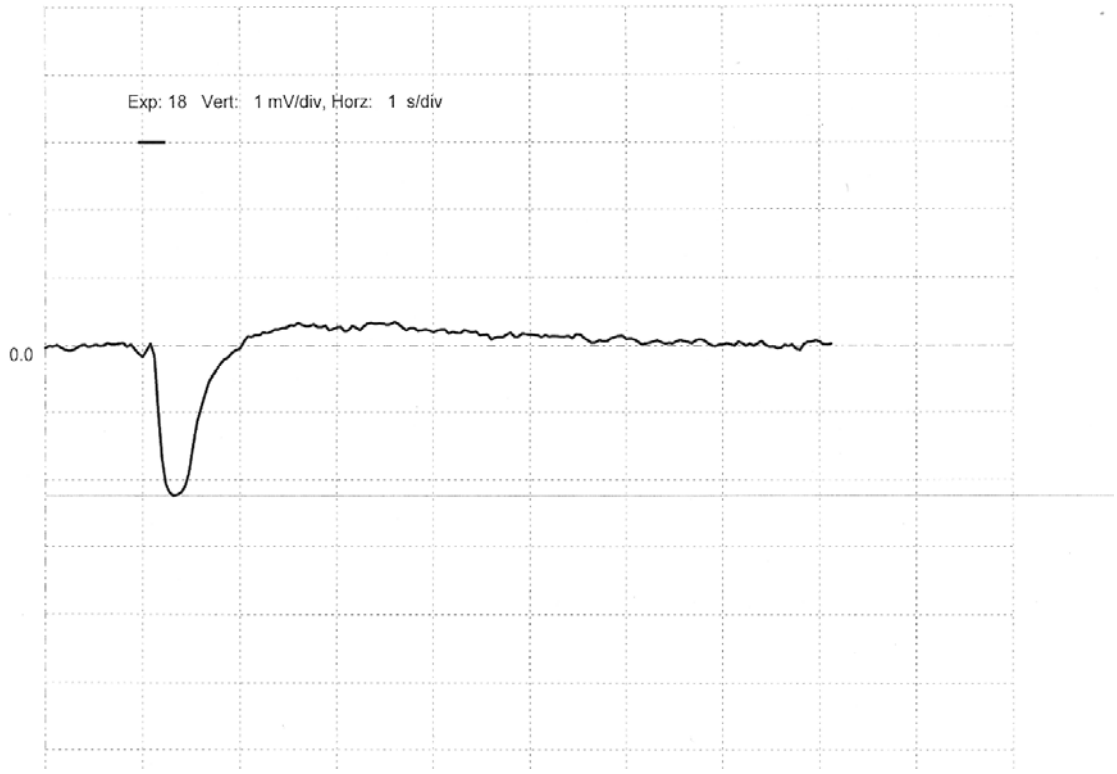


Figure 4.6: Typical electroantennogram (EAG) of an antenna towards airstream from a filter paper strip treated with a solution of known concentration.

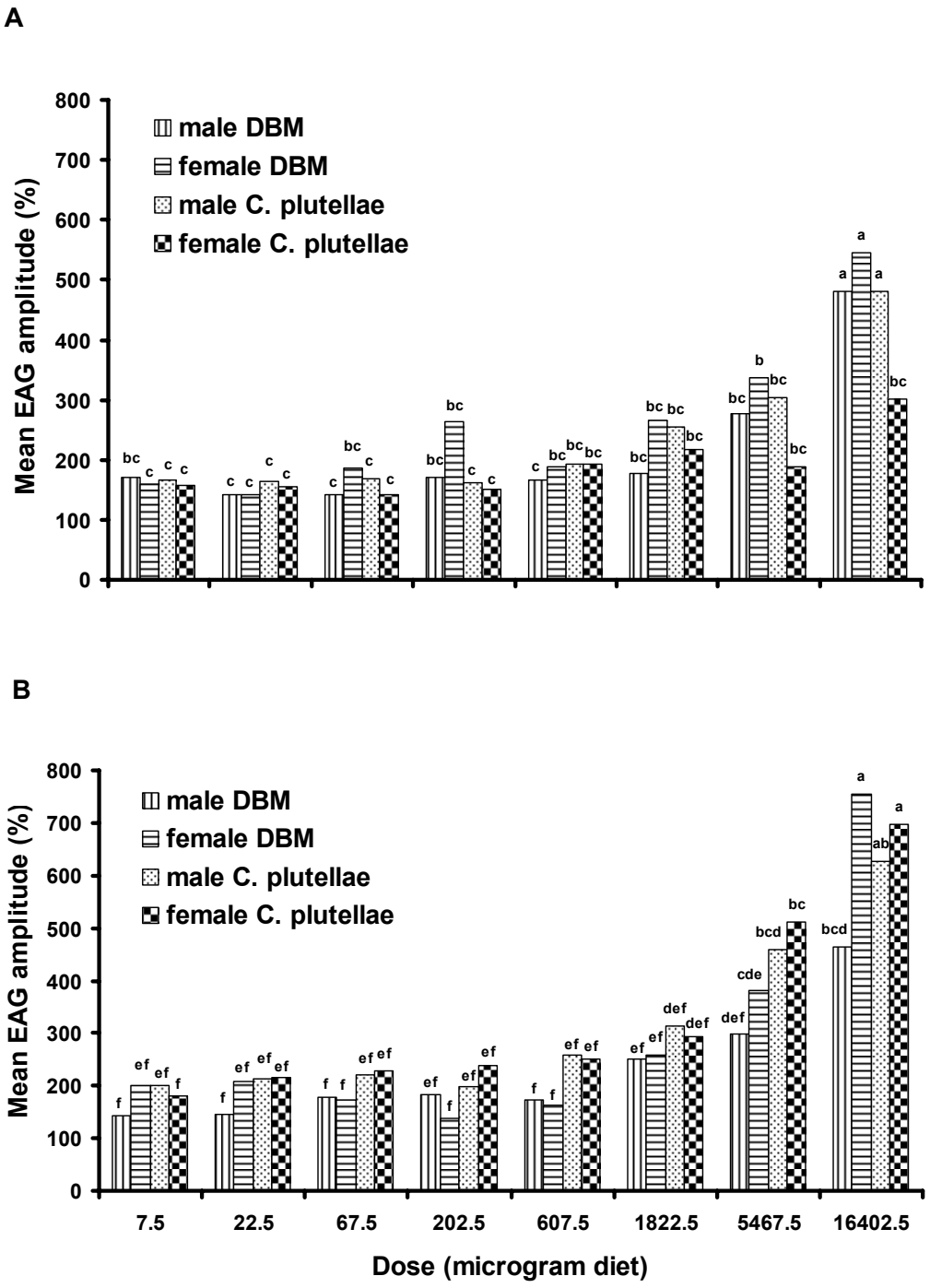


Figure 4.7: The electroantennogrm (EAG) responses of the diamondback moth, *Plutella xylostella,* and *Cotesia plutellae* to eight doses of honey (A) and honey-beebread (B). Percent EAG amplitude to clean air control was normalized as zero (0). Bars with the same letter are not significantly different at $P \leq 0.05$.

Table 4.3: Comparison of average electroantennogram responses to honey and honey-beebread by each sex of *Plutella xylostella* and *Cotesia plutellae*.

Male			
Species	Diet	EAG (%)	Mean separation
Plutella xylostella			
	Honey	215.48	t = 1.14, d.f. = 39, P = 0.261
	Honey-beebread	229.55	
Cotesia plutellae			
	Honey	236.41	t = 4.65, d.f. = 79, P = 0.001
	Honey-beebread	311.29	
Female			
Species	Diet	EAG (%)	Mean separation
Plutella xylostella			
	Honey	260.98	t = 1.25, d.f. = 39, P = 0.220
	Honey-beebread	284.50	
Cotesia plutellae			
	Honey	188.10	t = 6.04, d.f. = 79, P = 0.001
	Honey-beebread	327.11	

Table 4.4: Influence of sexual categories on electroantennogram responses of *Plutella xylostella* and *Cotesia plutellae* to honey and honey-beebread.

		Male	
Diet	Species	EAG (%)	Mean separation
Honey			
	P. xylostella	215.48	t = 1.50, d.f. = 79, P = 0.142
	C. plutellae	236.41	
Honey-beebread			
	P. xylostella	229.55	t = 5.75, d.f. = 79, P = 0.001
	C. plutellae	311.29	
		Female	
Diet	Species	EAG (%)	Mean separation
Honey			
	P. xylostella	260.98	t = 2.52, d.f. = 79, P = 0.016
	C. plutellae	188.10	
Honey-beebread			
	P. xylostella	284.50	t = 1.73, d.f. = 79, P = 0.047
	C. plutellae	327.11	

Table 4.5: Intraspecific variation in detected electroantennogram by *Plutella xylostella* and *Cotesia plutellae* for honey and honey-beebread.

Plutella xylostella			
Diet	Sex	EAG (%)	Mean separation
Honey			
	Male	215.48	t = 2.82, d.f. = 39, P = 0.008
	Female	260.98	
Honey-beebread			
	Male	229.55	t = 2.58, d.f. = 39, P = 0.014
	Female	284.50	
Cotesia plutellae			
Diet	Sex	EAG (%)	Mean separation
Honey			
	Male	236.41	t = 1.88, d.f. = 79, P = 0.064
	Female	188.10	
Honey-beebread			
	Male	311.29	t = 0.59, d.f. = 79, P = 0.560
	Female	327.11	

showed that female diamondback moth was more sensitive than the male in both honey and honey-beebread tests while there was no case of sexual dimorphism in the responses of *C. plutellae*.

4.4.5 Discussion

In the olfactometer and EAG experiments, *C. plutellae* showed stronger preference for the odour of honey-beebread over that of honey. In order to optimize its longevity and offspring production, *C. plutellae* needs to feed throughout its lifetime. Therefore, ability to perceive food odour in the airstream and to orientate toward such becomes crucial. The results from the current study show that the parasitoid is able to distinguish food odour from that of clean air and respond by moving toward the source. Its ability to discriminate between food and nonfood sources using only olfaction in Y-tube olfactometer suggests that olfaction plays a key role in the parasitoid's food-finding process.

Naïve *C. plutellae* showed no or only a marginal attraction to clean air whereas significant responses were shown toward honey and honey-beebread. This is an indication that *C. plutellae* shows innate response to odours from its food and it would be able to locate honey or honey-beebread if supplied in an agroecosystem. However, honey-beebread elicited a greater response from *C. plutellae* suggesting that a component of the beebread must have been responsible. Beebread is rich in amino acids which are either absent or present in traces in honey (FAO, 1996). In a feeding bioassay, Lanza (1988) observed that two formicid ants (*Crematogaster*

erecta Mayr and *Solenopsis geminata* Fabricius) preferred artificial diets containing both amino acids and sugars to artificial diets with only sugars. It was concluded that the amino acids made the diets more attractive to the insects. Although it has been reported that not all hymenopterans favour amino acid-fortified diets in *choice* tests (Lanza *et al*., 1993; Gardener *et al*., 2003; González-Teuber and Heil, 2009), clarification of the role of amino acids in food preference of *C. plutellae* would add to knowledge of its nutrition physiology.

Olfactory perception in insects is usually followed by orientation behaviour which eventually leads to target location. Decision making prior to target location entails movement of different parts of the body in order for the insect to interpret the perceived odour. In the present study, a complex series of movement of the antennae, wings, tarsi and other parts of the body was observed either when the insect was moving or when stationary. This is an indication that the antennae alone may not be sufficient in making appropriate food foraging decisions in *C. plutellae*. Time taken by the parasitoid for decision making varied among the resources tested. The wasps needed a short time inside the olfactometer to choose honey-beebread while they needed a longer time to decide for honey. However, parasitoids that chose clean air spent the longest time in the olfactometer. Similarly, it took the parasitoids less time to get to honey-beebread in the odour chamber (homing behaviour) than for them to do same on honey. This implies that type and intensity of odour may influence the rate at which odours are perceived, interpreted and responded to by insects.

The diamondback moth and *C. plutellae* responded to all the doses of honey and honey-beebread tested in the electrophysiology experiment. However, a dose-dependency was observed as the EAG response increased with increasing concentration. Male DBM responded equally to honey and honey-beebread and a similar outcome was observed when females were tested. On the other hand, male and female *C. plutellae* detected higher EAGs from honey-beebread than from honey. This corroborates observations made in the olfactometer experiment where *C. plutellae* showed preference for honey-beebread in *choice* tests. Also, when the two insect species were compared, male and female *C. plutellae* detected higher EAGs than their corresponding DBM counterparts in the presence of honey-beebread. The EAG results indicate that the parasitoid has a higher chance of locating honey-beebread more quickly than its host in an agroecosystem. Another advantage of this attribute is that the parasitoid would be able to utilize honey-beebread soon after application, before it degrades.

The case of sexual dimorphism in the response of DBM to honey and honey-beebread, and the lack of such among *C. plutellae* might be a hint that the dietary requirements of female DBM are greater than that of the males. However, the explanation may not be this straightforward. Other factors such as odour components of food and the number of receptor cells specialized to different components of the mixture may be responsible for the occurrence of sexual dimorphism in an insect species. Recording the response of the individual olfactory

cells by single sensilla recording technique may thus be necessary in order to make a comprehensive explanation.

In conclusion, this study shows that *C. plutellae* distinguished honey and honey-beebread from clean air and that the latter diet is preferred in *choice* experiments using an olfactometer. The parasitoid also detected higher EAGs from honey-beebread than it did from honey whereas the diamondback moth detected equal EAGs from the two diets. Hence, honey-beebread may be a supplementary food source with greater benefit to *C. plutellae*. Application of these diets in an agroecosystem may result in behavioural changes among the insects. Further investigation of the impact of honey and honey-beebread on the foraging behaviour of *C. plutellae* is conducted in Chapter 5.

CHAPTER 5:

Effects of associative learning on the foraging behaviour of *Cotesia plutellae* in the laboratory

5.1 Introduction

Learning is a change in the behaviour of an organism as a result of experience, and its ability to learn accounts for a large part of its behaviour. Insects are capable of recognizing and remembering cues. The physical storage locations of olfactory and visual cues have been suggested to be the mushroom bodies and optic lobes of the brain respectively (Klowden, 2007). Although insects rely on innate behavior to successfully manage many types of variation and unpredictability, learning may be superior to innate behavior when dealing with features unique to time, place, or individuals. Variation in learning ability exists among individual insects and those that are good learners may be at an adaptive advantage over those that are poor learners (Behmer, 2004).

There are non-associative and associative forms of learning in insects. The former is characterized by a gradual increase in response to a stimulus with repeated exposure to it (sensitization) even when it has not been paired with any other stimulus while the latter involves the establishment, through experience, of an association between two stimuli or between a stimulus and a response (Papaj and Prokopy, 1989). For example, Olson *et al*. (2003) reported that after training *Microplitis croceipes* Cresson (Hymenoptera: Braconidae) with chemicals in close association with either sugar water or host faeces, the parasitoid successfully linked each of the chemicals to either the food or host resource.

Female parasitoids deal with the often competing needs of host searching (for immediate fitness) and food searching (for survival and/or future reproductive output) by detecting and integrating information on resource availability and distribution. This ability is accompanied by flexible behavioural processes that allow them to take decisions based on their internal variables such as hunger level, mating status, egg load, and age, and by external variables such as adult food and host availability and detectability (Desouhant *et al*., 2005). The 'hunger hypothesis' (Jacob and Evans, 2001) states that a hungry female wasp will more likely search first for food for herself rather than searching for hosts for her offspring.

The present study was conducted to test the effects of non-associative learning on food- and host-searching activities of newly emerged female *C. plutellae*. Responses to food and host cues by wasps having different physiological status were observed in the Y-tube olfactometer and the following questions were addressed:

1. do initial food and host experiences have additive effects on the foraging performance of *C. plutellae*?, and
2. does *C. plutellae* obey the hunger hypothesis?.

5.2 Materials and methods

The procedure described by Faria (2005) was followed in this experiment.

Experiment 1: Food learning.

The following groups of mated female *C. plutellae* were tested:

(a) wasps starved for about 20 h from the period they emerged (no feeding, no odour experience),

(b) hungry wasps with brief experience of honey odour, without feeding,

(c) hungry wasps with brief experience of honey-beebread odour, without feeding,

(d) hungry wasps with brief feeding experience on honey, and

(e) hungry wasps with brief feeding experience on honey-beebread.

For odour experience, wasps were confined in a well ventilated cylindrical plastic container (18.5 cm height, 9.0 cm diameter) which was then placed inside an equally ventilated Perspex cage (30.0 × 21.5 × 21.5 cm) containing four vials of a food source. In this way, wasps were allowed to have access to the food odour for 15 s after which the container was removed. This training procedure was repeated after 20 min for another 15 s. The experience without food reward was to determine if mere exposure to food odour would increase the responsiveness of wasps to such cues.

For the training with a food reward, wasps were introduced directly into the ventilated Perspex cage and allowed to feed for 15 s, after which the food sources were removed. Twenty minutes later, the females were given the same experience for another 15 s.

After the training experiences, the wasps were confined in glass jars and their responses to odour sources were tested 30 min later using the Y-tube olfactometer. The following odour source choices were tested:

i. honey vs. clean air, and

ii. honey-beebread vs. clean air.

Experiment 2: Food versus host learning.

After it was confirmed that *C. plutellae* females can be sensitized with food odour, an experiment was designed to test if previous experience and hunger state of the parasitoid would affect choice between food foraging and host foraging cues. The training protocol for hungry females was the same as that described in the food learning experiment. To obtain control (satiated) females, wasps were placed in the same set up as described above, but they were allowed to feed for 20 min. For host experience, wasps were provided with second instar DBM larvae in ratio 1:4 (parasitoid: host) inside a glass jar. It was not confirmed if eggs were laid in parasitized larvae.

Wasps with the following combination of training experiences were used for the experiments:

(a) wasps with neither food nor host experience,

(b) hungry wasps with brief feeding experience on honey,

(c) hungry wasps with brief feeding experience on honey-beebread,

(d) wasps that fed on honey for 20 min,

(e) wasps that fed on honey-beebread for 20 min,

(f) hungry wasps with brief feeding experience on honey in addition to host experience,

(g) hungry wasps with brief feeding experience on honey-beebread in addition to host experience,

(h) wasps that fed on honey for 20 min and also had host experience, and

(i) wasps that fed on honey-beebread for 20 min and also had host experience.

The following odour source choices were tested:

i. honey vs. infested cabbage seedlings, and

ii. honey-beebread vs. infested cabbage seedlings.

Bioassays

A Y-tube olfactometer was used to test the attractiveness of food- and host-associated cues for *C. plutellae* as described in Section 4.3 except that the cup bearing infested seedlings was fastened to a bell-jar (24 cm height, 10 cm diameter) using Parafilm. The jar was in turn connected to an olfactometer arm with Teflon tube (diameter 0.5 cm). A similar jar was attached to the other arm of the olfactometer, in the same manner, to ensure that air flows through the odour sources at the same rate (Fig. 5.1). Group of wasps with similar experience(s) was tested individually and each group had at least 20 replicates.

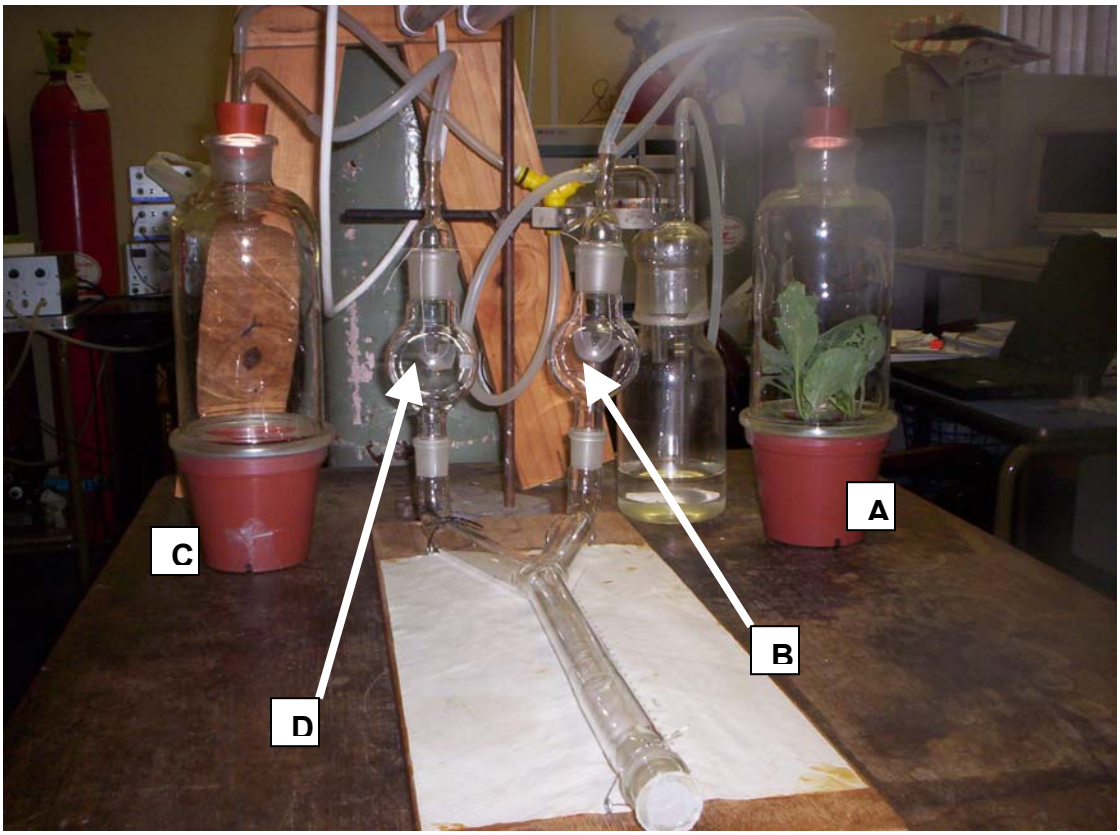


Figure 5.1: A photograph of the Y-tube olfactometer set-up used to test food versus host preference in *Cotesia plutellae*. The cup bearing infested cabbage seedlings (A) was fastened to the base of a bell-jar and it was connected to a side arm having a suspended container without food (B). The second cup (C) contained potting soil and it was connected to the arm having a suspended container with food (D).

5.3 Data analysis

Data for each wasp group were analyzed using Chi-Square test (SAS Institute, 1999) to determine significant differences between the number of insects choosing either of two test odours.

5.4 Results

Food learning

Female *C. plutellae* that were starved for about 20 h after emergence did not distinguish between food odour and clean air: the number of females that chose the arm carrying food odour was not different from the number that chose the arm carrying clean air (honey vs. clean air: χ^2 = 0.18, *n* = 22, *P* = 0.6698; honey-beebread vs. clean air: χ^2 = 0.93, *n* = 27, *P* = 0.3359). Wasps that had a brief experience of food odour without the food reward showed a weak preference for the food compared to clean air (honey vs. clean air: χ^2 = 4.24, *n* = 34, *P* = 0.0396; honey-beebread vs. clean air: χ^2 = 4.17, *n* = 29, *P* = 0.0411). However, wasps that had a brief feeding experience prior to the experiment showed a strong attraction to food odour (honey vs. clean air: χ^2 = 6.82, *n* = 33, *P* = 0.0090; honey-beebread vs. clean air: χ^2 = 7.76, *n* = 29, *P* = 0.0053) (Fig. 5.2).

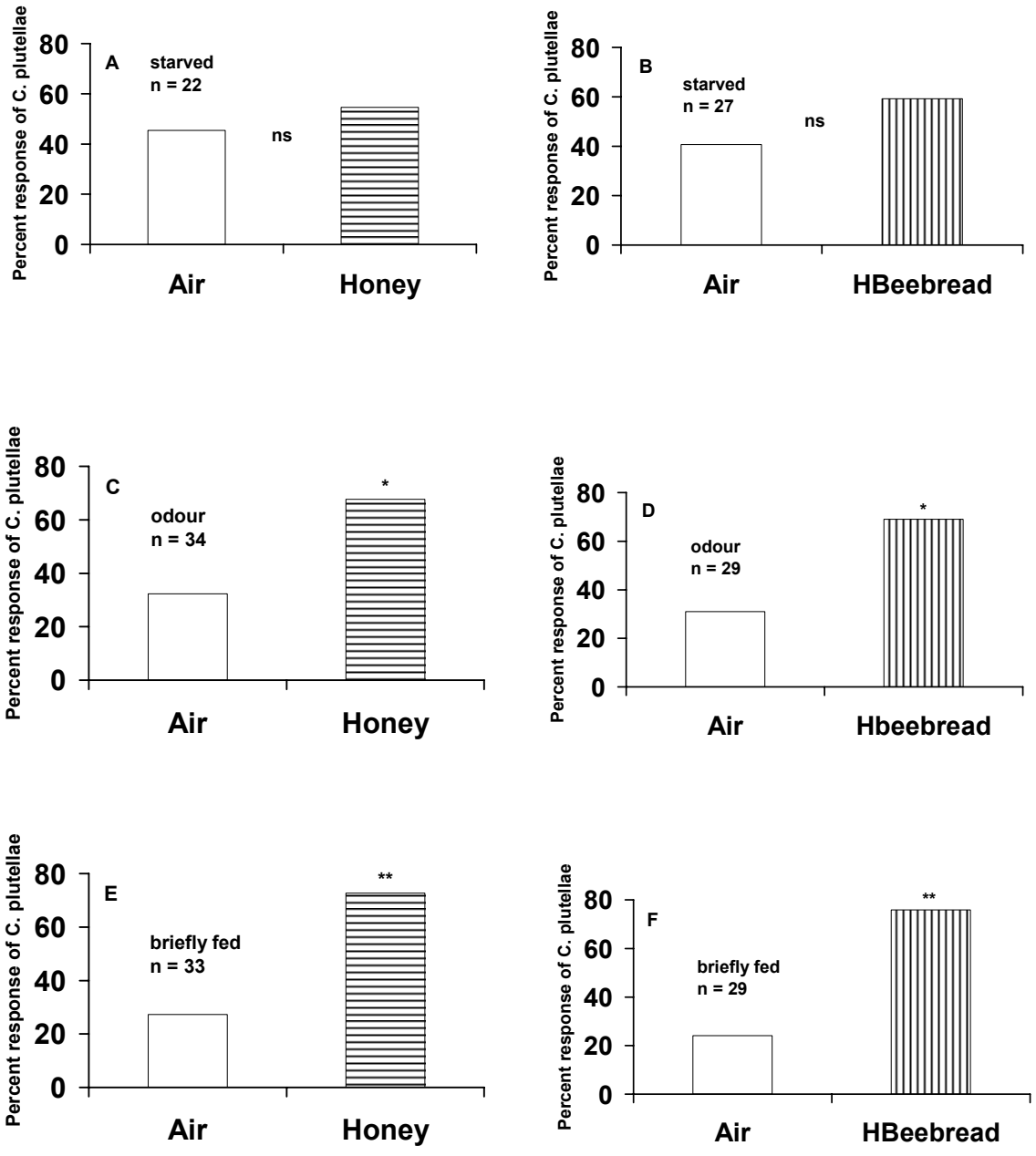


Figure 5.2: Responses of *Cotesia plutellae* females that were either starved for about 20 h from the period they emerged (A and B), allowed to have a brief experience of food odour (C and D), or briefly fed on a food type (E and F) prior to the trials. *, ** indicate significant χ^2-test at 0.05 and 0.01 levels of probability, respectively; 'ns' indicates insignificant treatment effect.

Food versus host learning

Hungry *C. plutellae* showed a weak attraction to odour emanating from food compared to that from host-infested cabbage seedlings (honey vs. host: χ^2 = 4.50, *n* = 32, *P* = 0.0339; honey-beebread vs. host: χ^2 = 4.83, *n* = 35, *P* = 0.0280) while the preference intensified among wasps that had a brief feeding experience before the experiment (honey vs. host: χ^2 = 7.76, *n* = 29, *P* = 0.0053; honey-beebread vs. host: χ^2 = 7.81, *n* = 37, *P* = 0.0052). On the contrary, satiated wasps showed a weak attraction for odour emanating from host-infested cabbage seedlings rather than food odour (honey vs. host: χ^2 = 4.50, *n* = 32, *P* = 0.0339; honey-beebread vs. host: χ^2 = 4.24, *n* = 34, *P* = 0.0396) (Fig. 5.3).

When given a choice between food odour and volatiles from host-infested cabbage seedlings, briefly-fed wasps with host experience responded to the two sources equally. There was no significant difference between the number of wasps that chose either of the two stimuli (honey vs. host: χ^2 = 0.50, *n* = 32, *P* = 0.4795; honey-beebread vs. host: χ^2 = 1.13, *n* = 32, *P* = 0.2888). However, satiated wasps with host experience showed a stronger preference for host-infested seedlings (honey vs. host: χ^2 = 8.53, *n* = 30, *P* = 0.0035; honey-beebread vs. host: χ^2 = 8.00, *n* = 32, *P* = 0.0047) (Fig. 5.4). These results were at variance with observations made among wasps without host experience.

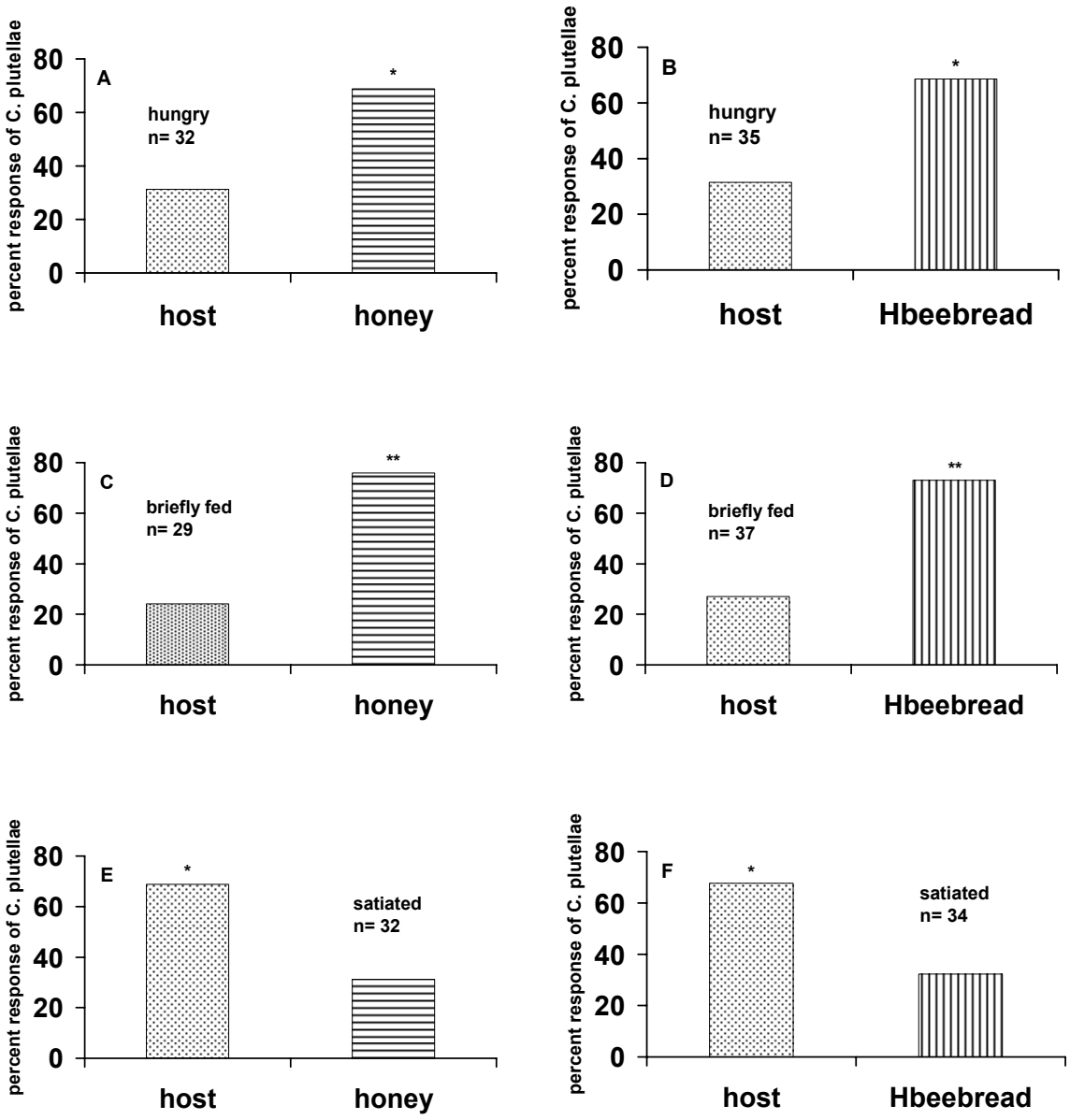


Figure 5.3: Responses of *Cotesia plutellae* females that were either hungry (A and B), briefly fed on a food type (C and D), or allowed to feed on a food type for about 20 min (E and F) prior to the trials.

*, ** indicate significant χ^2-test at 0.05 and 0.01 levels of probability, respectively.

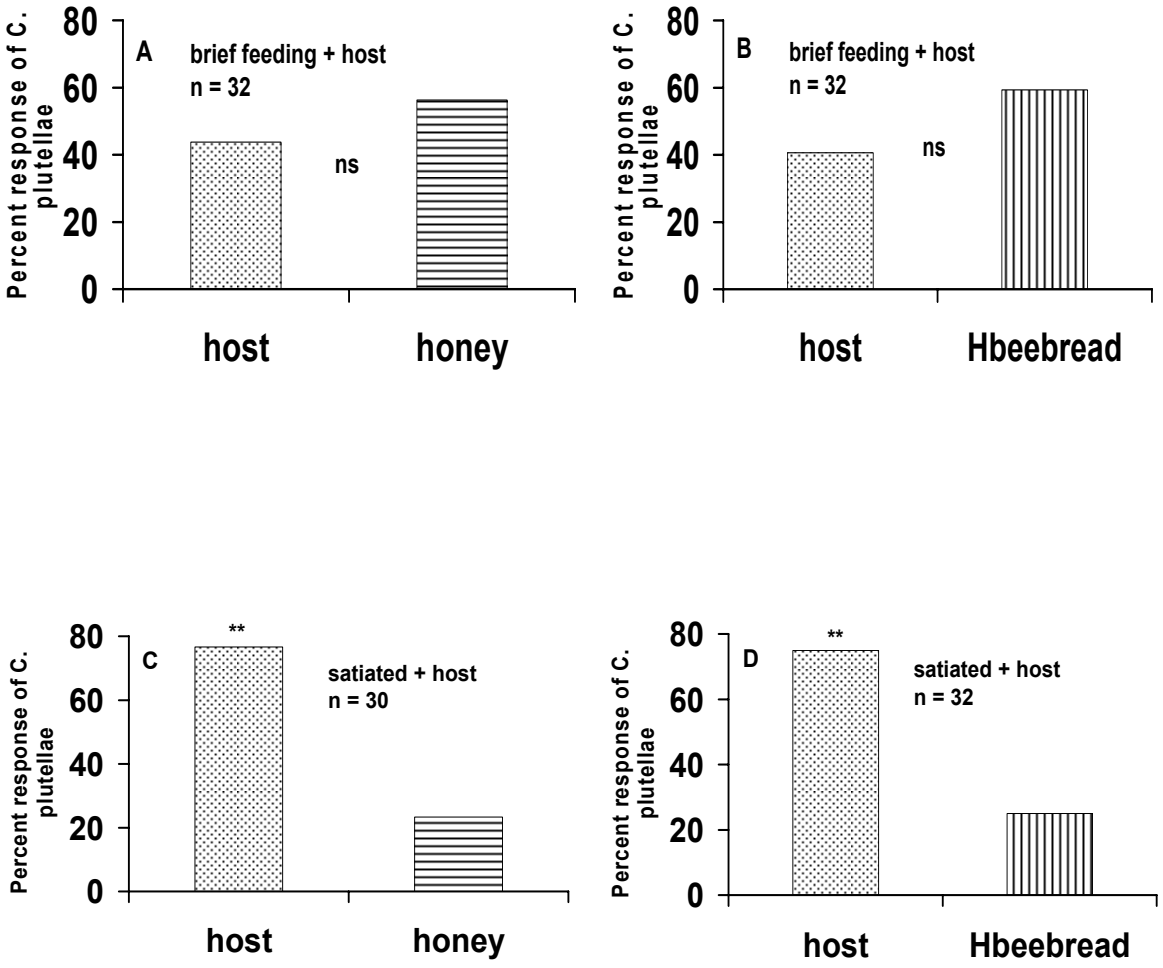


Figure 5.4: Responses of host-experienced *Cotesia plutellae* that were either briefly fed on a food type (A and B), or allowed to feed on a food type for about 20 min (C and D) prior to the trials.

** indicates significant χ^2-test at 0.01 level of probability; 'ns' indicates insignificant treatment effect.

5.5 Discussion

It is important for adult parasitoids to find hosts for reproductive purposes and locate food to meet their short-term nutritional needs. With such a requirement, ability to recognize food- and host-associated cues would help to optimize the use of time and energy when foraging for resources (Lewis *et al*., 1998; Faria, 2005). This attribute allows parasitoids to change their behaviour to semiochemical cues as a result of experiences obtained during foraging, either by responding to cues which were previously ignored (associative learning) or by heightening existing responses (sensitization) (Poppy *et al*., 1997). The results from the current study show that *C. plutellae* is capable of improving on existing responses depending on previous food and host experiences, and its physiological state. This enhances its decision making during foraging activities.

Food learning

Starved *C. plutellae* could not distinguish between food odour and clean air. Initial stages of starvation in insects have been found to increase sensitivity to odours whereas late stages of starvation have been reported to cause a reduction in sensitivity (Helms *et al*., 2003; Riddell and Mallon, 2006). Since starved *C. plutellae* would only live for an average of two days (Section 3.5), 20 h could be regarded as a late stage of starvation for this parasitoid, eventually leading to a reduction in the sensitivity of its chemoreceptors. There is indeed evidence that dramatic reduction in haemolymph glucose, occasioned by sugar deprivation, affects not only insect mobility, but also the functioning of the neural

network important for information processing, and the entire nervous system (Wegener *et al*., 2003; Lee and Park, 2004).

A weak attraction to food odour over that of clean air was observed when hungry wasps were given a brief experience of food odour prior to the experiment. This indicates that learning of food odour by *C. plutellae* could partially be the result of sensitization. In the field, *C. plutellae* females would probably first find these supplementary food sources by chance while searching for hosts. Once the food has been encountered, the wasps will learn to associate it with olfactory and/or visual cues and subsequently use the cues to search for it (Takasu and Lewis, 1996; Sato and Takasu, 2000).

Wasps with brief food reward, however, showed a stronger preference for food sources over that of clean air, suggesting that hungry wasps developed a stronger motivation to search for food after an initial food experience. This olfactometer report corroborates the wind tunnel experiments reported by Takasu and Lewis (1996) where female *M. croceipes* preferred food odour after an initial encounter with the food. These results indicate that when females experience an odour during feeding, they learn to associate the odour with the food and subsequently respond to the odour to find the food. Accuracy of females in choosing the experienced odour increases with an increasing number of odour experiences (Takasu and Lewis, 1996). With a progressive increase in the preference of *C. plutellae* for food, as a result of previous experiences, this study

has shown that the parasitoid is able to learn and associate odour with its food. This attribute makes *C. plutellae* a good candidate for physiological studies because its appetitive behaviour could be conditioned.

Food versus host learning

Hungry *C. plutellae* without host experience showed a weak preference for food over the host while the food preference became stronger when wasps were given a brief food reward before the experiment. In line with the hunger hypothesis (Jacob and Evans, 2001), it is expected that emerging parasitoid females will first engage in food searching rather than host searching activities as hunger reduces their responsiveness to host-associated cues and increases their responsiveness to food related ones. In *choice tests*, satiated *C. plutellae* without host experience preferred host to food. It appears that sufficient food reward is necessary for the parasitoid to show its innate responsiveness to host-associated cues. It is believed that after having a full meal, the parasitoid would generate the required energy and motivation for searching and handling of hosts.

Food-host experience interaction had a significant effect on the foraging behaviour of *C. plutellae.* In contrast to strong food preference that was observed among briefly fed wasps without host experience, briefly fed wasps with host experience were equally attracted to host and food sources. This indicates that host experience increases attraction to host-associated odours even when wasps are hungry. This is in agreement with the findings of Takasu and Lewis (1993)

which showed that intermediately fed females of the parasitoid *M*. *croceipes* without host experience were attracted to food odour, whereas attraction to host odour increased among wasps that had host experience in addition to brief food reward. Liu and Jiang (2003) also observed a tremendous increase in parasitism after *C. plutellae* was given an oviposition experience. Females learn to link chemicals with hosts during contact with host products or when ovipositing in a host and response to odour has been known to increase after such experience (Iizuka and Takasu, 1998; Takasu and Lewis, 2003).

When given a choice between food and hosts, satiated *C. plutellae* with host experience showed a strong preference for hosts which is in contrast to a weak host preference that was shown by satiated wasps without host experience. This difference could be explained in part by the contact ovipositor chemosensors made with host haemolymph during penetration. In an associative learning experiment (Takasu and Lewis, 2003), oviposition experience was linked with persistence of learned responses to host odour in *M. croceipes* and it was concluded that ovipositor contact with host haemolymph during oviposition was partially responsible for the enhanced retention of learned responses. Also, attraction to host-related odour can be expected to always occur (after a full meal) as finding hosts is the key to the reproductive success of parasitoids. These results corroborate several field studies (e.g., Baggen and Gurr, 1998; Jacob and Evans, 1998) in highlighting the importance of having nutritional resources close to hosts as an adequate amount of food in combination with a

host experience would have a significant effect on parasitoid efficacy. Food sources being close to host habitats will also decrease travel time, energy costs and the risks associated with switching from host to food searching.

Laboratory and field experiments have demonstrated that after learning host and food searching cues, females effectively forage for hosts and food to balance their needs for reproduction and survival depending on their nutritional state (Lewis *et al*., 1998; Iizuka and Takasu, 1999). Results for *C. plutellae* in the present study lend support to the general hypothesis that the hunger status and previous experience of a female wasp will influence her response to odour emanating from host and food sources and, therefore, affect her efficiency as a biological control agent. Due to its ability to learn and respond to honey, honey-beebread or host-related odours, *C. plutellae* would be able to switch between food and host searching (depending on its need) without losing time. A parasitoid that is able to exploit differences in food- and host-related cues has a higher chance of succeeding as a control agent.

CHAPTER 6:

General discussion

Cotesia plutellae is the most abundant parasitoid of the diamondback moth in South Africa and it accounts for more than 80% of total parasitism on the pest (Mosiane *et al.*, 2003; Smith and Villet, 2003). However, a major shortcoming of this parasitoid is that its population is usually low in cabbage fields during early spring, when cabbages need protection from an increasing population of the diamondback moth. Hence, efforts aimed at improving the fitness of *C. plutellae* become relevant. Food is a very important factor for biological success and because of this, the possibility of increasing the fitness of *C. plutellae* with a supplementary food source was investigated. A protein source (beebread) was included in the diet of *C. plutellae* and its effect on the performance of the parasitoid was investigated in the laboratory. Inclusion of beebread resulted in significant improvement in the biology, foraging behaviour and sensory activity of *C. plutellae*.

In this thesis, I addressed four general questions concerning the inclusion of beebread as a protein source in the diet of *C. plutellae*:

1. What is the effect of honey and honey-beebread on the performance of *C. plutellae* population under laboratory conditions?
2. How does each food source affect the biology of *C. plutellae*?
3. Do cues from honey and honey-beebread elicit similar behavioural responses in *C. plutellae*? and
4. Can *C. plutellae* associate food and host with their respective odour?.

In Chapter 2, beebread was successfully included in the diet of *C. plutellae* without any adverse effect on the performance of the parasitoid. The host-parasitoid colony thrived on honey-beebread as much as it did on honey. However, the parasitoid attained higher fitness on honey-beebread than on honey and this reflected in host suppression that occurred first in honey-beebread cages. It was shown that density-dependent parasitism, characteristic of parasitoids, kept the colonies running for the period of months that the study was conducted.

Chapter 3 focused on how beebread enhanced the biology of *C. plutellae* when included in its diet. Compared to pure honey, honey-beebread increased the fecundity and longevity of the parasitoid. It was shown that diet effect on fecundity of *C. plutellae* is more pronounced in the first three days of eclosion and it was suggested that test for diet suitability in this parasitoid should be limited to the first few days of eclosion. It was also observed that though *C. plutellae* is synovigenic, post-eclosion egg maturation did not depend on type of food given to the parasitoid but on maternal age. Maximum egg load was attained within 48 h of eclosion suggesting that the parasitoid is weakly synovigenic.

In Chapter 4, *C. plutellae* females showed excellent phagostimulatory responses to honey and honey-beebread, and to specific sugars (fructose, glucose, sucrose) that are dominant in honey. More time was needed to have a full meal

on honey and honey-beebread while less time was needed by the parasitoid on the specific sugars. It was pointed out that the high feeding duration on honey and honey-beebread could be a drawback if these are applied in the field as the parasitoid would have less time for host searching and egg laying activities. Therefore, application of dilute solutions was suggested as an alternative because a high amount of food can be consumed in a relatively short feeding time if a less viscous and less concentrated food is provided. The parasitoid also displayed innate attraction toward odours of honey and honey-beebread but when the two diets were presented in *choice* tests, the parasitoid showed preference for honey-beebread cues. What is not clear at this point is what was responsible for this preference. Further experiments showed that antennae of male and female *C. plutellae* showed stronger EAG reponses from honey-beebread than from honey. It was also observed that the parasitoid showed higher EAGs from honey-beebread than the diamondback moth. This indicated that the parasitoid stands a chance of benefiting more from honey-beebread than the diamondback moth.

Additional olfactometer experiments in Chapter 5 showed that *C. plutellae* is able to improve on its innate attraction to honey and honey-beebread with stepwise exposure to the diets. An increase in attraction was observed after the parasitoid was allowed to smell the food before trials in the olfactometer while greater attraction to food cues was recorded after *C. plutellae* was allowed to feed briefly before being tested. In tests involving choices between food- and host-related

cues, hunger status determined the decisions of *C. plutellae*. Attraction of hungry wasps to food cues increased after a brief feeding while satiated females showed a weak preference for host-related cues. However, after coming in contact with DBM larvae, satiated wasps showed a stronger attraction to host-related cues. *Cotesia plutellae* distinguished between food and host odours and its foraging decisions were enhanced by cue learning and host/food reward. Thus, the wasps switched between host and food foraging based on their physiological state (hungry or fed) and previous experiences with host and food. An important factor inducing variability in foraging behavior in parasitic wasps is experience gained by the insects. Together with the insect's genetic constitution and physiological state, experience ultimately defines the behavioral repertoire under specified environmental circumstances. Vet *et al*. (1990) presented a conceptual variable-response model based on several major observations of a foraging parasitoid's responses to stimuli involved in the host-finding process. These major observations are that (1) different stimuli evoke different responses or levels of response, (2) strong responses are less variable than weak ones, (3) learning can change response levels, (4) learning increases originally low responses more than originally high responses, and (5) host-derived stimuli serve as rewards in associative learning of other stimuli. Vet and van Opzeeland (1984) reported that oviposition experiences of two adult female *Asobara* spp. (Braconidae: Alysiinae) significantly altered their behavioural responses in microhabitat and host location. Females needed prior exposure to a host before they were capable of using volatile compounds related to the presence of their

hosts. *Asobara tabida* (Nees) attacks *Drosophila* in fermenting fruits and *A. rufescens* (Foerster) attacks Drosophilidae in decaying plant materials. Naïve females showed a strong preference for the odour of their own microhabitat. After experience with their own host and microhabitat, females were repelled by odours of the other microhabitat. 'Enforced' experience with this repellent microhabitat in the laboratory modified the olfactory response from repellency to attraction. It was shown that even the microhabitat odour preference pattern could be changed through experience. This kind of behavioural flexibility may be the rule rather than the exception in many other hymenopterous parasitoids.

The present work also highlights the importance of providing parasitoids with food sources close to the host sites, so that the wasps do not need to lose time and energy when switching from host to food foraging. One should consider that the presence of food sources may benefit not only the natural enemies, but also the pests (Baggen and Gurr, 1998), so the use of food sources that benefit only the natural enemies is desirable. In this context, honey-beebread may be of higher value to *C. plutellae* than to the diamondback moth. As in many Lepidoptera, *P. xylostella* adults do not feed on protein (Wheeler *et al*., 2000) and this may eliminate competition between the parasitoid and its host for beebread. In addition, *C. plutellae* showed stronger attraction to honey-beebread cues than the DBM in electrophysiological experiments. Future studies should investigate effect of protein levels on food preference of *C. plutellae*.

In summary, the studies presented in this thesis revealed that (1) protein is an important component of *C. plutellae* diet, (2) inclusion of beebread in the diet of *C. plutellae* can increase its longevity and reproductive output, (3) food odour is an adequate orientation cue for food foraging behaviour in *C. plutellae*, (4) associative learning enhances subsequent responses of *C. plutellae* to food and host related cues, and (5) after a previous experience, *C. plutellae* is able to choose between food and host foraging based on its physiological state. Further trials on a relatively bigger scale may be necessary to validate this report. Depending on the outcome, beebread may be incorporated into the diet of *C. plutellae* in state-initiated parasitoid multiplication program and resulting wasps could be used to augment in the field during early spring. Thus, adequate biological control would be achieved during this critical period.

REFERENCES

Abate, T., 1997. Integrated pest management in Ethiopia: an overview. In *Integrating biological control and host plant resistance: Proc. CTA/IAR/IIBC Semin.*, pp. 24-37. Wageningen, Netherlands: Tech. Coop. Rural Agric. Dev. 383 pp.

Abate, T., van Huis, A. and Ampofo, J.K.O., 2000. Pest management strategies in traditional agriculture: an African perspective. *Annual Review of Entomology* **45**: 631-659.

Akhtar, Y., Yeoung, Y.R. and Isman, M.B., 2008. Comparative bioactivity of selected extracts from Meliaceae and some commercial botanical insecticides against two noctuid caterpillars, *Trichoplusia ni* and *Pseudaletia unipuncta Phytochemistry Reviews* **7**: 77-88.

Albrecht, U., Wyler, T., Pfister-Wilhelm, R., Gruber, A., Stettler, P., Heiniger, P., Kurt, E., Schümperli, D. and Lanzrein, B., 1994. Polydnavirus of the parasitic wasp *Chelonus inanitus* (Braconidae): characterization, genome organization and time point of replication. *Journal of General Virology* **75**: 3353-3363.

van Alphen, J.J.M. and Jervis, M.A. 1996. Foraging behaviour. In Jervis, M.A. and Kidd, N. (eds.), *Insect Natural Enemies – practical approaches to their study and evaluation.* Chapman and Hall, London, pp. 1-62.

Andow, D.A., 1991. Vegetational diversity and arthropod population response. *Annual Review of Entomology* **36**: 561-586.

Annecke, D.P. and Moran, V.C., 1982. *Insects and Mites of Cultivated Plants in South Africa*. Butterworths, Pretoria, 383 pp.

Asgari, S., 2006. Venom proteins from polydnavirus-producing endoparasitoids: their role in host-parasite interactions. *Archives of Insect Biochemistry and Physiology* **61**: 146-156.

Åsman, K., 2002. Trap cropping effect on oviposition behaviour of the leek moth *Acrolepiopsis assectella* and the diamondback moth *Plutella xylostella. Entomologia Experimentalis et Applicata* **105**: 153-164.

Åsman, K. and Ekbom, B., 2006. Responses of ovipositing moths to host plant deprivation: life history aspects and implications for intercropping. *Agricultural and Forest Entomology* **8**(3): 213-219.

Åsman, K., Ekbom, B. and Rämert, B., 2001. Effect of intercropping on oviposition and emigration behavior of the leek moth (Lepidoptera: Acrolepiidae) and the diamondback moth (Lepidoptera: Plutellidae). *Environmental Entomology* **30**(2): 288-294.

Badenes-Perez, F.R., Shelton, A.M. and Nault, B.A., 2004. Evaluating trap crops for diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). *Journal of Economic Entomology* **97**: 1365-1372.

Badenes-Perez, F.R., Shelton, A.M. and Nault, B.A., 2005. Using yellow rocket as a trap crop for diamondback moth (Lepidoptera: Plutellidae). *Journal of Economic Entomology* **98**(3): 884-890.

Baggen, L.R. and Gurr, G.M., 1998. The influence of food on *Copidosoma koehleri* (Hymenoptera: Encyrtidae), and the use of flowering plants as a

habitat management tool to enhance biological control of potato moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae). *Biological Control* **11**: 9-17.

Baker, H.G. and Baker, I., 1983. A brief historical review of the chemistry of nectar. In Bentley, B. and Elias, T. (eds.), *The Biology of Nectaries*. Columbia University Press. New York, USA, pp. 126-152.

Beach, J.P., Williams III, L., Hendrix, D.L. and Price, L.D., 2003. Different food sources affect the gustatory response of *Anaphes iole*, an egg parasitoid of *Lygus* spp. *Journal of Chemical Ecology* **29**(5): 1203-1222.

Beck, M. and Strand, M.R., 2003. RNA interference silences *Microplitis demolitor* bracovirus genes and implicates *glc1.8* in disruption of adhesion in infected host cells. *Virology* **314**: 521-535.

Beck, M. and Strand, M.R., 2005. Glc1.8 from *Microplitis demolitor* bracovirus induces a loss of adhesion and phagocytosis in insect high five and S2 cells. *Journal of Virology* **79**(3): 1861-1870.

Beckage, N.E. and Gelman, D.B., 2004. Wasp parasitoid disruption of host development: implications for new biologically based strategies for insect control. *Annual Review of Entomology* **49**: 299-330.

Begum, M., Gurr, G.M., Wratten, S.D., Hedberg, P.R. and Nicol, H.I., 2006. Using selective food plants to maximize biological control of vineyard pests. *Journal of Applied Ecology* **43**: 547-554.

Behmer, S.T., 2004. Insect learning. In Capinera, J. (ed.), *Encyclopaedia of Entomology*. Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 1278-1283.

Bennett, R.N. and Wallsgrove, R.M., 1994. Secondary metabolites in plant defence mechanisms. *New Phytologist* **127**(4): 617-633.

Berndt, L.A. and Wratten, S.D., 2005. Effects of alyssum flowers on the longevity, fecundity, and sex ratio of the leafroller parasitoid *Dolichogenidea tasmanica*. *Biological Control* **32**: 65-69.

Berndt, L.A., Wratten, S.D. and Scarratt, S.L., 2006. The influence of floral resource subsidies on parasitism rates of leafrollers (Lepidoptera: Tortricidae) in New Zealand vineyards. *Biological Control* **37**: 50-55.

Bezemer, T.M., Harvey, J.A. and Mills, N.J., 2005. Influence of adult nutrition on the relationship between body size and reproductive parameters in a parasitoid wasp. *Ecological Entomology* **30**: 571-580.

Blackburn, T.M., 1991. Evidence for a `Fast-Slow' continuum of life-history traits among parasitoid Hymenoptera. *Functional Ecology* **5**(1): 65-74.

Boevé, J.L. and Wäckers, F.L., 2003. Gustatory perception and metabolic utilization of sugars by *Myrmica rubra* ant workers. *Oecologia* **136**: 508-514.

Bokonon-Ganta, A.H., Ramadan, M.M. and Messing, R.H., 2007. Reproductive biology of *Fopius ceratitivorus* (Hymenoptera: Braconidae), an egg–larval parasitoid of the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae). *Biological Control* **41**: 361-367.

Borrell, B.J., 2006. Mechanics of nectar feeding in the orchid bee *Euglossa imperialis*: pressure, viscosity and flow. *The Journal of Experimental Biology* **209**(24): 4901-4907.

Brindis, Y., Lachaud, J.P., Gómez, B.G.Y., Rojas, J.C., Malo, E.A. and Cruz-López, L., 2008. Behavioral and olfactory antennal responses of *Solenopsis geminata* (Fabricius) (Hymenoptera: Formicidae) workers to their Dufour gland secretion. *Neotropical Entomology* **37**(2): 131-136.

Broad, S.T., Schellhorn, N.A., Lisson, S.N. and Mendham, N.J., 2008. Host location and oviposition of lepidopteran herbivores in diversified broccoli cropping systems. *Agricultural and Forest Entomology* **10**: 157-165.

Brunherotto, R. and Vendramim, J.D., 2001. Bioactivity of aqueous extracts of *Melia azedarach* L. on tomato pinworm *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Neotropical Entomology* **30**(3): 455-459.

Bukovinszky, T., Tréfas, H., van Lenteren, J. C., Vet, L.E.M. and Fremont, J., 2004. Plant competition in pest-suppressive intercropping systems complicates evaluation of herbivore responses. *Agriculture, Ecosystems and Environment* **102**(2): 185-196.

Cai, J., Ye, G. and Hu, C., 2004. Parasitism of *Pieris rapae* (Lepidoptera: Pieridae) by a pupal endoparasitoid, *Pteromalus puparum* (Hymenoptera: Pteromalidae): effects of parasitization and venom on host hemocytes. *Journal of Insect Physiology* **50**: 315-322.

Cameron, P.J. and Walker, G.P., 1997. Host specificity of *Cotesia rubecula* and C*otesia plutellae*, parasitoids of white butterfly and diamondback moth.

Proceedings of the 50th New Zealand Plant Protection Conference **50**: 236-241.

Carpinella, C., Ferrayoli, C., Valladares, G., Defagó, M. and Palacios, S., 2002. Potent limonoid insect antifeedant from *Melia azedarach*. *Bioscience, Biotechnology and Biochemistry* **66**(8): 1731-1736.

Carson, R., 1962. *Silent Spring*. Mariner Books, Houghton Mifflin Company, USA, 400 pp.

Carton, Y. and Nappi, A.J., 1997. *Drosophila* cellular immunity against parasitoids. *Parasitology Today* **13**(6): 218-227.

Casas, J., Pincebourde, S., Mandon, N., Vannier, F., Poujo, R. and Giron, D., 2005. Lifetime nutrient dynamics reveal simultaneous capital and income breeding in a parasitoid. *Ecology* **86**(3): 545-554.

Chan, M.S. and Godfray, H.C.J., 1993. Host-feeding strategies of parasitoid wasps. *Evolutionary Ecology* **7**(6): 593-604.

Charleston, D.S., 2004. Integrating biological control and botanical pesticides for management of *Plutella xylostella.* PhD Thesis, Wageningen University, The Netherlands, 184pp.

Charleston, D.S., Gols, R., Hordijk, K.A., Kfir, R., Vet, L.E.M. and Dicke, M., 2006a. Impact of botanical pesticides derived from *Melia azedarach* and *Azadirachta indica* plants on the emission of volatiles that attract parasitoids of the diamondback moth to cabbage plants. *Journal of Chemical Ecology* **32**(2): 325-349.

Charleston, D.S. and Kfir, R., 2000. The possibility of using Indian mustard, *Brassica juncea*, as a trap crop for the diamondback moth, *Plutella xylostella*, in South Africa. *Crop Protection* **19:** 455-460.

Charleston, D.S., Kfir, R., Dicke, M. and Vet, L.E.M., 2005. Impact of botanical pesticides derived from *Melia azedarach* and *Azadirachta indica* on the biology of two parasitoid species of the diamondback moth. *Biological Control* **33**: 131-142.

Charleston, D.S., Kfir, R., Dicke, M. and Vet, L.E.M., 2006b. Impact of botanical extracts derived from *Melia azedarach* and *Azadirachta indica* on populations of *Plutella xylostella* and its natural enemies: a field test of laboratory findings. *Biological Control* **39**: 105-114.

Charnov, E.L., Los-den Hartogh, R.L., Jones, W.T. and van den Assem, J. 1981. Sex ratio evolution in a variable environment. *Nature* **289**: 27-33.

Chen, L. and Fadamiro, H.Y., 2006. Comparing the effects of five naturally occurring monosaccharide and oligosaccharide sugars on longevity and carbohydrate nutrient levels of a parasitic phorid fly, *Pseudacteon tricuspis. Physiological Entomology* **31**(1): 46-56.

Chesler, A. and Firestein, S., 2008. Current views on odour receptors. *Nature* **452**: 944.

Colinet, H., Boivin, G. and Hance, T., 2007. Manipulation of parasitoid size using the temperature-size rule: fitness consequences. *Oecologia* **152**: 425-433.

Corley, L.S. and Strand, M.R., 2003. Evasion of encapsulation by the polyembryonic parasitoid *Copidosoma floridanum* is mediated by a polar

body-derived extraembryonic membrane. *Journal of Invertebrate Pathology* **83**: 86-89.

Das, P.D., Raina, R., Prasad, A.R. and Sen, A., 2007. Electroantennogram responses of the potato tuber moth, *Phthorimaea operculella* (Lepidoptera; Gelichiidae) to plant volatiles. *Journal of Biosciences* **32**(2): 339-349.

Defagó, M., Valladares, G., Banchio, E., Carpinella, C. and Palacios, S., 2006. Insecticide and antifeedant activity of different plant parts of *Melia azedarach* on *Xanthogaleruca luteola. Fitoterapia* **77**: 500-505.

Desouhant, E., Driessen, G., Amat, I. and Bernstein, C., 2005. Host and food searching in a parasitic wasp *Venturia canescens*: a trade-off between current and future reproduction? *Animal Behaviour* **70**: 145-152.

Digilio, M.C., Pennacchio, F. and Tremblay, E. 1998. Host regulation effects of ovary fluid and venom of *Aphidius ervi* (Hymenoptera: Braconidae). *Journal of Insect Physiology* **44**: 779-784.

Eliopoulos, P.A., Stathas, G.J. and Bouras, S.I., 2005. Effects and interactions of temperature, host deprivation and adult feeding on the longevity of the parasitoid *Venturia canescens* (Hymenoptera: Ichneumonidae). *European Journal of Entomology* **102**: 181-187.

Ellers, J. and van Alphen, J.J.M., 1997. Life history evolution in *Asobara tabida*: plasticity in allocation of fat reserves to survival and reproduction. *Journal of Evolutionary Biology* **10**: 771-785.

Ellers, J., van Alphen, J.J.M. and Sevenster, J.G., 1998. A field study of size-fitness relationship in the parasitoid *Asobara tabida*. *The Journal of Animal Ecology* **67**(2): 318-324.

Ellers, J., Bax, M. and van Alphen, J.J.M., 2001. Seasonal changes in female size and its relation to reproduction in the parasitoid *Asobara tabida*. *Oikos* **92**: 309-314.

Ellers, J. and Jervis, M., 2003. Body size and the timing of egg production in parasitoid wasps. *Oikos* **102**: 164-172.

Ellers, J. and Jervis, M.A., 2004. Why are so few parasitoid wasp species pro-ovigenic? *Evolutionary Ecology Research* **6**: 993-1002.

Ellers, J., Sevenster, J.G. and Driessen, G., 2000. Egg load evolution in parasitoids. *The American Naturalist* **156**(6): 650-665.

Ellis, J.A., Walter, A.D., Tooker, J.F., Ginzel, M.D., Reagel, P.F., Lacey, E.S., Bennett, A.B., Grossman, E.M. and Hanks, L.M., 2005. Conservation biological control in urban landscapes: Manipulating parasitoids of bagworm (Lepidoptera: Psychidae) with flowering forbs. *Biological Control* **34**: 99-107.

Elmore, T., Ignell, R., Carlson, J.R. and Smith, D.P., 2003. Targeted mutation of a *Drosophila* odor receptor defines receptor requirement in a novel class of sensillum. *The Journal of Neuroscience* **23**(30): 9906-9912.

Endersby, N. and Cameron, P., 2004. Parasitism of *Nyctemera amica* (White) (Lepidoptera: Arctiidae) and *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) by *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae).

In Endersby, N.M. and Ridland, P.M. (eds.), *The management of diamondback moth and other crucifer pests.* Proceedings of the Fourth International Workshop, Melbourne, Australia, 26-29 November 2001, pp. 265-268.

English-Loeb, G., Rhainds, M., Martinson, T. and Ugine, T., 2003. Influence of flowering cover crops on *Anagrus* parasitoids (Hymenoptera: Mymaridae) and *Erythroneura* leafhoppers (Homoptera: Cicadellidae) in New York vineyards. *Agricultural and Forest Entomology* **5**: 173-181.

Evans, I.B.P., 1939. Solving the Union's pasture, crop and insect problems. *Annual Report of the Division of Plant Industry: Farming in South Africa*. The Government Printer, Pretoria, South Africa. **No. 117**, 22pp.

Fadamiro, H.Y. and Heimpel, G.E., 2001. Effects of partial sugar deprivation on lifespan and carbohydrate mobilization in the parasitoid *Macrocentrus grandii* (Hymenoptera: Braconidae). *Annals of the Entomological Society of America* **94**(6): 900-916.

FAO, 1996. Value-added products from beekeeping. *FAO Agricultural Services Bulletin No. 124* edited by Krell, R. http://www.fao.org/docrep/w0076e/w0076e00.htm#con

FAOSTAT, 2008. Provisional 2007 production data. Food and Agriculture Organization Online Statistical Databases. http://faostat.fao.org

Faria, C.A., 2005. The nutritional value of aphid honeydew for parasitoids of lepidopteran pests. PhD Thesis, University of Neuchâtel, Switzerland, 124pp.

Fauziah, H.I., Dzolkhifli, O. and Wright, D. J., 1992. Resistance to acylurea compounds in diamondback moth. In Talekar, N.S. (ed.), *Diamondback moth and other crucifer pests*. Proceedings of the Second International Workshop, Tainan, Taiwan, 10-14 December 1990. Asian Vegetable Research and Development Centre, Shanhua, Taiwan, AVRDC Publication No. 92-368, pp. 391-401.

Fellowes, M.D.E., van Alphen, J.J.M. and Jervis, M.A., 2007. Foraging behaviour. In Jervis, M.A. (ed.), *Insects as natural enemies: a practical perspective*. Springer, Netherlands pp. 1-71.

Ferreira, C., Torres, B.B. and Terra, W.R., 1998. Substrate specificities of midgut beta-glycosidases from insects of different orders. *Comparative Biochemistry and Physiology* **119B**: 219-225.

Finch, S. and Collier, R.H., 2003. Insects can see clearly now the weeds have gone. *Biologist* **50**(3): 132-135.

Fischer, S., Samietz, J., Wäckers, F.L. and Dorn, S., 2001. Interaction of vibrational and visual cues in parasitoid host location. *Journal of Comparative Physiology A* **187**: 785-791.

Fischer, S., Samietz, J., Wäckers, F.L. and Dorn, S., 2004. Perception of chromatic cues during host location by the pupal parasitoid *Pimpla turionellae* (L.) (Hymenoptera: Ichneumonidae). *Environmental Entomology* **33**(1): 81-87.

Fitton, M. and Walker, A., 1992. Hymenopterous parasitoids associated with diamondback moth: the taxonomic dilemma. In Talekar, N.S. (ed.),

Diamondback moth and other crucifer pests. Proceedings of the Second International Workshop, Tainan, Taiwan, 10-14 December 1990. Asian Vegetable Research and Development Centre, Shanhua, Taiwan, AVRDC Publication No. 92-368, pp. 225-232.

Fleming, J.G.W., 1992. Polydnaviruses: mutualists and pathogens. *Annual Review of Entomology* **37**: 401-425.

Forsse, E., Smith, S.M. and Bourchier, R.S., 1992. Flight initiation in the egg parasitoid *Trichogramma minutum*: effects of ambient temperature, mates, food, and host eggs. *Entomologia Experimentalis et Applicata* **62**: 147-154.

Frere, B., 1885. Historical sketch of South Africa. *Transactions of the Royal Historical Society* **2**: 1-60.

Fuchsberg, J.R., Yong, T.-H., Losey, J.E., Carter, M.E., Hoffmann, M.P., 2007. Evaluation of corn leaf aphid (*Rhopalosiphum maidis*; Homoptera: Aphididae) honeydew as a food source for the egg parasitoid *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae). *Biological Control* **40**: 230-236.

Gardener, M.C. and Rowe, R.J., 2003. Tropical bees (*Trigona hockingsi*) show no preference for nectar with amino acids. *Biotropica* **35**(1): 119-125.

GenStat, 2007. The GenStat Discovery Edition 3. VSN International, UK.

Giron, D. and Casas, J., 2003. Mothers reduce egg provisioning with age. *Ecology Letters* **6**: 273-277.

Giron, D., Dunn, D.W., Hardy, I.C.W. and Strand, M.R., 2004. Aggression by polyembryonic wasp soldiers correlates with kinship but not resource competition. *Nature* **430**: 676-679.

Godfray, H.C.J., 1994. *Parasitoids, behavioral and evolutionary ecology*. Princeton University Press, Princeton, 488 pp.

González-Teuber, M. and Heil, M., 2009. The role of extrafloral nectar amino acids for the preferences of facultative and obligate ant mutualists. *Journal of Chemical Ecology* **35**(4): 459-468.

Goudegnon, A.E., Kirk, A.A., Arvanitakis, L. and Bordat, D., 2004. Status of the Diamondback moth and *Cotesia plutellae* its main parasitoid in the Cotonou and Porto-Novo periurban areas of Benin. In Kirk, A.A., Bordat, D. (eds.), *Improving biocontrol of Plutella xylostella*. Proceedings of the International Symposium, Montpellier, France, 21-24 October, 2002, pp. 172-178.

Grainge, M., Ahmed, S., Mitchell, W.C. and Hylin, J.W., 1984. *Plant species reportedly possessing pest-control properties-A database*. Resource Systems Institute, East-West Center, Honolulu, Hawaii, U.S.A., 240 pp.

Greathead, D.J., 1971. A review of biological control in the Ethiopian region. *Technical Communication 5.* Commonwealth Institute of Biological Control, Wallingford, UK.

Gurr, G.M., Barlow, N.D., Memmott, J., Wratten, S.D. and Greathead, D.J., 2000. A history of methodological, theoretical and imperical approaches to biological control. In Gurr, G. and Wratten, S. (eds.), *Biological control:*

measures of success. Kluwer Academic, Publishers, Dordrecht, The Netherlands, pp. 3-38.

Gurr, G.M. and Wratten, S.D., 1999. Integrated biological control: a proposal for enhancing success in biological control. *International Journal of Pest Management* **45**: 81-84.

Habluetzel, A., Carnevali, F., Lucantoni, L., Grana, L., Attili, A.R., Archilei, F., Antonini, M., Valbonesi, A., Abbadessa, V., Esposito, F., van der Esch, S.A., 2007. Impact of the botanical insecticide Neem Azal® on survival and reproduction of the biting louse *Damalinia limbata* on angora goats. *Veterinary Parasitology* **144**: 328-337.

Hama, H., 1992. Insecticide resistance characteristics of diamondback moth. In Talekar, N.S. (ed.), *Diamondback moth and other crucifer pests*. Proceedings of the Second International Workshop, Tainan, Taiwan, 10-14 December 1990. Asian Vegetable Research and Development Centre, Shanhua, Taiwan, AVRDC Publication No. 92-368, pp. 455-463.

Hama, H., Kono, Y. and Sato, Y., 1987. Decreased sensitivity of central nerve to fenvalerate in the pyrethroid-resistant diamondback moth, *Plutella xylostella* Linne (Lepidoptera: Yponomeutidae). *Applied Entomology and Zoology* **22**: 176-180.

Hailton, W.D., 1967. Extraordinary sex ratios. *Science* **156**: 477-488.

Harvey, J.A., 2000. Dynamic effects of parasitism by an endoparasitoid wasp on the development of two host species: implications for host quality and parasitoid fitness. *Ecological Entomology* **25**: 267-278.

Harvey, J.A., Harvey, I.F. and Thompson, D.J., 2001. Lifetime reproductive success in the solitary endoparasitoid, *Venturia canescens. Journal of Insect Behavior* **14**(5): 573-593.

Harvey, J.A. and Vet, L.E.M., 1997. *Venturia canescens* parasitizing *Galleria mellonella* and *Anagasta kuehniella*: differing suitability of two hosts with highly variable growth potential. *Entomologia Experimentalis et Applicata* **84**: 93-100.

Helms, J.B., Booth, C.M., Rivera, J., Siegler, J.A., Wuellner, S. and Whitman, D.W., 2003. Lubber grasshoppers*, Romalea microptera* (Beauvois), orient to plant odors in a wind tunnel. *Journal of Orthoptera Research* **12**(2): 135-140.

Heyneman, A.J., 1983. Optimal sugar concentrations of floral nectars. Dependence on sugar intake efficiency and foraging costs. *Oecologia* **60**(2): 198-213.

Hines, R.L. and Hutchison, W.D., 2001. Evaluation of action thresholds and spinosad for lepidopteran pest management in Minnesota cabbage. *Journal of Economic Entomology* **94**(1): 190-196.

Hoferer, S., Wäckers, F.L. and Dorn, S., 1999. Measuring respiration rates in the parasitoid *Cotesia glomerata*. *Mitt. Dtsch. Ges. Allg. Angew. Ent.* **12**: 555-558.

Hokkanen, H.M.T., 1991. Trap cropping in pest management. *Annual Review of Entomology* **36**: 119-38.

Honěk, A., 1993. Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos* **66**(3): 483-492.

Hu, J., Zhu, X.X. and Fu, W.J., 2003. Passive evasion of encapsulation in *Macrocentrus cingulum* Brischke (Hymenoptera: Braconidae), a polyembryonic parasitoid of *Ostrinia furnacalis* Guenée (Lepidoptera: Pyralidae) *Journal of Insect Physiology* **49**: 367-375.

Huang, R.C., Okamura, H., Iwagawa, T., Tadera, K. and Nakatani, M., 1995. Azedarachin C, a limonoid antifeedant from *Melia azedarach. Phytochemistry* **38**(3): 593-594.

Huang, R.C., Tadera, K., Yagi, F., Minami, Y., Okamura, H., Iwagawa, T. and Nakatani, M., 1996. Limonoids from *Melia azedarach. Phytochemistry* **43**(3): 581-583.

Hutchison, W.D., Bolin, P.C. and Hines, R.L., 2004. Diamondback moth. *Vegetable Pest Factsheets*, University of Minnesota Extension Services, University of Minnesota, USA. http://www.vegedge.umn.edu/vegpest/colecrop/diamond.htm

Iizuka, T. and Takasu, K., 1998. Olfactory associative learning of the pupal parasitoid *Pimpla luctuosa* Smith (Hymenoptera: Ichneumonidae). *Journal of Insect Behavior* **11**(5): 743-760.

Iizuka, T. and Takasu, K., 1999. Balancing between host- and food-foraging by the host-feeding pupal parasitoid *Pimpla luctuosa* Smith (Hymenoptera: Ichneumonidae). *Entomological Science* **2**: 67-73.

Irvin, N.A. and Hoddle, M.S., 2007. Evaluation of floral resources for enhancement of fitness of *Gonatocerus ashmeadi*, an egg parasitoid of the glassy-winged sharpshooter, *Homalodisca vitripennis. Biological Control* **40**: 80-88.

Irvin, N.A., Hoddle, M.S. and Castle, S.J., 2007. The effect of resource provisioning and sugar composition of foods on longevity of three *Gonatocerus* spp., egg parasitoids of *Homalodisca vitripennis*. *Biological Control* **40**: 69-79.

Irvin, N.A., Scarratt, S.L., Wratten, S.D., Frampton, C.M., Chapman, R.B., Tylianakis, J.M., 2006. The effects of floral understoreys on parasitism of leafrollers (Tortricidae: Lepidoptera) on apples in New Zealand. *Agricultural and Forest Entomology* **8**: 25-34.

Iziko Museums, 2008. *Brassica oleracea* (Cabbage, Brussels sprouts, Broccoli, Kohlrabi, Cauliflower, Kale). *Biodiversity Explorer*, *Iziko Museums of Cape Town.*
http://www.biodiversityexplorer.org/plants/brassicaceae/brassica_oleracea.htm

Jacob, H. S. and Evans, E. W., 1998. Effects of sugar spray and aphid honeydew on field populations of the parasitoid *Bathyplectes curculionis* (Thomson) (Hymenoptera: Ichneumonidae). *Environmental Entomology* **27**: 1563-1568.

Jacob, H.S. and Evans, E.W., 2001. Influence of food deprivation on foraging decisions of the parasitoid *Bathyplectes curculionis* (Hymenoptera:

Ichneumonidae). *Annals of the Entomological Society of America* **94**(4): 605-611.

Jang, E.B., Messing, R.H., Klungness, L.M. and Carvalho, L.A., 2000. Flight tunnel responses of *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) to olfactory and visual stimuli. *Journal of Insect Behavior* **13**(4): 525-538.

Jervis, M.A., Boggs, C.L. and Ferns, P.N., 2005. Egg maturation strategy and its associated trade-offs: a synthesis focusing on Lepidoptera. *Ecological Entomology* **30**: 359-375.

Jervis, M.A., Copland, M.J.W. and Harvey J.A., 2007. The life-cycle. In Jervis, M.A. (ed.), *Insects as natural enemies: a practical perspective*. Springer, Netherlands, pp. 73-165.

Jervis, M.A. and Ferns, P.N., 2004. The timing of egg maturation in insects: ovigeny index and initial egg load as measures of fitness and of resource allocation. *Oikos* **107**: 449-460.

Jervis, M.A., Ferns, P.N. and Heimpel, G.E., 2003. Body size and the timing of egg production in parasitoid wasps: a comparative analysis. *Functional Ecology* **17**(3): 375-383.

Jervis, M.A. and Kidd, N.A.C., 1986. Host-feeding strategies in hymenopteran parasitoids. *Biological Reviews* **61**: 395-434.

Jervis, M.A. and Kidd, N.A.C., 1999. Parasitoid adult nutritional ecology: implications for biological control. In Hawkins, B.A., Cornell, H.V. (Eds.),

Theoretical Approaches to Biological Control. Cambridge University Press, Cambridge, pp. 131-151.

Jervis, M.A., Kidd, N.A.C., Fitton, M.G., Huddleston, T. and Dawah, H.A., 1993. Flower-visiting by hymenopteran parasitoids. *Journal of Natural History* **27**: 67-105.

Jervis, M.A., Kidd, N.A.C. and Heimpel, G.E., 1996. Parasitoid adult feeding behaviour and biocontrol – a review. *Biocontrol Newsletter and Information* **17**: 11-22.

Jervis, M.A., Kidd, N.A.C. and Walton, M., 1992. A review of methods for determining dietary range in adult parasitoids. *Entomophaga* **37**(4): 565-574.

Kapranas, A. and Luck, R.F., 2008. Egg maturation, host feeding, and longevity in two *Metaphycus* parasitoids of soft scale insects. *Biological Control* **47**: 147-153.

Karg, G., Suckling, D.M. and Bradley, S.J., 1997. Defining interaction between electroantennogram responses of *Epiphyas postvittana* (Lepidoptera: Tortricidae) to pheromone and other volatiles. *Journal of Insect Physiology* **43**(2): 179-187.

Keller, A. and Vosshall, L.B., 2003. Decoding olfaction in *Drosophila*. *Current Opinion in Neurobiology* **13**:103-110.

Ketseoglou, I. and Bouwer, G., 2008. A simple technique for the preliminary screening of parasitic wasps for polydnavirus-like particles. *African Entomology* **16**(2): 296-300.

Kfir, R., 1997. The diamondback moth with special reference to its parasitoids in South Africa. In Sivapragasam, A., Loke, W.H., Hussan, A.K. and Lim G.S. (eds.), *The management of diamondback moth and other crucifer pests*. Proceedings of the Third International Workshop, 29 October – 1 November 1996, Kuala Lumpur, Malaysia, Malaysian Agricultural Research and Development Institute (MARDI), pp. 54-60.

Kfir, R., 1998. Origin of the diamondback moth (Lepidoptera: Plutellidae). *Annals of the Entomological Society of America* **91**(2): 164-167.

Kfir, R., 2003. Biological control of the diamondback moth *Plutella xylostella* in Africa. In Neuenschwander, P., Borgemeister, C. and Langewald, J. (eds.), *Biological Control in IPM Systems in Africa.* CAB International pp. 363-375.

Kfir, R. and Thomas, J., 2001. Biological control of diamondback moth on St. Helena Island with parasitoids supplied by the ARC-PPRI. *Biocontrol News and Information* **22:** 76N.

Khaliq, A., Attique, M.N.R. and Sayyed, A.H., 2007. Evidence for resistance to pyrethroids and organophosphates in *Plutella xylostella* (Lepidoptera: Plutellidae) from Pakistan. *Bulletin of Entomological Research* **97**: 191-200.

Kidd, N.A.C. and Jervis, M.A., 2007. Population dynamics. In Jervis, M.A. (ed.), *Insects as natural enemies: a practical perspective*. Springer, Netherlands pp. 435-523.

Kimura, M.T., Awasaki, T., Ohtsu, T. and Shimada, K., 1992. Seasonal changes in glycogen and trehalose content in relation to winter survival of four temporate species of *Drosophila*. *Journal of Insect Physiology* **38**: 871-875.

Klowden, M.J., 2007. *Physiological Systems in Insects*, 2nd Edition. Academic Press, USA. 688 pp.

Koptur, S., 1992. Extrafloral nectary-mediated interactions between insects and plants. In: Bernays, E. (Ed.), *Insect–Plant Interactions*. CRC Press, Boca Raton, pp. 81-129.

Koul, O., Multani, J.S., Singh, G. and Wahab, S., 2002. Bioefficacy of toosendanin from *Melia dubia* (syn. *M. azedarach*) against gram pod-borer, *Helicoverpa armigera* (Hübner). *Current Science* **83**(11): 1387-1390.

Kretz, R., 1979. A behavioural analysis of colour vision in the ant *Cataglyphis bicolor* (Formicidae, Hymenoptera). *Journal of Comparative Physiology A* **131**: 217-233.

Landis, D.A., Wratten, S.D. and Gurr, G.M., 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annual Review of Entomology* **45**: 175-201.

Lanza, J., 1988. Ant preferences for passiflora nectar mimics that contain amino acids. *Biotropica* **20**(4): 341-344.

Lanza, J., Vargo, E.L., Pulim, S. and Chang, Y.Z., 1993. Preferences of the fire ants *Solenopsis invicta* and S. *geminata* (Hymenoptera: Formicidae) for

amino acid and sugar components of extrafloral nectars. *Environmental Entomology* **22**(2): 411-417.

Lavandero, B., Wratten, S., Shishehbor, P. and Worner, S., 2005. Enhancing the effectiveness of the parasitoid *Diadegma semiclausum* (Helen): movement after use of nectar in the field. *Biological Control* **34**: 152-158.

Lavine, M.D. and Beckage, N.E., 1996. Temporal pattern of parasitism-induced immunosuppression in *Manduca sexta* larvae parasitized by *Cotesia congregate*. *Journal of Insect Physiology* **42**(1): 41-51.

Leatemia, J.A., Laing, J.E. and Corrigan, J.E., 1995. Effects of adult nutrition on longevity, fecundity, and offspring sex ratio of *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae). *Canadian Entomologist* **127**: 245-254.

Lee, G. and Park, J.H., 2004. Hemolymph sugar homeostasis and starvation-induced hyperactivity affected by genetic manipulations of the adipokinetic hormone-encoding gene in *Drosophila melanogaster*. *Genetics* **167**(1): 311-323.

Lee, J.C. and Heimpel, G.E., 2008. Effect of floral nectar, water, and feeding frequency on *Cotesia glomerata* longevity. *BioControl* **53**(2): 289-294.

Lewis, W.J., Stapel, J.O., Cortesero, A.M. and Takasu, K., 1998. Understanding how parasitoids balance food and host needs: importance to biological control. *Biological Control* **11**: 175-183.

Lim, G. S., 1986. Biological control of diamondback moth. In Talekar, N.S. and Griggs, T.S. (eds.), *Diamondback Moth Management*. Proceedings of the

First International Workshop. Asian Vegetable Research and Development Center, Shanhua, Taiwan, pp. 159-171.

Lim, G.S., Sivapragasam, A. and Loke, W.H., 1997. Crucifer insect pest problems: trends, issues and management strategies. In Sivapragasam, A., Loke, W.H., Hussan, A.K. and Lim G.S. (eds.), *The management of diamondback moth and other crucifer pests*. Proceedings of the Third International Workshop, 29 October – 1 November 1996, Kuala Lumpur, Malaysia, Malaysian Agricultural Research and Development Institute (MARDI), pp. 3-16.

Liu, S. and Jiang, L., 2003. Differential parasitism of *Plutella xylostella* (Lepidoptera: Plutellidae) larvae by the parasitoid *Cotesia plutellae* (Hymenoptera: Braconidae) on two host plant species. *Bulletin of Entomological Research* **93**: 65-72.

Liu, Y.H. and Tsai, J.H., 2002. Effect of temperature on development, survivorship, and fecundity of *Lysiphlebia mirzai* (Hymenoptera: Aphidiidae), a parasitoid of *Toxoptera citricida* (Homoptera: Aphididae). Environmental Entomology **31**(2): 418-424.

Löhr, B., 2001. Diamondback moth on peas**,** really. *Biocontrol. News and Information* **19**: 38N-39N.

Löhr, B. and Gathu, R., 2002. Evidence of adaptation of diamondback moth, *Plutella xylostella* (L.), to pea, *Pisum sativum* L. *Insect Science and its Application* **22**(3): 161-173.

Long, R.F., Corbett, A., Lamb, C., Reberg-Horton, C., Chandler, J. and Stimmann, M., 1998. Beneficial insects move from flowering plants to nearby crops. *California Agriculture* **52**: 23-26.

Lucantoni, L., Giusti, F., Cristofaro, M., Pasqualini, L., Esposito, F., Lupetti, P. and Habluetzel, A., 2006. Effects of a neem extract on blood feeding, oviposition and oocyte ultrastructure in *Anopheles stephensi* Liston (Diptera: Culicidae). *Tissue and Cell* **38**: 361-371.

Mayhew, P.J. and Glaizot, O., 2001. Integrating theory of clutch size and body size evolution for parasitoids. *Oikos* **92**(2): 372-376.

McDougall, S.J. and Mills, N.J., 1997. The influence of hosts, temperature and food sources on the longevity of *Trichogramma platneri*. *Entomologia Experimentalis et Applicata* **83**: 195-203.

Mensah, R.K., 1997. Local density responses of predatory insects of *Helicoverpa* spp. to a newly developed food supplement ‘‘Envirofeast’’ in commercial cotton in Australia*. International Journal of Pest Management* **43**:221-25.

Mills, N.J. and Kuhlmann, U., 2000. The relationship between egg load and fecundity among *Trichogramma* parasitoids. *Ecological Entomology* **25**: 315-324.

Mitchell, E. R., Hu, G.Y. and Johanowicz, D., 2000. Management of diamondback moth (Lepidoptera: Plutellidae) in cabbage using collard as a trap crop. *HortScience* **35**: 875-879.

Mitsunaga, T., Shimoda, T. and Yano, E., 2004. Influence of food supply on longevity and parasitization ability of a larval endoparasitoid, *Cotesia*

plutellae (Hymenoptera: Braconidae). *Applied Entomology and Zoology* **39**(4): 691-697.

Morallo-Rejesus, B., 1986. Botanical insecticides against the diamondback moth. In Talekar, N.S. and Griggs, T.S. (eds), *Diamondback Moth Management*. Proceedings of the First International Workshop, 11-15 March, 1985. Asian Vegetable Research and Development Center, Shanhua Taiwan, pp. 241-255.

More, K., 2006. Response of cabbage (*Brassica oleracea* var. *capitata*) transplants to Nitrogen, Phosphorus and Potassium nutrition. MSc. Thesis, University of Pretoria, Pretoria, South Africa, 93pp.

Morris, M.C. and Li, F.Y., 2000. Coriander (*Cariandrum sativum*) "companion plants" can attract hoverflies, and may reduce pest infestations in cabbages. *New Zealand Journal of Crop and Horticultural Science* **28**: 213-217.

Morris, R.J. and Fellowes, M.D.E., 2002. Learning and natal host influence host preference, handling time and sex allocation behaviour in a pupal parasitoid. *Behavioral Ecology and Sociobiology* **51**: 386-393.

Morse, D.H., 1986. Predatory risk to insects foraging at flowers. *Oikos* **46**(2): 223-228.

Mosiane, M.S., Kfir, R. and Villet, M.H., 2003. Seasonal phenology of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), and its parasitoids on canola, *Brassica napus* (L.), in Gauteng Province, South Africa. *African Entomology* **11**(2): 277-285.

Musser, F.R., Nault, B.A., Nyrop, J.P. and Shelton, A.M., 2005. Impact of a glossy collard trap crop on diamondback moth adult movement, oviposition, and larval survival. *Entomologia Experimentalis et Applicata* **117**(1): 71-81.

Nakamatsu, Y. and Tanaka, T., 2003. Venom of ectoparasitoid, *Euplectrus* sp. near *plathypenae* (Hymenoptera: Eulophidae) regulates the physiological state of *Pseudaletia separata* (Lepidoptera: Noctuidae) host as a food resource. *Journal of Insect Physiology* **49**(2): 149-159.

Nakatani, M., Huang, R.C., Okamura, H., Iwagawa, T., Tadera, K. and Naoki, H., 1995. Three new antifeeding Meliacarpinins from Chinese *Melia azedarach* Linn. *Tetrahedron* **51**(43): 11731-11736.

Nation, J.L., 2008. *Insect Physiology and Biochemistry*. 2nd Edition. CRC Press Boca Raton, USA, 544 pp.

Noda, T. and Nakamura, A., 2004. Influences of adult body size on longevity and fecundity in *Oomyzus sokolowskii*, a larval-pupal parasitoid of *Plutella xylostella. Japanese Journal of Applied Entomology and Zoology* **48**(3): 226-229.

Nofemela, S.R., 2004. Studies on parasitoids of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), in South Africa. MSc. Thesis, Rhodes University, Grahamstown, South Africa, 92 pp.

Oliai, S.E. and King, B.H., 2000. Associative learning in response to color in the parasitoid wasp *Nasonia vitripennis* (Hymenoptera: Pteromalidae). *Journal of Insect Behavior* **13**(1): 55-69.

Olson, D.M., Fadamiro, H., Lundgren, J.G. and Heimpel, G.E., 2000. Effects of sugar feeding on carbohydrate and lipid metabolism in a parasitoid wasp. *Physiological Entomology* **25**: 17-26.

Olson, D.M., Rains, G.C., Meiners, T., Takasu, K., Tertuliano, M., Tumlinson, J.H., Wäckers, F.L. and Lewis, W.J., 2003. Parasitic wasps learn and report diverse chemicals with unique conditionable behaviors. *Chemical Senses* **28**: 545-549.

Onagbola, E.O., Fadamiro, H.Y. and Mbata, G.N., 2007. Longevity, fecundity, and progeny sex ratio of *Pteromalus cerealellae* in relation to diet, host provision, and mating. *Biological Control* **40**: 222-229.

Ooi, P.A.C., 1979. Incidence of *Plutella xylostalla* (L.) (Lepidoptera: Yponomeutidae) and its parasite *Apanteles plutellae* Kurdj. (Hymenoptera: Braconidae), in Cameron Highlands, Malays. *Malays. Applied Biology* **8**: 131-143.

Ozkan, C., 2007. Effect of food, light and host instar on the egg load of the synovigenic endoparasitoid *Venturia canescens* (Hymenoptera: Ichneumonidae). *Journal of Pest Science* **80**: 79-83.

Papaj, D.R. and Prokopy, R.J., 1989. Ecological and evolutionary aspects of learning in phytophagous insects. *Annual Review of Entomology* **34**: 315-350.

Parkinson, N., Richards, E.H., Conyers, C., Smith, I. and Edwards, J.P., 2002. Analysis of venom constituents from the parasitoid wasp *Pimpla*

hypochondriaca and cloning of a cDNA encoding a venom protein. *Insect Biochemistry and Molecular Biology* **32**: 729-735.

Pedigo, L.P., 2002. *Entomology and Pest Management*. 4th ed. Prentice Hall, Upper River, New Jersey, 741 pp.

Pelosse, P., Bernstein, C. and Desouhant, E., 2007. Differential energy allocation as an adaptation to different habitats in the parasitic wasp *Venturia canescens*. *Evolutionary Ecology* **21**: 669-685.

Pernal, S.F. and Currie, R.W., 2002. Discrimination and preferences for pollen-based cues by foraging honeybees, *Apis mellifera* L. *Animal Behaviour* **63**(2): 369-390.

Poelking, A., 1992. Diamondback moth in the Philippines and its control with *Diadegma semiclausum.* In Talekar, N.S. (ed.), *Diamondback moth and other crucifer pests*. Proceedings of the Second International Workshop, Tainan, Taiwan, 10-14 December 1990. Asian Vegetable Research and Development Centre, Shanhua, Taiwan, AVRDC Publication No. 92-368, pp. 271-278.

Pompanon, F., Fouillet, P. and Bolutreau, M., 1999. Physiological and genetic factors as a source of variation in locomotion and activity rhythm in a parasitoid wasp (*Trichogramma brassicae*). *Physiological Entomology* **24**: 346-357.

Poppy, G.M., Powell, W. and Pennacchio, F., 1997. Aphid parasitoid responses to semiochemicals – genetic, conditioned or learnt? *Entomophaga* **42**(1-2): 193-199.

Quicke, D. L. J., 1997. *Parasitic Wasps.* 1st Edition. Chapman and Hall, London, 470 pp.

Renwick, J.A.A. and Chew, F.S., 1994. Oviposition behaviour in Lepidoptera. A*nnual Review of Entomology* **39**: 377-400.

Riddell, C.E. and Mallon, E.B., 2006. Insect psychoneuroimmunology: Immune response reduces learning in protein starved bumblebees (*Bombus terrestris*). *Brain, Behavior, and Immunity* **20**: 135-138.

Riddick, E.W., 2005. Egg load of lab-cultured *Anaphes iole* and effects of mate presence and exposure time on load depletion? *BioControl* **50**: 53-67.

Riddick, E.W., 2006. Egg load and body size of lab-cultured *Cotesia marginiventris*. *BioControl* **51**(5): 603-610.

Riddick, E.W., 2007. Influence of honey and maternal age on egg load of lab-cultured *Cotesia marginiventris*. *BioControl* **52**: 613-618.

Rincon, C., Bordat, D., Löhr, B. and Dupas, S., 2006. Reproductive isolation and differentiation between five populations of *Cotesia plutellae* (Hymenoptera: Braconidae), parasitoid of *Plutella xylostella* (Lepidoptera: Plutellidae). *Biological Control* **36**(2): 171-182.

Rivero, A. and Casas, J., 1999. Incorporating physiology into parasitoid behavioral ecology: the allocation of nutritional resource. *Research in Population Ecology* **41**: 39-45.

Rivero, A. and West, S.A., 2002. The physiological costs of being small in a parasitic wasp. *Evolutionary Ecology Research* **4**: 407-420.

Rivers, D.B., Ruggiero, L. and Hayes, M., 2002. The ectoparasitic wasp *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) differentially affects cells mediating the immune response of its flesh fly host, *Sarcophaga bullata* Parker (Diptera: Sarcophagidae). *Journal of Insect Physiology* **48**(11): 1053-1064.

Roitberg, B.D., Boivin, G. and Vet, L.E.M., 2001. Fitness, parasitoids, and biological control: an opinion. *The Canadian Entomologist* **133**(3): 429-438.

Rojas, M.G., Vinson, S.B. and Williams, H.J., 1995. Supplemental feeding increases the utilization of a factitious host for rearing *Bracon thurberiphagae* Muesebeck (Hymenoptera: Braconidae) a parasitoid of *Anthonomus grandis* Boheman (Coleoptera: Curculionidae). *Biological Control* **5**(4): 591-597.

Romeis, J. and Wäckers, F.L., 2000. Feeding responses by female *Pieris brassicae* butterflies to carbohydrates and amino acids. *Physiological Entomology* **25**: 247-253.

Rosenheim, J.A., 1996. An evolutionary argument for egg limitation. *Evolution*, **50**(5): 2089-2094.

Rossbach, A., Löhr, B. and Vidal, S., 2005. Generalism versus specialism: responses of *Diadegma mollipla* (Holmgren) and *Diadegma semiclausum* (Hellen), to the host shift of the diamondback moth (*Plutella xylostella* L.) to peas. *Journal of Insect Behavior* **18**(4): 491-503.

Rossbach, A., Löhr, B. and Vidal, S., 2006. Host shift to peas in the diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae) and response to its parasitoid *Diadegma mollipla* (Hymenoptera: Ichneumonidae). *Bulletin of Entomological Research* **96**: 413-419.

Roush, R.T. 1997. Insecticide resistance management in diamondback moth: *quo vadis*? In Sivapragasam, A., Loke, W.H., Hussan, A.K. and Lim G.S. (eds.), *The Management of Diamondback Moth and Other Crucifer Pests*. Proceedings of the Third International Workshop, October 1996, Kuala Lumpur, Malaysia, Malaysian Agricultural Research Institute, pp. 21-24.

Roux, O., van Baaren, J., Gers, C., Arvanitakis, L. and Legal, L., 2005. Antennal structure and oviposition behavior of the *Plutella xylostella s*pecialist parasitoid: *Cotesia plutellae. Microscopy Research and Technique* **68**: 36-44.

Roux, O., Gers, C., Tene-Ghomsi, J.N., Arvanitakis, L., Bordat, D. and Legal, L., 2007. Chemical characterization of contact semiochemicals for host-recognition and host-acceptance by the specialist parasitoid *Cotesia plutellae* (Kurdjumov). *Chemoecology* **17**: 13-18.

Sagarra, L.A., Vincent, C., Peters, N.F. and Stewart, R.K., 2000. Effect of host density, temperature, and photoperiod on the fitness of *Anagyrus kamali*, a parasitoid of the hibiscus mealybug *Maconellicoccus hirsutus. Entomologia Experimentalis et Applicata* **96**: 141-147.

Sagarra, L.A., Vincent, C. and Stewart, R.K., 2002. Impact of mating on *Anagyrus kamali* Moursi (Hym., Encyrtidae) lifetime fecundity,

reproductive longevity, progeny emergence and sex ratio. *Journal of Applied Entomology* **126**(7-8): 400-404.

Sarfraz, M., Dosdall, L.M. and Keddie, B.A., 2006. Diamondback moth–host plant interactions: implications for pest management. *Crop Protection* 25: 625-639.

Sarfraz, M., Keddie, B.A. and Dosdall, L.M., 2005. Biological control of the diamondback moth, *Plutella xylostella*: A review. *Biocontrol Science and Technology* **15**(8): 763-789.

SAS Institute, 1999. *SAS/STAT user's guide*, Release V8. SAS Institute, Cary, North Carolina.

Sato, M. and Takasu, K., 2000. Food odor learning by both sexes of the pupal parasitoid *Pimpla alboannulatus* Uchida (Hymenoptera: Ichneumonidae). *Journal of Insect Behavior* **13**(2): 263-272.

Schiestl, F.P. and Marion-Poll, F., 2002. Detection of physiologically active flower volatiles using Gas Chromatography coupled with Electroantennagraphy. In Jackson, J.F., Linskens, H.F. and Inman, R.B., (eds.), *Analysis of taste and aroma. Molecular Methods of Plant Analysis Series v. 21*. Springer, Gernany, pp. 173-198.

Scott, S.M. and Barlow, C.A., 1984. Effect of prey availability during development on reproductive output of *Metasyrphus corollae* (Diptera: Syrphidae). *Environmental Entomology* **13**: 669-674.

Seal, D.R., Stansly, P.A. and Schuster, D.J., 2002. Influence of temperature and host on life history parameters of *Catolaccus hunteri* (Hymenoptera: Pteromalidae). *Environmental Entomology* **31**(2): 354-360.

Sereda, B., Basson, N.C.J. and Marais, P., 1997. Bioassay of insecticide resistance in *Plutella xylostella* (L.) in South Africa. *African Plant Protection* **3**: 67-72.

Sevenster, J.G., Ellers, J. and Driessen, G., 1998. An evolutionary argument for time limitation. *Evolution* **52**(4): 1241-1244.

Shafir, S., 1996. Color discrimination conditioning of a wasp, *Polybia occidentalis* (Hymenoptera: Vespidae). *Biotropica* **28**(2): 243-251.

Shelton, A.M. and Nault, B.A., 2004. Dead-end trap cropping: a technique to improve management of the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). *Crop Protection* **23**(6): 497-503.

Shiojiri, K., Takabayashi, J., Yano, S. and Takafuji, A., 2000. Flight response of parasitoids toward plant-herbivore complexes: A comparative study of two parasitoid-herbivore systems on cabbage plants. *Applied Entomology and Zoology* **35**(1): 87-92.

Siekmann, G., Tenhumberg, B. and Keller, M.A., 2001. Feeding and survival in parasitic wasps: sugar concentration and timing matter. *Oikos* **95**(3): 425-430.

Silva-Aguayo, G., 2004. Botanical insecticides. In Radcliffe, E.B. and Hutchison, W.D. (eds.), *Radcliffe's IPM World Textbook*, *University of Minnesota's Electronic Textbook of Integrated Pest Management*, USA.

http://ipmworld.umn.edu/chapters/SilviaAguayo.htm

Singer, M.C., 1986. The definition and measurement of oviposition preference in plant-feeding insects. In Miller, T.A. and Miller, J.A. (eds.), *Insect-Plant Interactions*. Springer-Verlag, New York, pp. 65-94.

Sirot, E. and Bernstein, C., 1996. Time sharing between host searching and food searching in parasitoids: State-dependent optimal strategies. *Behavioral Ecology* **7**: 189-194.

Sivinski, J., Vulinec, K. and Aluja, M., 2001. Ovipositor length in a guild of parasitoids (Hymenoptera: Braconidae) attacking *Anastrepha* spp. fruit flies (Diptera: Tephritidae) in Southern Mexico. *Annals of the Entomological Society of America* **94**(6): 886-895.

Smith, T.J. and Villet, M.H., 2003. Parasitoids associated with the diamondback moth, *Plutella xylostella* (L.), in the Eastern Cape, South Africa. In Endersby, N. (ed.), *The management of Diamondback moth and other crucifer pests*. Proceedings of the Fourth International Workshop, Melbourne, Australia, November 26-29, 2001.

StatsDirect 2009. *StatsDirect Statistical Software* Version 2.7.3. StatsDirect Ltd., England. http://www.statsdirect.com

Stapel, J.O., Cortesero, A.M., De Moraes, C.M., Tumlinson, J.H. and Lewis, W.J.,1997. Extrafloral nectar, honeydew, and sucrose effects on searching behavior and efficiency of *Microplitis croceipes* (Hymenoptera: Braconidae) in cotton. *Environmental Entomology* **26**: 617-623.

Stemele, M.A., 2005. The diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae) and its natural enemy complex: Responses to pesticide treatments and semiochemicals. MSc Thesis, University of Fort Hare, Alice, South Africa, 63 pp.

Stephens, M.J., France, C.M., Wratten, S.D. and Frampton, C., 1998. Enhancing biological control of leafrollers (Lepidoptera: Tortricidae) by sowing buckwheat (*Fagopyrum esculentum*) in an orchard. *Biocontrol Science and Technology* **8**: 547-558.

Steppuhn, A. and Wäckers, F.L., 2004. HPLC sugar analysis reveals the nutritional state and the feeding history of parasitoids. *Functional Ecology* **18**: 812-819.

Stireman III, J.O., 2002. Host location and selection cues in a generalist tachinid parasitoid. *Entomologia Experimentalis et Applicata* **103**: 23-34.

Strand, M.R., Clark, K.C. and Gardiner, E.M., 1999. Plasmatocyte spreading peptide does not induce *Microplitis demolitor* polydnavirus-infected plasmatocytes to spread on foreign surfaces. *Archives of Insect Biochemistry and Physiology* **40**(1): 41-52.

Strand, M.R. and Pech, L.L., 1995. Immunological basis for compatibility in parasitoid-host relationships. *Annual Review of Entomology* **40**: 31-56.

Sun C.N., 1992. Insecticide resistance in diamondback moth. In Talekar, N.S. (ed.), *Diamondback moth and other crucifer pests*. Proceedings of the Second International Workshop, Tainan, Taiwan, 10-14 December 1990.

Asian Vegetable Research and Development Centre, Shanhua, Taiwan, AVRDC Publication No. 92-368, pp. 419-426.

Tabashnik, B.E., 1994. Evolution of resistance to *Bacillus thuringiensis*. *Annual Review of Entomology* **39**: 47-79.

Tabashnik, B.E., Groeters, F.R., Finson, N. and Johnson, M.W., 1994. Instability of resistance to *Bacillus thuringiensis. Biocontrol Science and Technology* **4**(4): 419-426.

Takasu, K. and Lewis, W.J., 1993. Host- and food-foraging of the parasitoid *Microplitis croceipes*: learning and physiological state effects. *Biological Control*. **3**: 70-74.

Takasu, K. and Lewis, W.J., 1995. Importance of adult food sources to host searching of the larval parasitoid *Microplitis croceipes*. *Biological Control* **5**: 25-30.

Takasu, K. and Lewis, W.J., 1996. The role of learning in adult food location by the larval parasitoid, *Microplitis croceipes* (Hymenoptera: Braconidae). *Journal of Insect Behavior* **9**: 265-281.

Takasu, K. and Lewis, W.J., 2003. Learning of host searching cues by the larval parasitoid *Microplitis croceipes*. *Entomologia Experimentalis et Applicata* **108**: 77-86.

Talekar, N.S. and Shelton, A.M., 1993. Biology, ecology, and management of the diamondback moth. *Annual Review of Entomology* **38**: 275-301.

Talekar, N.S. and Yang, J.C., 1991. Characteristics of parasitism of diamondback moth by two larval parasites. *Entomophaga* **36**(1): 95-104.

Talekar, N.S. and Yang, J.C., 1993. Influence of crucifer cropping system on the parasitism of *Plutella xylostella* (Lep., Yponomeutidae) by *Cotesia plutellae* (Hym., Braconidae) and *Diadegma semiclausum* (Hym., Ichneumonidae). *Entomophaga* **38**(4): 541-550.

Talekar, N.S., Yang, J.C. and Lee, S.T., 1992. Introduction of *Diadegma semiclausum* to control diamondback moth in Taiwan. In Talekar, N.S. (ed.), *Diamondback moth and other crucifer pests*. Proceedings of the Second International Workshop, Tainan, Taiwan, 10-14 December 1990. Asian Vegetable Research and Development Centre, Shanhua, Taiwan, AVRDC Publication No. 92-368, pp. 263-270.

Tamò, C., Roelfstra, L., Guillaume, S. and Turlings, T.C.J., 2006. Odour-mediated long-range avoidance of interspecific competition by a solitary endoparasitoid: a time-saving foraging strategy. *Journal of Animal Ecology* **75**: 1091-1099.

Tanaka, K., Matsumoto, H. and Hayakawa, Y., 2002. Detailed characterization of polydnavirus immunoevasive proteins in an endoparasitoid wasp. *European Journal of Biochemistry* **269**: 2557-2566.

Taylor, A.D., 1988. Host effects on larval competition in the gregarious parasitoid *Bracon hebetor*. *Journal of Animal Ecology*, **57**: 163-172.

Thompson, S. N., 1986. Nutrition and *in vitro* culture of insect parasitoids. *Annual Review of Entomology* **31**: 197-219.

Thompson, S.N., 1999. Nutrition and culture of entomophagous insects. *Annual Review of Entomology* **44**: 561-592.

Tiwari, K.N., Singh, A. and Mal, P.K., 2003. Effect of drip irrigation on yield of cabbage (*Brassica oleracea* L. var. *capitata*) under mulch and non-mulch conditions. *Agricultural Water Management* **58**: 19-28.

Tran, T.V. and Takasu, K., 2000. Life History of the pupal parasitoid *Diadromus subtilicornis* (Gravenhorst) (Hymenoptera: Ichneumonidae) as influenced by temperature, photoperiod, and availability of food and hosts. *Entomological Science* **3**(2): 255-264.

Tsunoda, S., 1980. Eco-physiology of wild and cultivated forms in Brassica and allied genera. In Tsunoda, S., Hinata, K. and Gomez-Campo, C. (eds.), *Brassica crops and wild allies: biology and breeding*. Japan Scientific Societies Press, Tokyo, Japan, pp. 109-120.

Tylianakis, J.M., Didham, R.K. and Wratten, S.D., 2004. Improved fitness of aphid parasitoids receiving resource subsidies. *Ecology* **85**(3): 658-666.

Uçkan, F., Ergin, E., Sinan, S. and Sak, O., 2003. Morphology of the reproductive tract and ovariole histology of *Apanteles galleriae* (Hymenoptera: Braconidae) reared on two host species. *Pakistan Journal of Biological Sciences* **6**(16): 1389-1395.

Ueno, T., 1999. Reproduction and host-feeding in the solitary parasitoid wasp *Pimpla nipponica* (Hymenoptera: Ichneumonidae). *Invertebrate Reproduction and Development* **35**: 231-237.

Ueno, T., 2000. Host concealment: a determinant of host acceptance and host-feeding in an ectoparasitoid wasp. *OIKOS (Copenhagen)* **89**(2): 223-230.

Vandermeer, J.H., 1992. The ecology of intercropping. Cambridge University Press, Cambridge, 237 pp.

Vasquez, B.L., 2001. Resistant to most insecticides. In Walker, T.J. (ed.), *University of Florida Book of Insect Records*. http://ufbir.ifas.ufl.edu/.

Ventura, M.U. and Ito, M., 2000. Antifeedant activity of *Melia azedarach* (L.) extracts to *Diabrotica speciosa* (Genn.) (Coleoptera: Chrysomelidae) beetles. *Brazilian Archives of Biology and Technology* **43**(2): 215-219.

Verkerk, R.H.J. and Wright, D.J., 1996. Multitrophic interactions and management of the diamondback moth: A review. *Bulletin of Entomological Research* **86**(3): 205-216.

Vet, L.E.M., Lewis, W.J., Papaj, D.R. and van Lenteren, J.C., 1990. A variable-response model for parasitoid foraging behavior. *Journal of Insect Behavior* **3**(4): 471-490.

Vet, L.E.M. and van Opzeeland, K., 1984. The influence of conditioning on olfactory microhabitat and host location in *Asobara tabida* (Nees) and *A. rufescens* (Foerster) (Braconidae: Alysiinae) larval parasitoids of Drosophilidae. *Oecologia* **63**(2): 171-177.

Visser, J.H., 1986. Host odor perception in phytophagous insects. *Annual Review of Entomology* **31**: 121-144.

Vuorinen, T., Nerg, A., Ibrahim, M.A., Reddy, G.V.P. and Holopainen, J.K., 2004. Emission of *Plutella xylostella*-induced compounds from cabbages grown at elevated CO_2 and orientation behavior of the natural enemies. *Plant Physiology* **135**: 1984-1992.

Waage, J., 2000. Foreword. In Gurr, G. and Wratten, S. (eds.), *Biological control: Measures of success.* Kluwer Academic, Publishers, Dordrecht, The Netherlands, pp. v-vi.

Waage, J. K. and Cherry, A., 1992. Quantifying the impact of parasitoids on diamondback moth. In Talekar, N.S. (ed.), *Diamondback moth and other crucifer pests*. Proceedings of the Second International Workshop, Tainan, Taiwan, 10-14 December 1990. Asian Vegetable Research and Development Centre, Shanhua, Taiwan, AVRDC Publication No. 92-368, 245-253.

Wäckers, F.L., 1994. The effect of food-deprivation on the innate visual and olfactory preferences in the parasitoid *Cotesia rubecula*. *Journal of Insect Physiology* **40**: 641-649.

Wäckers, F.L., 1999. Gustatory response by the hymenopteran parasitoid *Cotesia glomerata* to a range of nectar and honeydew sugars. *Journal of Chemical Ecology* **25**: 2863-2877.

Wäckers, F.L., 2000. Do oligosaccharides reduce the suitability of honeydew for predators and parasitoids? A further facet to the function of insect synthesized honeydew sugars. *Oikos* **90**: 197-201.

Wäckers, F.L., 2001. A comparison of nectar and honeydew sugars with respect to their utilization by the hymenopteran parasitoid *Cotesia glomerata*. *Journal of Insect Physiology* **47**: 1077-1084.

Wäckers, F.L., 2005. Suitability of (extra-) floral nectar, pollen and honeydew as insect food sources. In Wäckers, F.L., van Rijn, P.C.J. and Bruin, J.

(Eds.), *Plant-provided food for carnivorous insects: a protective mutualism and its applications*. Cambridge: Cambridge University Press 356 pp.

Wäckers, F.L. and Lewis, W.J., 1994. Olfactory and visual learning and their combined influence on host site location by the parasitoid *Microplitis croceipes* (Cresson). Biological Control **4**(2): 105-112.

Wäckers, F.L., Romeis, J. and van Rijn, P., 2007. Nectar and pollen feeding by insect herbivores and implications for multitrophic interactions. *Annual Review of Entomology* **52**: 301-323.

Waladde, S.M., Leutle, M.F. and Villet, M.H., 2001. Parasitism of *Plutella xylostella* (Lepidoptera: Plutellidae): Field and Laboratory Observations. *South African Journal of Plant and Soil* **18**(1): 32-37.

Wang, X.G. and Messing, R.H., 2003. Egg maturation in the parasitoid *Fopius arisanus* (Hymenoptera: Braconidae): do host-associated stimuli promote ovarian development?. *Annals of the Entomological Society of America* **96**(4): 571-578.

Wanner, H., Gu, H. and Dorn, S., 2006. Nutritional value of floral nectar sources for flight in the parasitoid wasp, *Cotesia glomerata*. *Physiological Entomology* **31**: 127-133.

Wardle, A.R., 1990. Learning of host microhabitat colour by *Exeristes roborator* (F.) (Hymenoptera: Ichneumonidae). *Animal Behaviour* **39**(5): 914-923.

Webb, B. A. and Luckhart, S., 1994. Evidence for an early immunosuppressive role for related *Campoletis sonorensis* venom and ovarian proteins in

Heliothis virescens. Archives of Insect Biochemistry and Physiology **26**: 147-163.

Webb, B.A. and Luckhart, S., 1996. Factors mediating short- and long-term immune suppression in a parasitized insect. *Journal of Insect Physiology* **42**(1): 33-40.

Webb, B.A., Strand, M.R., Dickey, S.E., Beck, M.H., Hilgarth, R.S., Barney, W.E., Kadash, K., Kroemer, J.A., Lindstrom, K.G., Rattanadechakul, W., Shelby, K.S., Thoetkiattikul, H., Turnbull, M.W. and Witherell, R.A., 2006. Polydnavirus genomes reflect their dual roles as mutualists and pathogens. *Virology* **347**: 160-174.

Wegener, G., Tschiedel, V., Schlöder. P. and Ando, O., 2003. The toxic and lethal effects of the trehalase inhibitor trehazolin in locusts are caused by hypoglycaemia. *The Journal of Experimental Biology* **206**(7): 1233-1240.

Wheeler, D.E., Tuchinskaya, I., Buck, N.A. and Tabashnik, B.E., 2000. Hexameric storage proteins during metamorphosis and egg production in the diamondback moth, *Plutella xylostella* (Lepidoptera). *Journal of Insect Physiology* **46**: 951-958.

Whitfield, J.B., 2003. Phylogenetic insights into the evolution of parasitism in Hymenoptera. *Advances in Parasitology* **54**: 69-100.

Williams III, L. and Roane, T.M., 2007. Nutritional ecology of a parasitic wasp: food source affects gustatory response, metabolic utilization, and survivorship. *Journal of Insect Physiology* **53**: 1262-1275

Winkler, K., Wäckers, F.L., Bukovinski-Kiss, G. and van Lenteren, J.C., 2006. Nectar resources are vital for *Diadegma semiclausum* fecundity under field conditions. *Basic and Applied Ecology* **7**: 133-140.

Winkler, K., Wäckers, F.L., Stingli, A. and van Lenteren, J.C., 2005. *Plutella xylostella* (diamondback moth) and its parasitoid *Diadegma semiclausum* show different gustatory and longevity responses to a range of nectar and honeydew sugars. *Entomologia Experimentalis et Applicata* **115**: 187-192.

Wyckhuys, K.A.G., Strange-George, J.E., Kulhanek, C.A., Wäckers, F.L. and Heimpel, G.E., 2008. Sugar feeding by the aphid parasitoid *Binodoxys communis*: how does honeydew compare with other sugar sources? *Journal of Insect Physiology* **54**: 481-491.

van Wyk, B., van Heerden, F. and van Oudtshoorn, 2002. *Poisonous plants of South Africa*. 1st Edition. Briza Publications, Gauteng, South Africa, 288 pp.

Zaviezo, T. and Mills, N., 2000. Factors influencing the evolution of clutch size in a gregarious insect parasitoid. *Journal of Animal Ecology* **69**(6): 1047-1057.

Zhang, Z., Ye, G., Cai, J. and Hu, C., 2005. Comparative venom toxicity between *Pteromalus puparum* and *Nasonia vitripennis* (Hymenoptera: Pteromalidae) toward the hemocytes of their natural hosts, non-target insects and cultured insect cells. *Toxicon* **46**: 337-349.

Zhao, J.Z., Collins, H.L., Li, Y.X., Mau, R.F.L., Thompson, G.D., Hertlein, M., Andaloro, J.T., Boykin, R. and Shelton, A.M., 2006. Monitoring of

diamondback moth (Lepidoptera: Plutellidae) resistance to spinosad, indoxacarb, and emamectin benzoate. *Journal of Economic Entomology* **99**(1): 176-181.

Ziegler, R. and Van Antwerpen, R., 2006. Lipid uptake by insect oocytes. *Insect Biochemistry and Molecular Biology* **36**(4): 264-272.

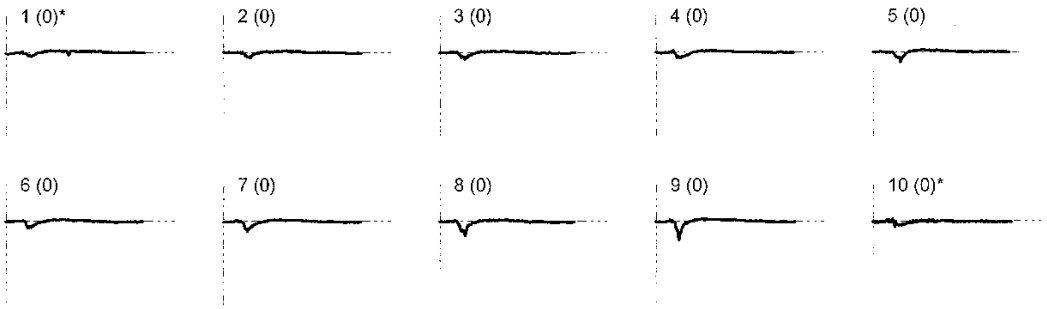
APPENDICES

Appendix 1: Composition of Yeager's physiological salt per liter of distilled water.

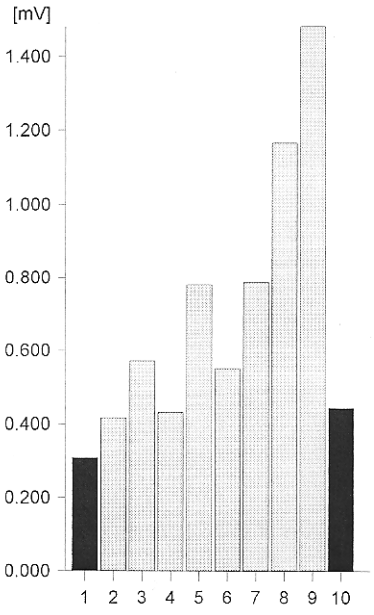
Salt		Quantity (g)
Common name	Chemical formula	
Sodium chloride	NaCl	10.93
Potassium chloride	KCl	1.57
Calcium chloride	$CaCl_2$	0.85
Magnessium chloride	$MgCl_2$	0.17
Sodium bicarbonate	$NaHCO_3$	0.17

Appendix 2: A representative of electroantennographs (A) with corresponding mV (B) and normalized percent (C) values from eight stimuli. Black bars (1 and 10) represent control observations before and after test stimuli (2–9) were applied.

A



B



C