
Food foraging in adult parasitoid *Cotesia rubecula*:

how sugar sources contribute to survival and reproduction

by

Gitta Siekmann

Dipl. – Biol., University of Hanover, Germany

Thesis submitted for the degree of

Doctor of Philosophy

at Adelaide University

Department of Applied and Molecular Ecology

Faculty of Agricultural and Natural Resource Sciences

Adelaide University

Australia

April, 2002

To Michael R. Sincock, my ‘Wunderlich’

Table of Contents

Table of Figures

List of Tables

Abstract

Sugar intake can extend the lifespan and fecundity of adult parasitic wasps. How often parasitoids in the field encounter sugar and how this contributes to their lifetime reproduction remains largely unknown. Laboratory studies providing easy access to abundant sugar sources seem to largely overestimate parasitoid longevity and fecundity. This study aimed to explore various aspects of sugar foraging in the field and its consequences for a female parasitoid. As a study system the solitary endoparasitoid *Cotesia rubecula* (Hymenoptera: Braconidae) was chosen. It parasitises larvae of the cabbage white butterfly, *Pieris rapae* (Lepidoptera: Pieridae). Without sugar, female *C. rubecula* live only 2-3 days, during which time they have approximately 100 eggs available to deposit. This non-host feeding species is believed to be time- rather than egg-limited in its reproduction. The need for food may be related to the rate and kind of energy depletion during foraging. A quantification of energy reserves after different foraging activities (flight and oviposition) identified carbohydrates as the major flight fuel for an adult parasitoid. The deposition of a relatively high number of eggs (oviposition) hardly depleted any energy reserves. Parasitoids that have to travel large distances between host encounters may benefit greatly from sugar consumption in respect to reproductive success.

With a short life span of only 2-3 days under favourable foraging conditions and an even shorter time available for coordinated searching behaviour, female *C. rubecula* seem to have only little time available for host foraging until starvation sets in and limits them to random search for hosts or food in their immediate environment. However, feeding experience modified the flower foraging behaviour of *C. rubecula* by enhancing its response to distant nectar sources. It is estimated that parasitoids such as *C. rubecula* need to feed once every one or two days on a sugar source such as floral nectar to survive in the field. However, when provided with flowers in large field cages, survival was only marginally affected. Reproduction was significantly higher in the presence of flowers not only due to higher lifespan but also higher foraging activity of wasps. Yet, under the given conditions *C. rubecula* realised only a fraction of its potential fecundity. Other factors such as temperature had a stronger influence on lifespan and foraging behaviour than the availability of food.

The need for food has to be closely studied in relation to a species' adaptation to its environment. The availability of both, hosts and food, as well as food quality and extrinsic mortality factors, influence the benefits and risks of sugar foraging. The costs of finding sufficient sugar to increase expected lifetime may often outweigh the benefits from sugar foraging. Information on the phylogenetic history with sugar sources and the life history evolution of a parasitoid species are basic requirements in evaluating present parasitoid sugar foraging. The findings of this study have practical implications for the conservation of natural enemies in the biological management of insect pests.

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is given in the text.

I consent to this thesis being made available for photocopying or loan when deposited in the University Library.

23.04.2002

Candidate's signature Date

Acknowledgements

Firstly, I would like to thank my supervisors Dr Michael A. Keller and Dr Brigitte Tenhumberg for their guidance, encouragement, support and fruitful discussions throughout the course of this study.

Special thanks to past and present members of the Mike Keller Laboratory/ Entales Group. Ana Lilia, Cate, Darryl, Dawn, Jeanine, Kaye, Megan, Steve, Vanessa and Xin Geng shared most of my time at the Waite Campus and will always be warmly remembered.

Prof Dr Otto Schmidt and Prof Dr Stefan Vidal played a crucial role in my decision to join to the Department of Applied and Molecular Ecology (formerly: Crop Protection) and to start a PhD course. Thank you for your support and encouragement.

Members of the Department of Applied and Molecular Ecology, Terry Feckner, Gary Taylor, Heather Fraser, Nicky Featherstone and Sue Eaton provided assistance with laboratory equipment and administrative matters. Anke Johnsen has provided generous help and support within and outside the Department. Margaret Cargill (ACUE) taught an excellent English writing and speaking course. Scott Field provided 'last minute' editorial input.

Members of the Otto Schmidt Laboratory provided space and assistance for biochemical extraction work.

I am grateful to my fellow students and the academic staff that I have met during my time at the Waite Campus and who became friends, especially Sara Kamaludeen, Dawn Hawthorn-Jackson, Ana Lilia Alfaro Lemus, Cate Paull, Nancy Schellhorn and Anuja Kumaratilake.

Special thanks to my friends in Germany that kept contact and provided constant encouragement.

I would also like to acknowledge the Australian Government for sponsoring my study and stay at Adelaide University by providing an International Postgraduate Research Scholarship (IPRS) and Adelaide University Scholarship (AUS).

Finally, this work would not have been possible without my family Tata and Hanna and my partner Mike. I would not have come this far without you.

Chapter 1 General introduction

Parasitoids are insects whose larvae develop by feeding in or on the bodies of other arthropods, usually insects (Godfray 1994). Larval feeding results in the death of the parasitoid's hosts. Parasitoid research is warranted for several reasons. Parasitoids are rich in species; they might comprise up to 20%-25% of all insect species (LaSalle and Gauld 1991; Godfray 1994) and up to 10% of all metazoan animal species (Hassel 1978). They can be considered as keystone species in many terrestrial ecosystems since they interact with a large number of species of insect herbivores (LaSalle and Gauld 1991, 1993). Parasitoids hold ecological and economic importance in agro-ecosystems, as they are able to suppress insect pest populations (Greathead 1986). Research on parasitoids also contributes to the understanding on the evolution of life history traits and behavioural adaptations (Hubbard and Cook 1978; Van Alphen and Vet 1986; Godfray 1994). As there is a direct link between host foraging behaviour and reproduction, parasitoids are an ideal study system to study the evolution and adaptation of behaviour (Van Alphen and Vet 1986). Being insects they are usually easy to rear and have short generation times, adding to their suitability in research studies.

This study deals with various aspects of the sugar foraging behaviour in adult insect parasitoids. Sugar intake can have a substantial impact on the lifespan of many species of parasitoids (Jervis *et al.* 1992; Jervis *et al.* 1993; Jervis *et al.* 1996). The lifespan of adult parasitoids might increase from a few days when kept with water only to several weeks when caged with honey or a sugar sources of similar quality (Leius 1961b; Hohmann *et al.* 1988; Wäckers and Swaans 1993; Morales-Ramos *et al.* 1996; Heimpel *et al.* 1997c; McDougall and Mills 1997a; Thompson 1999). An increase in lifespan is desirable if the natural lifespan of an unfed parasitoid might be too short to lay all its eggs. This might be the case for many species of parasitoids (Ellers 1998; Sevenster *et al.* 1998; Ellers *et al.* 2000). There is little known about how much sugar sources in the field contribute to parasitoid survival and reproduction. The major aim of this thesis was to explore the role of sugar foraging in the life of the insect parasitoid *Cotesia rubecula* (Hymenoptera: Braconidae) by employing elements from insect physiology, behavioural ecology and life history theory. Therefore, relevant literature was reviewed in the appropriate chapters for continuity and completeness of a given research subject. The remainder of this introductory section consists of a brief overview of parasitoid feeding ecology, a description of the insect species used in the experiments, the aims of this thesis, and a brief overview of the methodological approach used in the individual chapters.

1.1 Sugar feeding in parasitoids

Parasitoids feed on several different types of food (Jervis and Kidd 1986; Jervis *et al.* 1992). The majority of parasitoid species readily feed on sugary food such as nectar and honeydew, and many feed on their hosts as well (Jervis and Kidd 1986). Host feeding allows the female to obtain proteins to meet the amino acid demands associated with egg production, while feeding from non host sources provides the female with sugars to meet the body's energetic demands (Jervis and Kidd 1986; Rivero and Casas 1999a). Thus, host feeding behaviour is linked to the way parasitoids develop their eggs. Syn-ovigenic parasitoids eclose with none or a fraction of their potential complement of mature eggs and require proteinaceous food material such as host hemolymph to mature their eggs (Flanders 1950; Jervis and Kidd 1986; Van Lenteren *et al.* 1987; Rivero and Casas 1999a). Pro-ovigenic parasitoids eclose with more or less their full complement of mature eggs and require little or no food for egg production during their adult life and do not host feed (Fletcher *et al.* 1994; Rivero and Casas

1999a). Maintaining basic body functions is especially important during periods where hosts are scarce or hard to find. Sugar intake increases life expectancy and therefore a parasitoid's chances of finding more hosts. Understanding the role of sugar foraging in insect parasitoids also has practical application in biological control programs that aim to conserve and support natural antagonists of insect pests in agro-ecosystems (Wäckers 1994; Jervis *et al.* 1996; Takasu and Lewis 1996).

1.1.1 Life-history

The study of life-history is concerned with those traits of an organism that influence lifetime reproductive success such as age and size at maturity, potential fecundity, number and sex ratio of offspring and adult lifespan (Roff 1992; Stearns 1992). Predictions in life-history are based on the allocation of resources that lead to trade-offs between traits, such as between survival and reproduction (Reznik 1985; Ellers 1998). For example, energy and time invested into reproduction are not available to maintain survival. Sugar intake in parasitoids relaxes the constraints of limited energy reserves and influences life span directly. With a longer lifespan parasitoids can potentially encounter more hosts and achieve a higher reproduction.

Sugar availability is crucial for the lifespan of parasitoids. Laboratory studies have demonstrated an enormous increase (5- 10 fold) in the lifespan when parasitoids were held with sugar sources in comparison to unfed parasitoids (Leius 1961a, b; Hohmann *et al.* 1988; Wäckers and Swaans 1993; Dyer and Landis 1996; Morales-Ramos *et al.* 1996; England and Evans 1997; McDougall and Mills 1997b). To what extent the lifespan of parasitoids in the field is influenced by sugary food is practically unknown as it is difficult to track individuals and their foraging history over their lifetime. Laboratory studies where sugar sources are readily available are bound to overestimate the lifespan of parasitoids in the field because frequent food encounters may be rare in nature.

The frequency of sugar feeding in the field depends on the availability of sugar sources and the behaviour of parasitoids. For example, sugar sources can be encountered through active food foraging or opportunistically during the time parasitoids search for hosts.

1.1.2 Behavioural ecology

The way in which behaviour contributes to survival and reproduction depends on the animal's ecology (Krebs and Davies 1993). Behavioural ecology is about the fitness value of behaviour. Although the benefits of sugar intake seems to be clearly positive (as a direct investment to maintain or increase energy reserves), sugar foraging has to be viewed as an integrated part of parasitoid ecology. How much sugar foraging contributes to an individual's survival and whether sugar foraging is favoured or penalised by selection (Krebs and Davies 1993) depends on ecological factors including a parasitoid's ecology, the availability of sugar sources, the availability of hosts, and the risk of predation.

There exists a wealth of information on parasitoid optimal foraging behaviour for hosts. The link between successful oviposition and fitness has inspired many researchers to develop optimal foraging models for parasitoids (Bailey *et al.* 1962; Cook and Hubbard 1977; Waage 1979; Van Alphen and Galis 1983; Bernstein *et al.* 1988; Driessen *et al.* 1995; Tenhumberg *et al.* 2001). The fact that parasitoids might be foraging for sugary food as well has been largely neglected and only recently a few researchers have started to acknowledge food foraging as a complementary strategy to maximise fitness (Sirot and Bernstein 1996; Krivan and Sirot 1997). Sugar foraging can be viewed as an investment into future reproduction since a higher life expectancy might result in more host encounters. However, the farther a sugar source is away from host occupied sites, the higher is the risk to loose track of host sites, to deplete energy reserves after encountering a poor or depleted food source, and to encounter predators. For example, nectar producing plants are attractive to all kinds of arthropods (Morse 1986; Maingay *et al.* 1991) and a wasp might be killed or injured by predatory wasps, ants or spiders when visiting flowers. The detection of sugar sources using visually or olfactory receptors and their neural processes might be costly to develop and exercise. An analysis of all the associated costs and benefits is necessary to understand food foraging behaviour.

1.1.3 Energy metabolism

Insect physiology and in particular energy metabolism, provides information about the needs and constraints of an adult parasitoid in regard to food intake and utilisation. Adult parasitoids eclose with some energy reserves, mainly stored in the fat body in the form of carbohydrates and lipids carried over from their larval stage (Wheeler 1989). Parasitoids do not depend on sugar intake to complete their life cycle but may benefit from sugar encounters to increase their reproduction. Consumed sugar (=carbohydrates) affects a parasitoid's energy reserves. Without sugary food, carbohydrate reserves are used up first during an insect's life, followed by lipid reserves (Olson *et al.* 2000). Carbohydrates are important to fuel the nervous system, general somatic maintenance and flight (Wigglesworth 1972; Chippendale 1978). Insect flight is a highly energy demanding behaviour and will play a key role in the metabolic energy balance and rate of energy depletion (Wheeler 1989).

In parasitoids, flight is an essential means to locate resources but decreases the potential lifespan by rapidly depleting energy reserves. Sugar intake contributes to the energy reserves needed to fuel flight. These vital energy reserves can be quantified using biochemical extraction method (Van Handel 1985a, b). Using such a method, one can study energy dynamics with and without sugar intake. The extent of sugar foraging could be influenced by the amount of energy a forager spends on flying. For example, parasitoids that fly a lot during foraging for hosts may start to search for food earlier than those less active.

1.1.4 Relevance to biological control programs

In times when serious pest problems are prominent in modern agriculture and chemical control has become the dominant strategy, alternatives such as the promotion of biological control strategies within integrated pest management are urgently needed (Wilson and Huffaker 1976; DeBach and Rosen 1991; Van Driesche and Bellows Jr 1996). Pesticide use has attracted a growing concern due to toxicity hazards for farmers and other people dealing with their production and application, contamination of soils and waterways, and the infiltration of the food chain (Wilson and Huffaker 1976; Van Driesche and Bellows Jr 1996). The term "biological control" was first used by Smith (1919) to describe the use of natural enemies through introduction or other manipulation to control insect pests (Simmonds *et al.* 1976). In an applied sense, biological control is defined as the utilisation of natural enemies to reduce the damage caused by noxious organisms to tolerable levels (DeBach and Rosen 1991). Natural enemies can be utilised in three major ways: 1) importation of exotic species (= "classical" biological control), 2) augmentation of established species through direct supplementation of their populations (insectary mass production and periodic colonisation) and 3) the conservation of natural enemies via manipulation of their environment (DeBach and Rosen 1991). Within the strategy of conserving natural enemies such as parasitic wasps, biological control practitioners and ecologists advocate the propagation of flowers or use of food sprays to feed natural enemies in agro-ecosystems (Rabb *et al.* 1976; Altieri and Whitcomb 1979; Powell 1986; Van Emden 1990; Wratten and van Emden 1995; Jervis *et al.*

1996; Jervis and Kidd 1999). Several studies, mostly from the past, have reported an increased parasitism due to the presence of adult food sources (Tab. 1.1).

Table 1.1 Examples of increased parasitism when natural sugar sources were present (modified after Powell 1985)

Parasitoids	Pests	Crops	Food sources	References
Tiphia vernalis (Tiphiidae)	Japanese beetle (*Popillia japonica*)	Various pastures	Aphid honeydew	Gardner (1938)
Tiphia popilliavora (Tiphiidae)	White grubs (*Phyllophaga* spp.)	Various crops	Nectar from weeds, honeydew from scale insects	Wolcott (1942)
Aphelinus mali (Aphelinidae)	Aphids	Apple	Nectar from Phacelia and Eryngium	Telenga (1958)
Apantelis medicaginis (Braconidae)	Lepidoptera (*Colias philodice*)	Alfalfa	Nectar from weeds and aphid honeydew	Allen and Smith (1958)
Aphytis proclia (Aphelinidae)	Scale insects (*Quadraspidiotus perniciosus*	Orchards	Phacelia -nectar	Chumakova (1960)
Various species	Apple codling moth (*Carpocosa pomonella*) Tent caterpillar (*Malocosoma americanum*)	Apple	Nectar from weeds	Leius (1967)
Lixophaga sphenophori (Tachinidae)	Sugar cane weevil (*Rhabdoscelus obscurus*)	Sugar cane	Euphorbia -nectar	Topham and Beardsley (1975)
Campoletis sonorensis (Ichneumonidae)	Tobacco budworm (*Heliothis virescens*)	Cotton	Extrafloral nectar from cotton	Lindgren and Lukefahr (1977)
Copidosoma koehleri (Encyrtidae)	Potato moth (*Phtorimaea opperculla*)	Potato	Coriander- and faba bean - nectar	Baggen and Gurr (1998)
Copidosoma koehleri (Encyrtidae)	Potato moth (*Phtorimaea opperculla*)	Potato	Borage- or buckwheat-nectar	Gurr et al. (1998)

At the same time concerns are voiced that the evidence from the field of improved biological control from supplemental food sources is still largely anecdotal and properly replicated field tests are still in the minority (Jervis *et al.* 1996). To date, the hypothesis that the consumption of sugary food ultimately leads to improved pest mortality remains unconfirmed (Jervis and Kidd 1999). There exists also a range of more recent field studies that could not detect an effect of the presence of flowers on parasitism of insect pests (Kloen and Altieri 1990; Cowgill 1995; Bigger and Chaney 1998; Cappuccino *et al.* 1999; Nicholls *et al.* 2000). Although demonstrating a promising increase in the potential of parasitoid fecundity when caged with sugary food sources (Leius 1961a, b; Van Lenteren *et al.* 1987; Hagley and Barber 1992; England and Evans 1997; Heimpel *et al.* 1997a; Teraoka and Numata 2000), parasitoid reproduction in the field is difficult to manipulate.

Each cropping system, with its pest and natural enemy- complex, sugar producing sources, population densities and climate, will have different outcomes in pest mortality (Jervis *et al.* 1996; Lewis *et al.* 1998). A step towards the understanding why the propagation of flowers in agro-ecosystems sometimes increases pest mortality and sometimes not, may lay in the knowledge of the selective use of flowers by pest insects and their natural enemies (Baggen *et al.* 1999). Another important step in evaluating the success of flower propagation may lay in the research of the fundamentals of sugar foraging behaviour of parasitoids.

1.2 Biology of the parasitoid *Cotesia rubecula* and its host *Pieris rapae*

Cotesia rubecula Marshall (Hymenoptera: Braconidae) was selected as the focus of this study because it feeds solely on sugar, has a high potential fecundity, and female life expectancy is greatly influenced by sugar consumption (Nealis 1986, 1990; Sengonca and Peters 1993;

Wäckers and Swaans 1993). Starved *C. rubecula* have been found to respond to flowers in the presence of hosts in olfactometer experiments and to yellow colours in flight chambers (Wäckers 1994). Females feed readily on sugar sources such as honey, honeydew and floral nectar, thereby increasing their lifespan up to 2-10 fold (Sengonca and Peters 1993; Wäckers and Swaans 1993). Without feeding, females usually die after 2 to 3 days at 25°C (Wäckers and Swaans 1993). Host feeding does not occur in this species. Female *C. rubecula* have predominantly pro-ovigenic (a large number of small, hydropic eggs, no protein source required for maturation) and some synovigenic characteristics (not all eggs fully mature at eclosion) (i.e. as *Venturia canescens* in Fletcher *et al.* 1999), maturing about 100 eggs within their first three days of life (Nealis 1990). Jervis *et al.* (2001) recently reviewed the mode of egg development and defined all parasitoid species that emerge with at least some immature eggs as 'synovigenic'. Female *C. rubecula* deposit a maximum number of 10 eggs per day at high host densities under semi -field conditions (Nealis 1990). *C. rubecula* is an endoparasitic koinobiont. The host larva continues to live during parasitoid development but dies shortly after the parasitoid larva has left the host's body. This species is a solitary parasitoid of the cabbage white butterfly *Pieris (*syn. *Artogeia) rapae* Lineaus (Lepidoptera: Pieridae) feeding on Brassica plants (see refs. in Cameron *et al.* 1995). Females usually oviposit a single egg inside the host's body and the developing parasitoid larva consumes host hemolymph and tissue. Larvae pupate in a cocoon outside the host's body. If more than one egg is deposited in the same host (superparasitism), only one parasitoid larva can successfully complete development. *C. rubecula* originated from Europe and was introduced into USA, Canada, New Zealand and Australia (Nealis 1985; Cameron *et al.* 1995). Egg deposition is most successful in early instars (2^{nd} instar) of the host and the mature parasitoid larva generally emerges from the fourth instar (M. A. Keller, unpubl. data.). *C. rubecula* exhibits a short day photoperiodic response to trigger its winter diapause. In South -Australia it enters diapause if the day length is less than 13 hours and remains in diapause from March/April to October (Nealis 1985; M.A. Keller, pers. communication).

The small cabbage white butterfly *P. rapae* is now a cosmopolitan species (Hern *et al.* 1996). Adult butterflies feed on floral nectar and larvae feed exclusively on cruciferous species with the exceptions of mignonette *(Reseda* spp.., Resedaceae*)* and nasturtium *(Tropaeolum* ssp., Tropaeolaceae*)*. During their lifetime females lay 100 – 750 eggs singly on the under-surface of leaves (Jones 1981). In large Brassica plantings the distribution of larval *P. rapae* throughout a habitat usually follows a poisson or negative binomial distribution (Kobayashi 1966; Jones 1981; Vos 1999), mostly with a mean density of one or two larvae per plant (Harcourt 1961; Kobayashi 1966; Jones 1977). However, on small patches of host plants or isolated plants, densities of 5 larvae per plant are not uncommon (M. A. Keller, pers. communication). The damage to foliage caused by *P. rapae* is insignificant, although it can be severe in seasons with high populations (Hern *et al.* 1996). The pest status of this species arises primarily, because of the contamination of plants by its frass rendering them unmarketable, rather than the amount of crop consumed (Finch and Thompson 1992).

1.3 Aims and methodological approach

This study explored the role of sugar foraging in female *C. rubecula* and aimed to answer the following questions

- What are the energetic costs of oviposition and flight in adult *C. rubecula*?
- What are the constraints on parasitoid sugar foraging in the field?
- Does sugar foraging contribute to parasitoid fitness?

Sugar foraging was aimed to be investigated as an integrated part of the parasitoid's adult lifespan in relation to its energy metabolism and its host foraging behaviour (Fig. 1.1). Initially, a newly emerged female parasitoid has limited energy reserves which she expends on foraging for hosts. Flying within and between host occupied sites and laying eggs may consume a significant amount of energy. The potential lifespan is not long enough to lay all or most of the egg complement unless food is consumed. Therefore, sugar intake contributes to maintain or increase energy reserves and increases life expectancy, consequently increasing opportunities for host encounters. Increasing their life expectancy is of particular importance if hosts are scarce (spatially or temporarily) or hard to find. If food cannot be found where hosts live, parasitoids have to "decide" what to forage for: food or hosts. A trade-off between host and sugar foraging arises. Sugar foraging can be risky in terms of poor energy return or increased predation on the food source (Morse 1986; Maingay *et al.* 1991). Host foraging consumes energy and decreases life expectancy if not enough food is encountered.

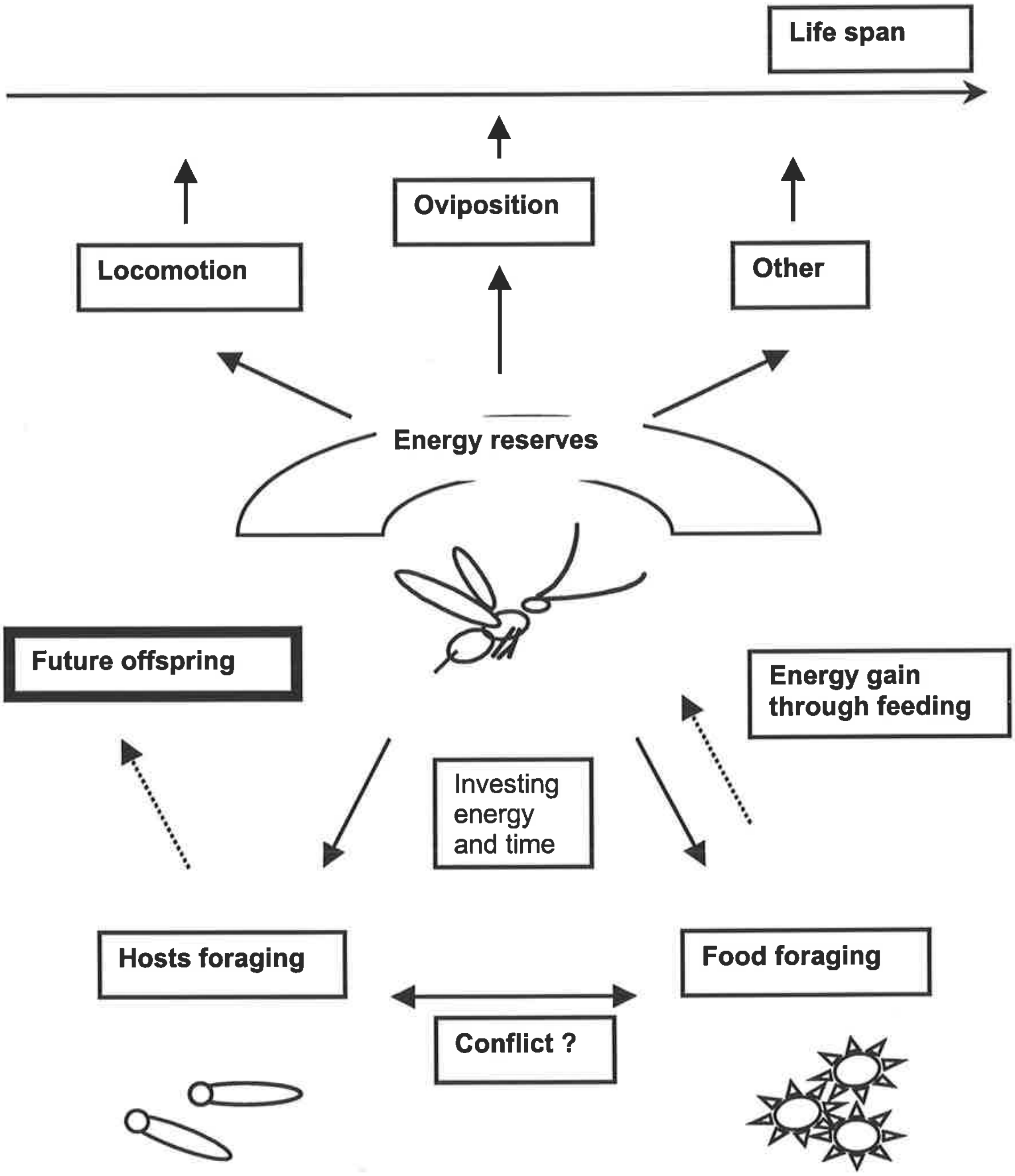


Figure 1.1 Selected relations between host and food foraging in female *C. rubecula* in terms of energy expenditure, energy gain and fitness gain (future offspring).

In the following six chapters experimental manipulation and a mathematical model, addressing the aforementioned questions, are described. Sugar intake maintains or increases energy reserves. The need for sugar can be quantified, and even predicted, if the energy depletion by major behavioural foraging activities such as flight is determined. In CHAPTER 2 the energy expenditure of female *C. rubecula* on flight and oviposition was firstly identified (depletion of lipid or carbohydrate reserves) and then quantified using a biochemical extraction method.

In the following three chapters constraints on the sugar foraging behaviour of female *C. rubecula* in the field were identified and discussed. Responsiveness to distant food sources, the 'choice' of foraging for food or for hosts, access to food and quantity of sugar intake were tested to which extent they would constrain sugar foraging in the field. In CHAPTER 3, the response of hungry parasitoids to flowers in the presence of hosts was investigated in a wind tunnel. The choice of location for foraging was documented and also the wasp's behaviour during the time locating a particular resource, nectar or hosts, to determine if parasitoids search directly or randomly for a distant flower. To test and compare the ability to provide food by naturally occurring sugar sources such as floral nectar and honeydew with honey, experiments described in CHAPTER 4 were designed to evaluate various flower species and a homopteran honeydew for the survival of parasitoids. In CHAPTER 5 the survival gain from a single meal was examined, an important factor if sugar encounters in nature are sporadic events in a life of a parasitoid. Knowing the lasting effect from a single meal, the minimum feeding frequency to avoid starvation in the field can be estimated. The minimum feeding frequency needed to survive can be compared with the chances to find sufficient food on a regular basis. If extrinsic mortality factors are not dominating parasitoid survival, then the lifespan of parasitoids in the field can be estimated.

The ultimate function of sugar foraging is assumed to increase parasitoid fitness. Field trials and a mathematical model were used to estimate the contribution of food sources to survival and reproduction of *C. rubecula*. To estimate the effect of flowers on the longevity and reproduction of individual wasps in the field, experiments conducted in a semi-field set up are described in CHAPTER 6. Small groups of female wasps were released into large field cages with a daily exchange of plants with new hosts. These releases were repeated over two foraging seasons. To predict why and when a parasitoid will forage for food instead for hosts, physiological and environmental factors have to be considered. Optimality modelling in form of a stochastic dynamic model was adopted in CHAPTER 7 to test the hypothesis that food foraging is shaped by the availability of food and hosts as well as by the energetic value of food and the risk of predation. The optimal behavioural strategy was calculated by choosing the behavioural choice (food foraging or host foraging) that yielded the highest fitness output. By examining the energy metabolism and the constraints on sugar foraging of female *C. rubecula*, the need for sugar and the chances of finding sufficient food to increase life expectancy in the field can be assessed. Ultimately, this project aimed to evaluate the contribution of sugar foraging to parasitoid lifetime reproductive success.

Chapter 2 Energy reserves and costs of foraging

2.1 Introduction

Under the assumption that animals optimise their behaviour to achieve maximum fitness (Krebs and Davies 1993), the allocation of energy reserves, especially in short lived organisms such as insects, should be well organised. This study is concerned with the identification of energy reserves and energy expenditure in an insect parasitoid.

Adult parasitoids offer a good study system to study the linkage of energy reserves with behaviour, lifespan and reproduction. Parasitoids not only search for hosts but also for food such as nectar and honeydew to maintain or increase their energy reserves (Leius 1961a; Lewis *et al.* 1998). Parasitoids have been widely used to study the adaptive nature of behavioural decisions (Godfray 1994) and the effect of behaviour on population dynamics (Thompson 1987; Ives 1995; Krivan and Sirot 1997). But parasitoid physiology in regard to energy allocation is still poorly understood and a call for more physiological realism in parasitoid behaviour has been voiced (Jervis and Kidd 1995; Rivero and Casas 1999a). For example, parasitoids might make decisions such as searching for food instead searching for hosts or superparasitise due to low energy reserves (Sirot and Bernstein 1997). The quantification of energy reserves in parasitoids and its rate of depletion can be a useful tool in understanding host and food foraging behaviour.

Energy reserves in insects are usually stored as lipids and carbohydrates (glycogen and free sugars) and, to a lesser degree, as proteins (Wigglesworth 1972) . The extraction of lipids in parasitoids has been suggested as an alternative method not only to measure expected survival (instead of observing lifespan) but also to measure the costs of dispersal (flight) during an individual's lifetime (Ellers 1996; Ellers *et al.* 1998). However, a large body of literature states that many of species of Diptera and Hymenoptera use carbohydrates as the major, if not sole, flight fuel (Beenakkers 1969; Nayar and Van Handel 1971; Wigglesworth 1972; Steele 1981; Neukirch 1982; Friedman 1985; Candy 1989). It is fair to assume that hymenopteran parasitoids might be included in this group as well. Many parasitoids, host-feeding and non-host feeding species alike, can greatly increase their lifespan when kept with a sugar source reviewed by (Jervis *et al.* 1996). As a conversion of ingested sugars into lipids does not occur (Ellers 1996; Olson *et al.* 2000), parasitoids seem to depend more on carbohydrates than on lipids. Therefore, the link between carbohydrate dynamics and foraging behaviour deserves attention.

Foraging for hosts is likely to consume a large amount of energy during the life of a parasitoid. Host-foraging can be basically characterised as flying between patches of hosts, moving within a patch, handling hosts and laying eggs. Flying is one of the most energy demanding processes in insects (Wigglesworth 1972) and energy depletion through flight could be used as a measure to characterise foraging intensity. When measuring the energetic costs of foraging, it is important to distinguish between the costs of flight and the loss of energy rich substances such as lipids by the deposition of eggs. For example, if the total lipid content of a parasitoid can be reduced by both flight activity and by developing and depositing lipid-rich eggs, flight and oviposition have to be measured separately. Habitats are often unpredictable in the density and distribution of hosts: describing two extremes of a possible range, host foraging could be characterised by extensive travelling and rare host encounters or little travelling and abundant host encounters. A way to identify energy expenditure on activities such as egg laying and flight is the extraction of lipids and carbohydrates of individuals that were subjected to such foraging activities under controlled conditions in the laboratory. A colorimetric technique to extract total lipids and total carbohydrates from the same individual was devised by Van Handel (1985a, b) on mosquitos. Since then, this method has been widely used on a variety of dipteran and to a lesser degree hymenopteran species (Yuval and Schlein 1986; Van Handel and Day 1988; Passera and Keller 1990; Sartori *et al.* 1992; Warburg and Yuval 1996). To date only a single study has reported the extraction of lipids and carbohydrates in parasitoids and documented lifetime storage of sugars, glycogen and lipids of starved and sugar-fed adults of the parasitoid *Macrocentrus grandii* (Olson *et al.* 2000).

The aim of this experiment was to quantify energy reserves in the female parasitoid *Cotesia rubecula* Marshall (Hymenoptera: Braconidae) and to identify the costs of flight and oviposition. In this study total lipids and carbohydrates of females were extracted from newly emerged and starved to death individuals to identify their energy reserves. To determine how energy reserves might change after feeding, a third group was held with continuous access to honey for 1-3 days. After determining the initial energy reserves of newly emerged females, a subsequent cohort of females was subjected for one day to different activity treatments to identify the energetic costs of oviposition and flight.

2.2 Materials and method

2.2.1 Insect and plants – standard culturing practice

C. rubecula–females used in the experiments originated from strains collected from commercial broccoli fields nearby Adelaide, South Australia. In the rearing procedure females were allowed to parasitise second-instar larvae of *P. rapae*, feeding on cabbage plants. Cabbage plants (*Brassica oleracea* L. cv. “Green Coronet”, Yates) were grown in 12.5cm pots under glasshouse conditions until carrying 4-6 mature leaves. To obtain host larvae for *C. rubecula*, cabbage plants were left in a cage with *P. rapae* – butterflies until each leaf carried approx. 10 eggs. After a period of 8-10 days, plants with 2nd instar *P. rapae* were made available in 25 cm wide and 35 cm high rectangular cages (made out of aluminium frames covered with gauze) for wasps to parasitise. Light and temperature conditions were set to 14L:10D and 25°C. Wasp cocoons were collected from the rearing cages and kept in a clear, air-ventilated 2 l plastic container until adult emergence. As about 90% of adults emerge within a few hours after onset of light in the morning, newly emerged females were separated from the cocoon stock about 3 hours after this event to allow time for mating and to obtain a uniform age group. After this procedure females were held as pairs in clear 0.7 l plastic containers, each with a cotton wick soaked in water and a piece of paper towel as resting place until used for the experiment.

2.2.2 Carbohydrate and lipid assays

Initial energy reserves were determined by extracting total carbohydrates and lipids from female *C. rubecula* upon eclosion and after starvation to death. The difference between these quantities was assumed to be available as energy reserves. The change in energy reserves after sugar feeding was analysed in females that spent 1 day or 3 days with continuous access to honey.

Emerging adults were subjected to one of four treatments. To obtain total lipid and carbohydrate levels upon adult emergence, 36 newly emerged females were killed by freezing approximately 1-3 hours after eclosion (= eclosion treatment, final sample size after biochemical analysis for carbohydrate assay n = 36, for lipid assays n = 29). In the starvation treatment 37 wasps were held in groups of three or four in a clear, air-ventilated 2 l plastic container with water and a piece of paper towel as a resting place until they died (= starvation treatment, final sample size after biochemical analysis for carbohydrate assays n= 37, for lipid assays n= 34). Containers were monitored three times a day to collect dead wasps. Wasps in the feeding treatments were held in the same way but were provided with drops of canola-honey (Kangeroo Island, SA). Sixteen wasps were either collected after 1 day (= 1d food treatment) and 9 wasps after 3 days (= 3d food treatment). Wasps collected from the treatments were individually put into 1.5ml microcentrifuge tubes (Eppendorff) and stored at –30°C until biochemical analysis.

A quick and easy way to determine initial energy reserves might be their estimation via body size parameters such as dry body weight and wing length (van Handel *et al.* 1988; M.A. Keller pers.comm.). Prior to the biochemical analysis, wasps were dried at 80°C for 3 days to obtain their dry weight (Microbalance, LM 600, Beckmann). The drying procedure also conserves specimens and allows storage at room temperature. Dry weight as an estimator can be used with newly emerged wasps but not with older wasps as weight changes with time. To test the degree of correlation between energy reserves and conservative body size parameters, wing length was measured to the nearest 0.05mm. Initial measurements of hind tibia length showed less size variation than wing length and were therefore discarded as an alternative size parameter. After weighing, one forewing was pulled off the body using fine forceps and smoothed out within a small drop of water in a glass petri-dish. Its length was measured from where the wing rooted in the muscle tissue to its longest point at the tip.

Analysis of sugar, glycogen and lipids from the same individual was conducted as described in Van Handel (1985a,b). After weighing and measuring, females were transferred (together with their severed forewing) back into the 1.5 ml centrifuge tube (where they had been stored) and crushed in 0.2 ml sodium sulfate solution (2%) with a small plastic pestle. An empty tube was treated in the same way to obtain a blank reading for each assay. After crushing the insect, 1.3 ml chloroform-methanol (1:2) was added and the solution mixed for 1 min. After that, the tubes were centrifuged for 3 min at 5000 g^{-1}. For the sugar and lipid assay 0.5 ml of the supernatant was transferred to two glass test tubes (12mm x 100mm). The absorbance read from these aliquots was adjusted to the initial volume (1.7ml) of the solution to obtain the total quantity of lipids and sugars. The rest of the supernatant was discarded and the precipitate, which contained all glycogen, was dissolved in 1 ml anthrone reagent as prepared in Van Handel (1985a) and transferred into a third glass tube. Tubes containing the supernatant were heated in a block heater at 100°C until only 0.1 – 0.2 ml were left in the sugar assay-tubes and all solution was evaporated from the tubes for the lipid assay.

Glycogen and sugar assay Glass tubes containing the precipitate were topped up to 5ml with anthrone reagent and mixed on a vortex mixer. Then the tubes were heated at 100°C for 17 min, allowed to cool at room temperature and then mixed again. After heating, the solution had turned green and the glass tube could be inserted into a in a spectro-photometer (Model 330, 400-680nm, Turner) where its absorbance was read at 625 nm. Tubes previously allocated to sugar assays were topped up with anthrone reagent to 5 ml and treated as in the previously described glycogen assay.

Lipid assay 0.2 ml sulfuric acid was added to glass tubes, previously allocated to the lipid assay, and heated at 100°C for 10 min. The solution was allowed to cool at room temperature and topped up to 5ml with a vanillin phosphoric acid reagent as prepared in Van Handel (1985b). The solution was mixed and left for 5 min to react. The pink colour complex stays stable for up to 30 min (Van Handel 1985b). The colour solution was mixed again and its absorbance read at 525nm. In rare cases where absorbance would exceed the absorbance or optical density (OD) of 1, solutions were diluted to half the original concentration and read again. Samples were run in batches of 11 insects.

Standard curves Known quantities of carbohydrate and lipids were prepared as described in Van Handel (1985a, b). For the carbohydrate assays 100 mg glucose was dissolved in 100 ml ethanol (70%). For the lipid assay 100 mg canola oil was dissolved in 100 ml chloroform. Aliquots of 50, 100, 150 and 200 µl of these solutions were dispensed into glass test tubes and treated in the same way as described above for carbohydrate and lipid assays. Each sample was replicated three times and its mean used to calculate a linear regression equation (Fig. 2.1).

2.2.3 Measuring costs of flight and oviposition

To identify the energetic costs of foraging activity, 69 newly emerged female *C. rubecula* were subjected randomly over a period of several months, either to a flight treatment (n= 23), oviposition treatment (n= 22) or a control (n= 24). After biochemical analysis, the final carbohydrate sample size remained unchanged and the final lipid sample size (due to loss of original samples) for the flight/oviposition/control – treatments were n = 19, 19 and 21, respectively. Treatments lasted for one day at a constant temperature of 25°C. After the experimental procedure, wasps were transferred singly to a 1.5 ml microcentrifuge tube and stored at –30°C until further analysis.

Oviposition treatment Three times during one day, female *C. rubecula* were exposed to abundant hosts. Within such a period, a wasp was placed into a rectangular cage measuring 25 x 25cm at the base and 35cm high (aluminium frame covered with gauze) and allowed to climb onto the wall. A fine painting brush was moistened with water and dabbed on an area of a leaf with host damage and host frass. After that, a 2nd instar *P. rapae* was taken up with this brush from its feeding site and held right in front of the wasp. Usually the wasps started immediately antennating the larva and subsequently stung it. This procedure was repeated at intervals of approx. 30 secs to 60 secs, each time with a new host until the wasp refused to sting anymore. Such a "host exposure-period" was repeated three times per day with approx 3 hours resting time in between. During resting periods a wasp was held singly in a 0.7 ml clear and air ventilated plastic container with water and a piece of paper towel. At the end of the day wasps were killed by freezing them at –30°C.

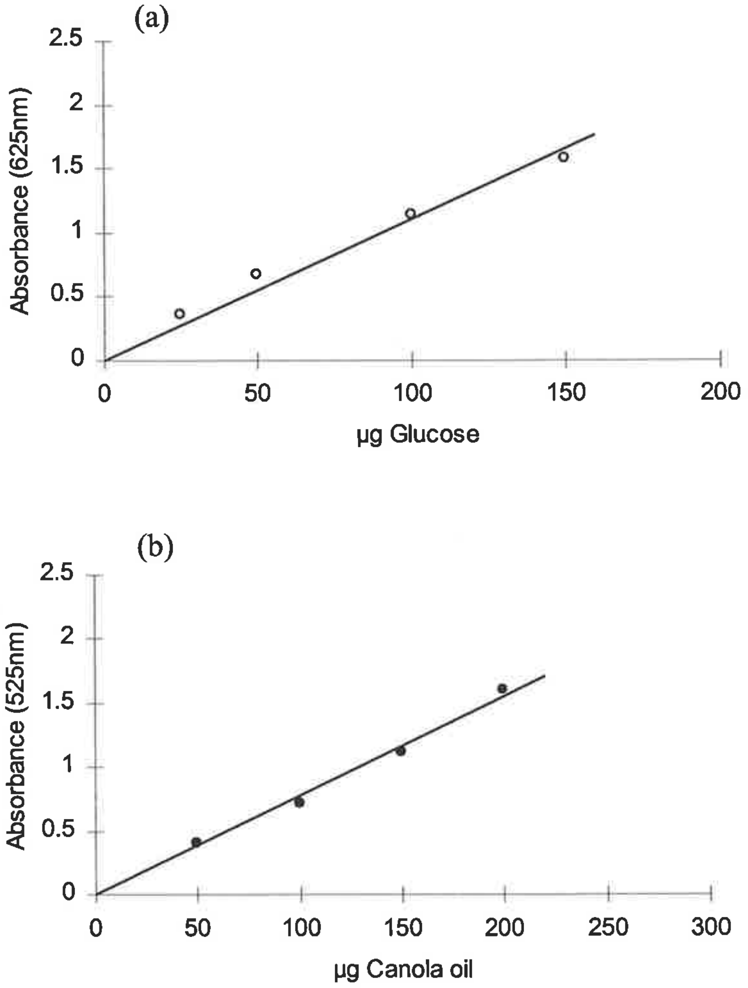


Figure 2.1 Standard curves for **(a)** carbohydrates (free sugars and glycogen) in form of glucose using the hot anthrone test and **(b)** lipids in form of canola oil using the vanillin phosphoric acid test. One data point represents the mean of three repeated samples. Readings over 1 were obtained by dilution (see text). Linear regression equation for carbohydrate curve is $y = 0.011x$, $R^2 = 0.99$ and for lipid curve $y = 0.0077x$, $R^2 = 0.97$

Flight treatment Female *C. rubecula* on the day of emergence were forced to fly extensively three times for a limited period in a wind tunnel. One day prior to the treatment a cabbage plant with approx. 4-6 leaves was "infested" with three 2nd instar *P. rapae* larvae on three different leaves. On the day of experimentation, hosts were removed from the leaves and the plant placed into a wind tunnel. Wind speed was initially set to 25 cm/s. The wasp was briefly allowed to antennate feeding damage and then placed 1m downwind into an open-ended glass vial. From there, wasps usually took off and flew to the plant. After the wasp had landed, the wind speed was increased to 40 – 50 cm/s to increase the wasp's energy expenditure while flying (Keller 1990). The wasp was taken up with an aspirator and re-released 1m downwind of the cabbage plant into free air from where she usually continued to fly to the plant. This procedure was repeated until the sum of all "flight bouts" reached 3 minutes (timed with a stop watch). Wasps that continuously flew to the illuminated ceiling and did not respond to the cabbage plant, were eliminated from the experiment. In between periods of flying, wasps were held in containers to rest as described in the oviposition treatment. Within the resting period hosts were put back onto the plant to obtain fresh feeding damage. At the end of the day wasps were killed by freezing them at –30°C. Wasps assigned to the control treatment were kept all day with water and a piece of paper towel in the same type of containers as used in the oviposition treatment.

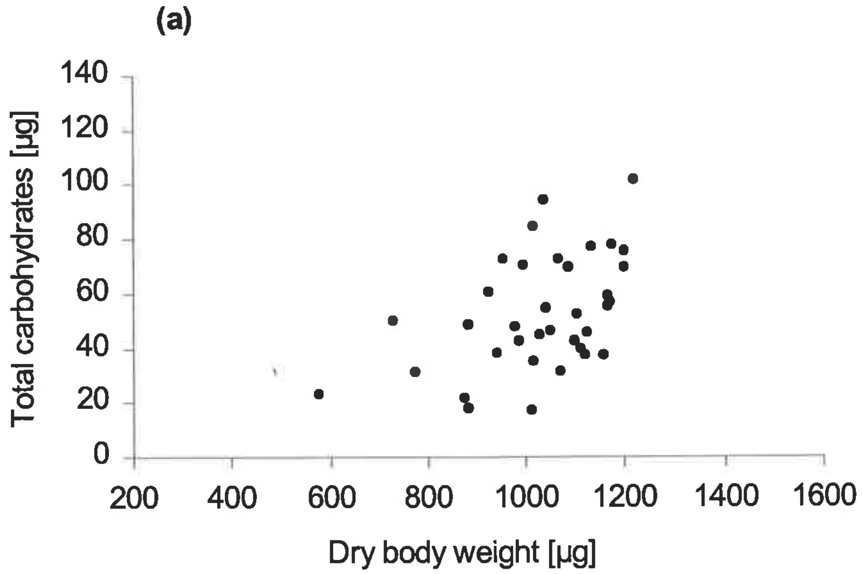
Data analysis Total lipid and carbohydrate quantities of the three different treatments were analysed with a one-way ANOVA followed by a multiple comparison of the means using the Tukey-Kramer Test (JMP-Version 3.1, SAS Institute 1995). Data were log transformed if unequal variances occurred indicated by O'Brian Test (JMP-Version 3.1, SAS Institute 1995).

2.3 Results

2.3.1 Profile of carbohydrate and lipids in female *C. rubecula*

Carbohydrate profile Total carbohydrates were estimated to comprise 4.5% - 5.5% of the dry body weight of newly emerged female *C. rubecula* reared in the laboratory (Fig.2.2 a). About 64 % of the total carbohydrates in newly emerged and unfed females were estimated to be depletable energy reserves, the rest structural components of the insect body such as in chitin (Fig. 2.3). In unfed wasps the major fraction of carbohydrates was comprised of glycogen and a minor fraction of free sugars. This composition changed in honey-fed females: their fraction of free sugars increased by more than 10-fold, whereas the amount of glycogen remained more or less constant after one day and even decreased after 3 days. With access to honey, 1 day and 3 day old wasps maintained a constant amount of carbohydrates. Within this period no diversion of ingested sugars into glycogen occurred but a decrease in glycogen was recorded (ANOVA on log-transformed data, DF = 60, F= 5.81, *P*= 0.005).

Lipid profile Total lipids were estimated to comprise about 10% - 12% of the dry weight of newly emerged female *C. rubecula* (Fig. 2.2b). About half of the extracted lipids were metabolised during the lifetime of unfed females (Fig. 2.4). Honey feeding did not increase the amount of lipids in the insect body, indicating that a transformation of ingested sugars into lipids (lipogenesis) does not occur. At eclosion total lipids are positively correlated with total carbohydrates (r = 0.47) and carbohydrate levels can be used to predict lipid levels ($y_{lipids}= 0.94x_{carbo} + 66.52$, linear regression analysis, DF = 28, F= 12.9, *P*= 0.0013). After wasps had starved to death no significant correlation between lipids and carbohydrates could be detected anymore (r = 0.11, linear regression analysis, DF= 31, F= 0.14, *P*= 0.562).



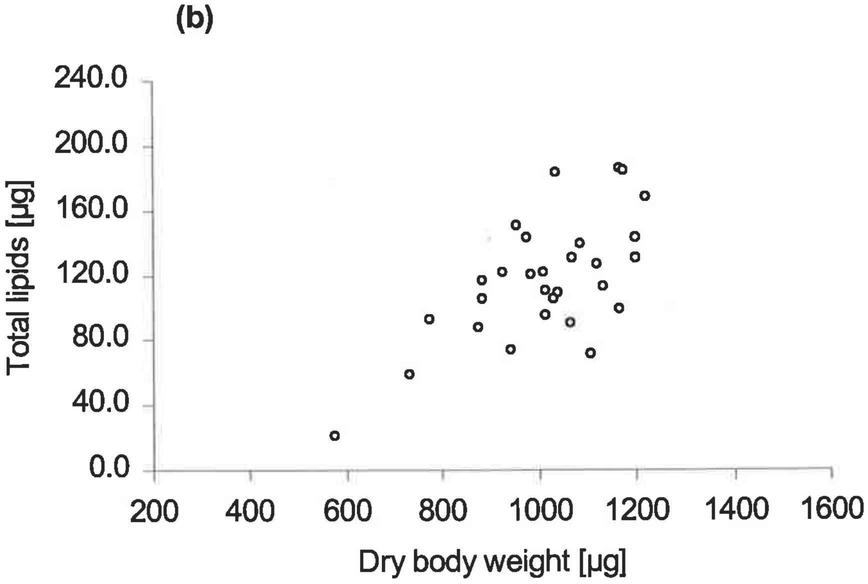


Figure 2.2 Relationship between dry body weight and **(a)** total carbohydrates and **(b)** total lipids in newly emerged female *C. rubecula.*

Pearson correlation coefficient for carbohydrate and lipid reserves are r= 0.47 (n= 36) and r= 0.66 (n= 29) respectively

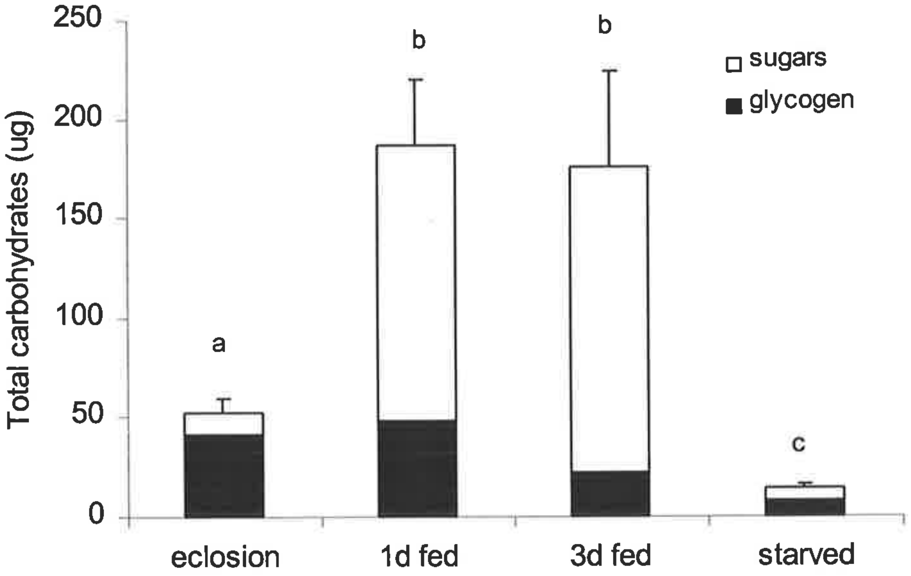


Figure 2.3 Total carbohydrates (mean ± 95% conf. int.) extracted from laboratory- reared female *C. rubecula* upon adult eclosion (n = 36) and death after starvation (n = 37). A third group was held with honey and analysed after 1 day (n = 16) or 3 day food exposure (n = 9).

Data log-transformed before ANOVA, DF = 97, F = 170.35, $P < 0.0001$; different letters above columns indicate significant difference after Tukey test $P \leq 0.05$.

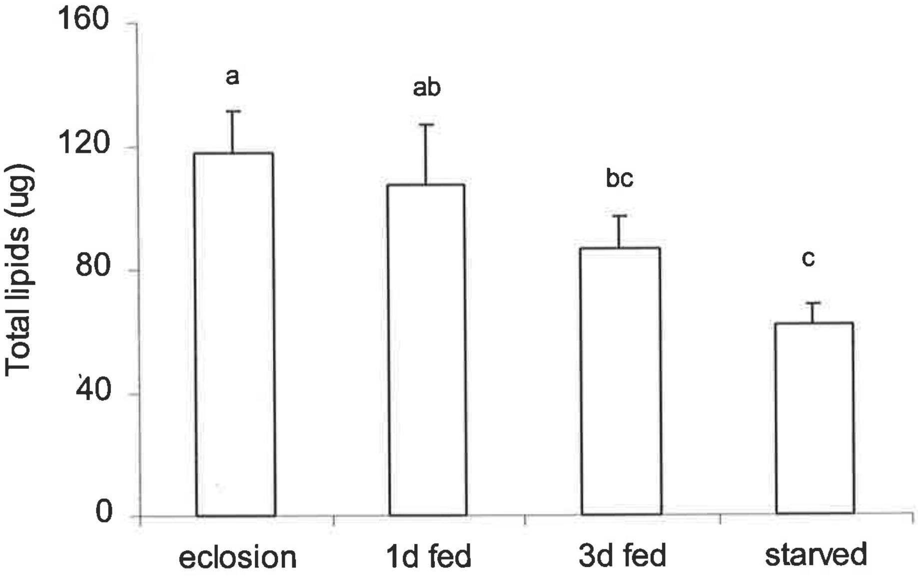


Figure 2.4 Total lipids (mean ± 95% conf. int.) extracted from laboratory- reared female *C. rubecula* females upon adult eclosion (n = 29) and death after starvation (n = 34). A third group was held with honey and analysed after 1 day (n = 16) or 3 day food exposure (n = 9).

ANOVA (DF = 87, F = 20.24, P < 0.0001); different letters above columns indicate significant difference after Tukey test $P \leq 0.05$.

Correlations between body size and energy reserves Dry weights of newly emerged females were moderately correlated with energy reserves (Fig. 2.2). Wing length was moderately correlated with lipid reserves and weakly correlated with carbohydrate reserves (Fig. 2.5 a,b). Variation within wing length was low as 90% of records were within 3.30 mm – 3.55 mm. The low variation in body size is probably a result of laboratory rearing where host size and host plant quality are uniform. As a result, body size parameters cannot be used to estimate energy reserves and energy expenditure.

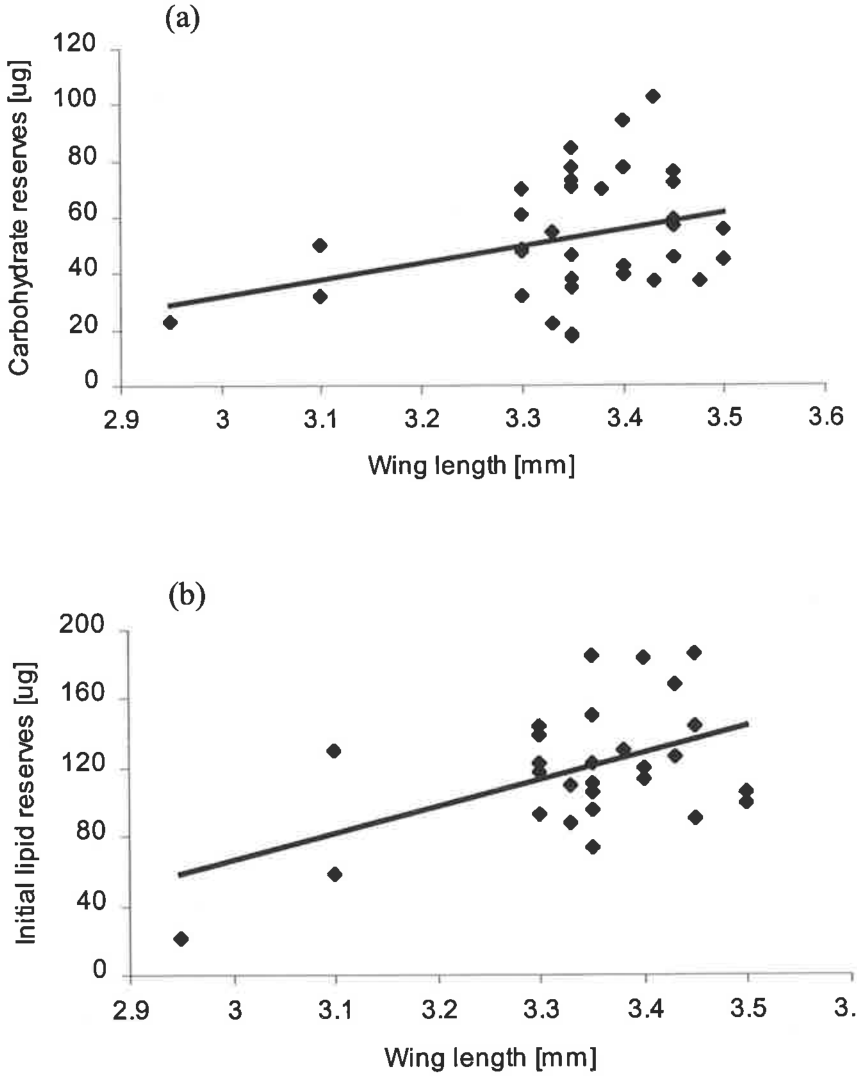


Figure 2.5 Correlation between wing length and **(a)** total carbohydrates or **(b)** lipids in newly emerged *C. rubecula* females reared in the laboratory.

Pearson correlation coefficients for carbohydrates are 0.31 (n = 36) and for lipids 0.50 (n = 29).

2.3.2 Energy costs of foraging

Wasps in the flight treatment depleted a higher amount of carbohydrate reserves than those in the oviposition treatment (Fig. 2.6) (ANOVA, df = 68, F = 5.93, *P* = 0.0043). During the flight treatment wasps flew distances between 27 m – 87 m (mean = 51.2 m) in the wind tunnel arena. Flight distance was not a good indicator of carbohydrate expenditure (Linear regression analysis, df = 15, F = 2.42, *P* = 0.1422). No significant difference in total carbohydrates could be detected between the control and the flight treatment, suggesting that wasps confined in a small container have a high rate of activity. It is estimated that female *C. rubecula* held for one day in a container without oviposition opportunities might deplete on average 9.3 μg (24 %) of their initial carbohydrate reserves and when forced to fly in a wind tunnel for one day as much as 18.4 μg (48.0 %) using the following approximation:

$$\text{mean}_{\text{carbohydrate expenditure}} = \text{mean}_{\text{initial total carbohydrates at eclosion}} - \text{mean}_{\text{total carbohydrates after treatment}}$$

Carbohydrate reserves were identified as the major fuel for flight, as no difference in lipid reserves was detected among the treatment groups (Fig. 2.7) (ANOVA df = 59, F = 0.5313, p = 0.59). The deposition of a relatively high number of eggs did not affect carbohydrate and lipid reserves, indicating that *C. rubecula* eggs are not rich in carbohydrates and lipids. Moreover, wasps with the opportunity to oviposit hardly spent any energy as their reserves were not significantly different from initial energy reserves at the time of emergence (Fig. 2.6 and Fig. 2.7). In the oviposition treatment, where females were stimulated to lay their maximum number of eggs per day, on average 19.9 eggs (9– 33 eggs) were laid. As hardly any energy was spent in ovipositing wasps no regression analysis between the number of eggs laid and energy spent was carried out.

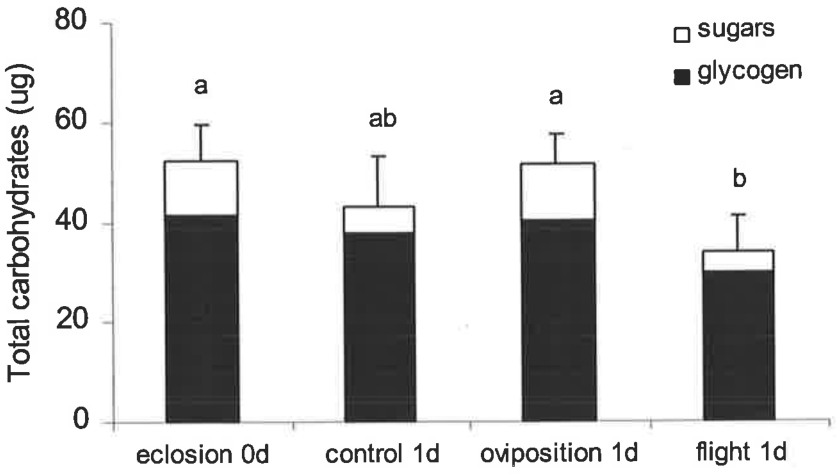


Figure 2.6 Total carbohydrates (mean ± 95 % conf. interval) extracted from female *C. rubecula* after one day of flying (n = 23), ovipositing (n = 22) or untreated (n = 24). For comparison total carbohydrates at eclosion are added.

ANOVA, DF = 104, F = 5.66, *P* = 0.003; Different letters above columns indicate statistically different means after Tukey –test, *P* < 0.05.

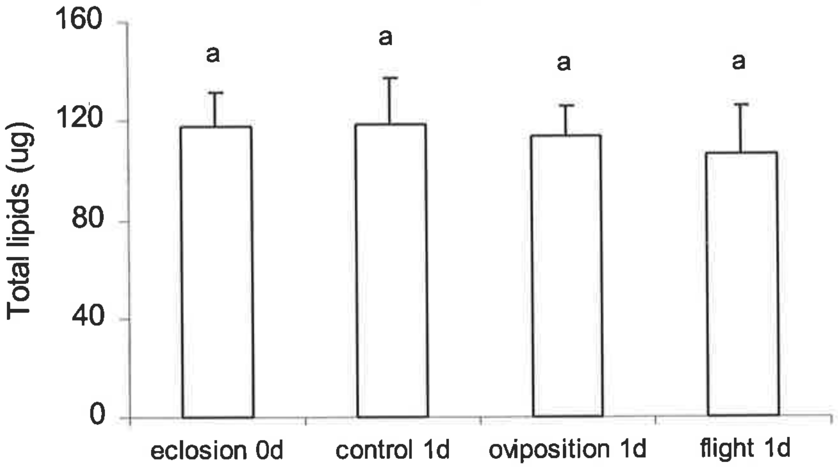


Figure 2.7 Total lipids (mean ± 95 % conf. interval) extracted from female *C. rubecula* during one day of flying (n = 19), ovipositing (n = 19) or untreated (n = 21). For comparison total lipids at eclosion are added.

No statistical difference between mean values detected (ANOVA, DF = 87, F= 0.45, P = 0.717)

2.4 Discussion

The quantification of energy reserves in parasitic wasps contributes to the understanding of their foraging behaviour and life-history traits such as lifespan and numbers of eggs laid. The aim of this study was the identification of energy reserves in an adult parasitoid and its energy expenditure during foraging. During the first day of life (which is already 1/3 of the lifespan of an unfed wasp) in female *C. rubecula*, lipid reserves declined slowly and independently of locomotive activity and numbers of eggs laid. A high rate of locomotion, particularly flying, is estimated to consume a large fraction of carbohydrate reserves.

2.4.1 Energy reserves in female *C. rubecula*

The energy reserves in adult stages of insects are a result of the feeding history of its larval form and often diminish quickly during the adult life (Foster 1995). Therefore, hungry parasitoids feed on sugar sources when given the opportunity and thus, are able to replenish some of their depleted reserves. When female *C. rubecula* in this study were kept with honey, their initial sugar levels (but not glycogen) increased by at least ten times, indicating a great sugar storage capacity. There was no evidence of a conversion of carbohydrates into lipids. A conversion of ingested sugars into glycogen was also not evident, suggesting that food is stored in the crop and/or gut system of the wasp. From there sugars may be directly released into the hemolymph as required. Only a small amount of sugars can diffuse into the insect's hemolymph as osmotic properties restrict a large influx of sugar molecules (Chippendale 1978).

Carbohydrate and lipid reserves seem to be concomitantly used but at a different rate. In female *C. rubecula,* carbohydrate reserves seem to be consumed more rapidly than lipid reserves. The high amount of lipids left in dead unfed parasitoids (53% of total lipids) indicates that apart from structural lipids, a fraction of lipid reserves might still remain intact. Although the egg complement was not removed before the analysis of energy reserves, it should make no or little difference to the lipid content. Firstly, eggs of pro-ovigenic parasitoids, such as *C. rubecula*, are usually poor in nutrients (Clausen 1940; Fletcher *et al.* 1994; Rivero and Casas 1999b). Secondly, a high oviposition rate in this experiment that depleted the wasps initial egg complement did not affect its lipid levels. The assumption of an incomplete lipid depletion is supported by studies that identified that greater usage of lipid reserves is dependent on a relatively long lifespan due to sugar feeding (Olson *et al.* 2000) or low temperatures (Nilssen 1997). This implies that the depletion of lipids depends on carbohydrate metabolism. When attempting to correlate energy reserves with the expected lifespan of wild parasitoids in the field, it is important to consider their temperature and feeding history.

2.4.2 On the correlation between body size, foraging activity and energy reserves

When measuring energy expenditure via quantitative biochemical extractions, the initial energy reserves of the animal have to be estimated via correlated body parameters. This assumes a quantitative relationship between energy reserves and body size. Unfortunately, in this study, body size parameters such as wing length and hind tibia length did not show a strong correlation with initial lipid and carbohydrate levels. This could be due to a low variation in body size and energy reserves. As the experimental wasps were reared in the laboratory and their size was not manipulated, the range of different body sizes was very small when initial energy reserves were estimated. Therefore, energy expenditure could not be calculated by using body size as an estimator for initial reserves. However, other studies using a wider range of wing lengths or hind tibia lengths in estimating initial energy reserves yielded a satisfactory correlation (Van Handel and Day 1988; Ellers 1996). Before embarking on quantitative measurements on energy expenditure, it is important to identify a body size parameter that correlates well with initial energy reserves.

2.4.3 Carbohydrates as flight fuel

The usage of carbohydrates as flight fuel in insect parasitoids emphasised the importance of sugar feeding. Wasps with feeding opportunities in their habitat have a chance to maintain or replenish carbohydrate reserves and as a consequence, sustain body maintenance and the ability to disperse over longer distances during their life than unfed wasps can. The importance of sugar intake to sustain locomotion highlights that sugar foraging itself may play a larger role in parasitoid foraging behaviour than previously assumed.

The storage of lipids has the advantage of delivering two times more energy than stored carbohydrates given the same quantity (Beenakkers 1969). However, carbohydrates as a flight fuel seem to be optimal for insects such as parasitic wasps that feed on sugar and fly for relatively short periods of time. In contrast, insects with migratory flight phases are known to switch from carbohydrate to lipid depletion or rely solely on lipids to fuel flight (Crabtree and Newsholme 1975). Given the nature of parasitoid foraging it is unlikely that long, migratory flights (such as in butterflies, aphids and locusts) occur but rather frequent and short flights within or between host occupied sites. Carbohydrates can be replenished directly through sugar intake and this provides almost immediate energy for flight if initial reserves are exhausted (Wheeler 1989). The carbohydrate storage in insects appears to provide sufficient energy for a maximum of about 30 to 45 min of continuous flight but food in the crop may substantially increase the duration of flight (see refs. in Wheeler 1989).

2.4.4 Energy reserves, dispersal and host encounters

A surprising discovery during the analysis of data of this study was the similar carbohydrate depletion of wasps held as a control and those that were flown extensively in the wind tunnel. Wasps caged in small containers remained active, perhaps due to stress, thereby expending a lot of energy. Estimated longevities of wasps held under these conditions could be too short as wasps might be more active under confined conditions than when freely foraging in the field.

The estimation of dispersal ability via carbohydrate reserves differs from Ellers and co-workers (Ellers 1996; Ellers and Van Alphen 1997; Ellers *et al.* 1998) which have identified lipid reserves as predictor of dispersal ability and habitat adaptation in a parasitoid species. Parasitoids that live in habitats with a high degree of host patchiness and low host density per patch have to travel a lot between patches. Therefore they have a higher amount of lipid reserves that is available for dispersal (and survival in general) than parasitoids in less patchy and "richer" host habitats (Ellers and Van Alphen 1997). These parasitoids have less lipid reserves available for dispersal because they allocated more energy resources to the production of a higher egg loads. However, the analysis in this study suggests that carbohydrates are better suited to analyse dispersal ability, survival and habitat adaptation in parasitoids than lipid reserves. As the initial size of lipid reserves is positively correlated with the size of glycogen and sugar reserves, the quantity of lipid reserves might be initially correlated with dispersal ability and lifespan as well.

This study suggests that depletion of carbohydrates and lipids happens at a different rate (carbohydrates are depleted faster), therefore lipids are not a good predictor for dispersal and lifespan. In this study female *C. rubecula* that were starved to death might have had still some lipid reserves left. This has important consequences as the allocation of lipid reserves is currently discussed as evidence for a trade-off between survival and reproduction (lipids invested into eggs are not available for survival and vice versa). The question arises how the mode of egg development in parasitoids (being syn- or pro-ovigenic) influences their energy expenditure on foraging as those lipid studies were conducted with a synovigenic species and this experiment with a pro-ovigenic species. Lipid reserves are likely to be larger in syn-ovigenic than in pro-ovigenic parasitoids because a large fraction is incorporated into eggs (Ellers and Van Alphen 1997; Rivero and Casas 1999b). However, the allocation of remaining lipid reserves to support survival seems to depend strongly on carbohydrate reserves and temperature as discussed earlier. The dynamics of lipid and carbohydrate reserves as well as energy requirements of egg development should be unambiguously identified before analysing the role of energy reserves in trade-offs between life history traits such as survival and reproduction.

Chapter 3 State- dependent responses to flowers and hosts

3.1 Introduction

Life histories of animals are shaped by compromises as time and energy is often allocated to one process at the expenses of other processes. A classical example is the trade-off between reproduction and survival (Reznik 1985). For example, under limited energy conditions nutrients invested into eggs are not available as survival reserves and *vice versa* (Van Noordwijk and de Jong 1986; Tatar and Carey 1995). A trade-off in regard to time allocation arises when animals search for nesting or egg sites and cannot forage for food in this time (Krivan and Sirot 1997).

Parasitic wasps are an ideal study system to study the trade-off between two competing foraging strategies in regard to reproduction and survival. Parasitic wasps lay eggs in or on a discrete host and often rely on sugary food to extend their longevity. A conflict between searching for oviposition or feeding sites arises when both resources are found on different plants and a female parasitoid has exhausted her energy reserves but still has eggs left to lay. If both, food sources and hosts are available, female wasps should alternately search for food and hosts to aim for maximum lifetime reproduction (Sirot and Bernstein 1996). The decision to search for food instead of hosts will depend on factors such as energy state, experience in food searching, egg load, distance to and abundance of food sources (Jervis *et al.* 1996; Lewis *et.al.* 1998). In this study energy state and food experience are investigated because they had been identified as major driving forces on the preference for hosts or food in parasitic wasps (Lewis and Takasu 1990; Wäckers 1994).

Nectar and honeydew appear to be the most commonly exploited sugar sources under field conditions (Jervis *et al* 1996). In regard to lifetime extension floral nectar seems to be of higher quality than honeydew (Elliott *et al.* 1987; Wäckers and Swaans 1993; Stapel *et al.* 1997) which can be frequently found on the same plant and consequently encountered by random search. In contrast, floral nectar is usually located on a different plant, which might require a directed search (Wäckers and Swaans, 1993). Field observation on flowers yielded a high number of hymenopteran parasitoid species indicating that parasitoids are attracted to them (Elliott *et al.* 1987; Jervis *et al.* 1993). To determine the magnitude of attraction of flowering plants to parasitoids in the field, it is important to know if female wasps would ignore the presence of hosts in favour of a nectar source on a different plant.

As females emerge with sufficient energy reserves they will initially concentrate on host-searching and with decreasing energy reserves will become more responsive to food cues. Parasitic wasps use olfaction to locate nectar sources (Leius 1967; Lewis and Takasu 1990; Wäckers and Lewis 1994; Patt *et al.* 1999) and to some degree visual cues (Wäckers 1994). There are only a few studies on how parasitic wasps locate potential nectar sources in the presence of hosts. Given the choice between odour cues from sugar sources or hosts, the majority of starved parasitic wasps were moving towards a flower (Wäckers 1994) or scented artificial sugar solutions (Lewis and Takasu 1990; Takasu and Lewis 1993; Takasu and Lewis 1996). These choice experiments were carried out in an olfactometer apparatus with flowers and hosts in walking distance of nectar-naïve wasps (Wäckers 1994) or in a wind tunnel arena within flying distance of food experienced wasps (Lewis and Takasu 1990; Takasu and Lewis 1993). The first case reveals insight into the wasp's preferences in a location that provides both, hosts and nectar at the same time. However, nectar and hosts are often found on different plants and require the wasp to travel between those locations.

The process of flying to a nectar plant makes decisions more complex because (1) there is a risk to loose track of the host site, (2) flying to the food source costs precious energy and (3) the energy reward of the food source might be very low or lacking at all. Takasu and Lewis (1993, 1996) have demonstrated in their wind tunnel studies that hungry, food experienced parasitic wasps still prefer food over hosts even if they have to fly to the resources. However, no research has been done on the response of nectar-naive and nectar-experienced wasps given a choice of a flowering plant and a plant with hosts in flying distance. This situation might not only resemble a more natural setting a wasp would encounter in the field but also reveals the choices of nectar-naïve wasps when resources are within flying distance. Parasitic wasps often forage in agricultural areas where hosts and food are separated by distance: hosts occur on crop plants while nectar producing plants might occur at some distance, perhaps only at the margins of crop fields.

This study aims to investigate the influence of energy state and nectar experience of a parasitic wasp on her choice to fly to a plant with hosts or a plant with flowers. To analyse the choices made, the sequence and duration of behavioural acts was recorded as well. For example, the extent to which a wasp would fly, walk or sit still can indicate if coordinated searching behaviour rather than random behaviour is employed. Also, flowers may be located in a different way than hosts which could be traced by a comparison of the frequency and duration of certain behavioural acts employed in foraging behaviour. Sugar-fed wasps were compared to starved wasps that were either nectar-naïve or nectar experienced. Preferences were counted as choices of wasps landing and remaining on the flower plant or host plant or somewhere else in the wind tunnel. The behaviour leading to a given location was also recorded in order to compare the response to hosts and nectar within and between females of different treatments.

3.2 Materials and method

Cabbage plants and insects were obtained using standard culturing practice (see Materials and method in Chapter 2). Pak choi plants (*Brassica rapa* var. *chiniensis*) were grown in 10cm pots in a glass house room fitted with a plant light (1000W, American metal multi-vapour halide lamp). As the initiation of flowering in pak choi plants can be accelerated by long day light-conditions, natural daylight was extended to 16 hours. Pak choi was chosen as a food plant in the experiment as it produces fragrant flowers and can support the longevity of *C. rubecula*-wasps up to 19 days (unpubl. data).

3.2.1 Experimental procedure

The maximum longevity of female *C. rubecula* without food, is only 2-3 days under the given temperature conditions and therefore, 1 day old females were chosen for the experiment. On the day prior to the experiment, all wasps were allowed to antennate feeding damage, frass and subsequently to sting one host. During this procedure a second instar *P. rapae*-larva was transferred from its feeding site onto an up side down plastic cup together with a piece of a cabbage leaf with feeding damage and frass. A female was removed from a cage with an aspirator and allowed to climb onto the cup. After the host was stung, the wasp was returned to her container and considered as host-experienced.

To obtain fed females, 1/3 of the wasps were held in containers that contained a 10ml plastic container with four dots of honey (Kangaroo Island "Canola"). Starved wasps were held in containers that contained only water and a piece of paper towel. In order to obtain nectar-experienced wasps, individuals were removed from their container, allowed to climb onto a pak choi flower and feed for 10s on its nectar. These wasps experienced for an additional 20s the scent of the flowers by holding the wasp close to the flowers without allowing her to climb onto the flower. Starved-naïve wasps experienced no feeding or flower scent. Also, one day prior to the experiment cabbage plants with 4-6 mature leaves were infested with three 2nd-instar *P. rapae*-larvae on three different leaves each. This was to have feeding damage and frass present in the experiment.

The observations took place in an open wind tunnel arena. The experimental arena consisted of a rectangular 60 cm wide and 140 cm long wooden panel covered with coarse sand, a fan and circular tunnel construction. Two vertical wooden frames covered with Terylene voil were fitted to the ends of the arena. An ordinary fan with a diameter of 40 cm situated in one wooden frame, generated circular air movement by blowing air into a polyethylene tunnel under the arena that connected the bottom end with the top end of the experimental area. Air was passing through a "honey comb" resembling cardboard, fitted into the other wooden frame, to generate a laminar airflow in the experimental area. Average wind speed was 22.4 cm/s and temperature was 25^{o} C (±1.5 C). Lights consisted of 4 "cool white" fluorescent light tubes (52W, Phillips*)* fixed 1 m above the floor of the arena producing a light intensity of 8000 Lux.

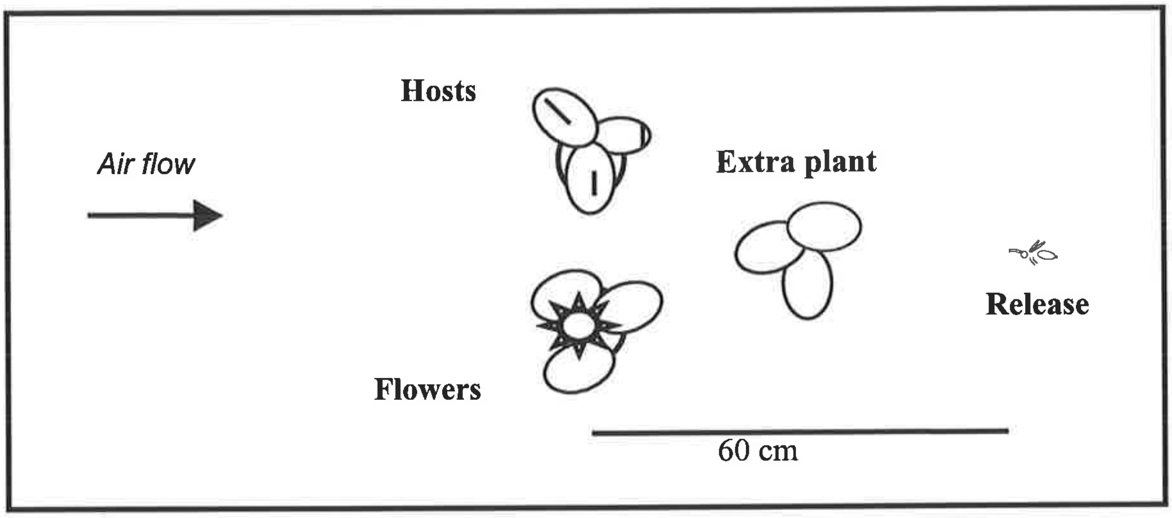


Figure 3.1 Positioning of plants and release of individual wasps in the wind tunnel arena.

Thirty minutes prior to the experiment the containers with wasps were placed into the experimental arena to allow the wasps to adapt to the environmental conditions. Two potted cabbage plants, one plant with hosts prepared one day earlier (=host plant) and another plant carrying a stem of pak choi flowers (=food plant) were positioned side by side 10 cm apart and within 60 cm of the release position of the wasp (Fig. 3.1). As flowering pak choi plants grow 2-3 times higher than cabbage plants, a stem with flowers was cut and assembled with a cabbage plant in a manner that flowers were at the same height as the feeding damage on the host plant. A pak choi flower stem with approx. 10-15 single opened flowers was cut, put into a glass vial containing water, sealed with a cotton ball and inserted into the soil of a potted cabbage plant. To control for volatiles emanating from the cut stem, a pak choi leaf was prepared in the same manner as the flower stem and added to the cabbage plant with hosts. To reduce the possibility of wasps landing randomly on one of the plants a third cabbage plant was placed in between the test plants and the position of release. The extra plant was slightly shorter than the test plants so that it would not block the odour plume from hosts and flowers. All replications (35 per treatment) had a balanced number of both resource plants being either on the left or right side in the arena. To focus the attention of the wasp on the experimental set up, the lip of a release vial was slightly rubbed against a cabbage leaf before a wasp was put in there to be released. Additionally before a release, the wasp was taken up by an aspirator, held close to feeding damage and flowers for 5 seconds each and then transferred to the release vial. This glass vial (2cm in diameter and 10 cm in length) was held to a metal stand at height of hosts and flowers. The experiment started when the wasp moved to the lip of the vial and her antennae appeared outside the vial.

Behaviours and locations of the wasp were recorded with a hand held computer equipped with the software "The Observer" (Noldus Technology, 3.1). The preferred location was defined as the plant or location where the observed wasp settled either directly after take-off from the release vial or after a brief "stop-over" on another location and exhibited searching behaviour that either led to feeding or stinging a host; if neither of these events occurred the observation was terminated after 10 min and the preferred location recorded as the location where she spent most of the observational time. Locations were recorded as a) cabbage plant next to release vial (=extra plant), b) cabbage plant with feeding damage and hosts (=host plant), c) cabbage plant with flower stem (=food plant) and d) the rest of the arena (=other). Behaviours recorded with "The Observer" Software are described in Table 3.1.

Table 3.1 Behaviour catalogue used for *C. rubecula* (M. Keller, unpubl. data) responding to nectar and hosts in a wind tunnel arena

Behaviour	Description
Flying	Any airborne activity
Walking	Wasp moves forward usually alternately touching the antennae to the surface
Grooming	Any action which appeared to involve cleaning of the body
Pointing	Stationary behaviour of wasp usually facing into the wind, the antennae raised and moving
Standing still[1]	Stationary behaviour with no movement of any body parts, usually longer than 30 s
Feeding	Wasp consumed liquid or solid substances by moving mouthparts over the surface either during walking or standing, antennae slightly curled inwards
Attacking	Wasp contacts host with her antennae, curled her abdomen under the body and ovipositor pierces cuticle of host

1Distinction between 'pointing' and 'standing still' obtained by plotting log survivor values of durations. Visual scan of turning point in curve indicates separation in time, here 30s (convex shape indicated mixture of two exponentials, test for exponentiality, (Haccou and Meelis 1995).

3.2.2 Data analysis

Logistic regression was used to analyse the choices of location between different treatment groups (SAS Institute 1995). Two independent binomial tests within each treatment group were carried out for, a) the choice between resource plants and other locations, and b) the choice between food and host plant.

Latency of flight and the latency of arrival on a plant with resources were analysed using univariate survival analysis (SAS Institute 1995). The majority of wasps displayed a latency of flight only a few seconds long, therefore Wilcoxon Test Statistic was used in favour of the Log-rank Test as it stresses early differences rather than late differences between flight latencies. In respect to the latency of arrival on a resource plant, differences became apparent over longer time periods, therefore the Log-rank Test was selected.

The frequencies of transition from one type of behaviour to another were constructed as matrices and tested against expected frequency (as in Field and Keller 1993). Due to low occurrences of the first, the behaviours 'standing still' and 'pointing' were pooled and called "stationary" so that no expected values were less than 1 and no more than 20% of the expected values less than 5. The expected values of the matrix cells were found using the iterative proportional fitting method of Goodman (1968). The deviations in the overall table were found to be statistically different. So significant transitions were analysed by reducing the table into a 2 x 2 matrix around each transition and performing a *G* test. The significance of these individual tests was adjusted to a table-wide level of 5%, using the sequential Bonferroni method (Rice 1989). The results of analysis of transition matrices are presented graphically in flow diagrams. Areas of circles in the diagrams are proportional to the overall frequency of each behaviour and the width of arrows are proportional to the standardised residual of significant positive deviations from expected values. Dotted arrows present marginal significance as original *P*-values for transition were $P \leq 0.05$ before Bonferroni adjustment but not after. Durations of behaviours were expressed in proportion of time until settling on the preferred location. These data had been arcsine-transformed before an Analysis of Variance was carried out (Sokal and Rohlf 1981).

3.3 Results

3.3.1 Choice of location

The internal state of female *C. rubecula* lead to different responses to hosts and flowers (logistic regression, DF = 4, L-R $\chi^2 = 26.95$, $P < 0.0001$). The majority of fed wasps settled on the host plant whereas a higher proportion of starved wasps flew to the flowers (Fig.3.2a). A preference for either the flowers or hosts could not be detected. A comparison between starved and starved-experienced wasps revealed that nectar-experience increased the response to both resource plants (logistic regression, DF = 2, L-R $\chi^2 = 8.81$, $P = 0.012$). The majority of starved-experienced wasps flew to the resource plants settled on the host plant and food plant with nearly equal proportions. In contrast, nearly half of the number of starved-naive wasps remained in other areas of the wind tunnel such as on the release vial, the floor of the arena or on the extra plant (Fig.3.2a). As a consequence of the internal state and chosen location less wasps from the starvation treatments attacked hosts than fed wasps, but rather exhibited feeding behaviour within 10 min of observation (logistic regression, DF = 4, L-R $\chi^2 = 37.59$, $P < 0.001$, Fig. 3.2b). Interestingly, hungry wasps had been found feeding not only on the nectar of the pak choi flowers, but also scraping their mouthparts over areas apparently free of sugars such as cabbage leaves, the floor of the wind tunnel arena and the stand of the release vial.

3.3.2 Effect of starvation and nectar experience on searching behaviour

Initially, the location of resources was conducted as a function of internal state and chosen location (food plant or host plant). As no significant differences could be found in regard to location, data were pooled to compare the behaviour as a function of the wasp's state only. Generally, fed and starved-experienced wasps shared most of the behavioural pattern shown in locating the resource plants. Starved-naive wasps behaved differently in comparison to starved-experienced and fed wasps, as they showed a longer latency of flight (univariate survival analysis, Wilcoxon test, DF = 2 , χ^2 =15.31 , *P = 0.0005,* Fig. 3.3) and needed more time to arrive on a plant with resources (univariate survival analysis, Wilcoxon test, DF = 2 , χ^2 =5.057 , *P= 0.079*, but see results of pairwise comparison in Fig. 3.4). This indicates an increasingly delayed response to cues from flowers or hosts in starved-naïve wasps, but not in starved-experienced wasps. Starved-naïve wasps spent also less time flying in comparison to fed and starved-experienced wasps (Fig. 3.5), indicating a behaviour reducing energy expenditure. The most coordinated behaviour in response to plants with resources was exhibited by starved-experienced wasps, followed closely by fed wasps (Fig. 3.6). Starved-naïve wasps exhibited increasingly random transitions between behaviours, most apparent was the random transition of flight to any of the other behaviours, which in fed and starved-experienced wasps is generally followed by walking.

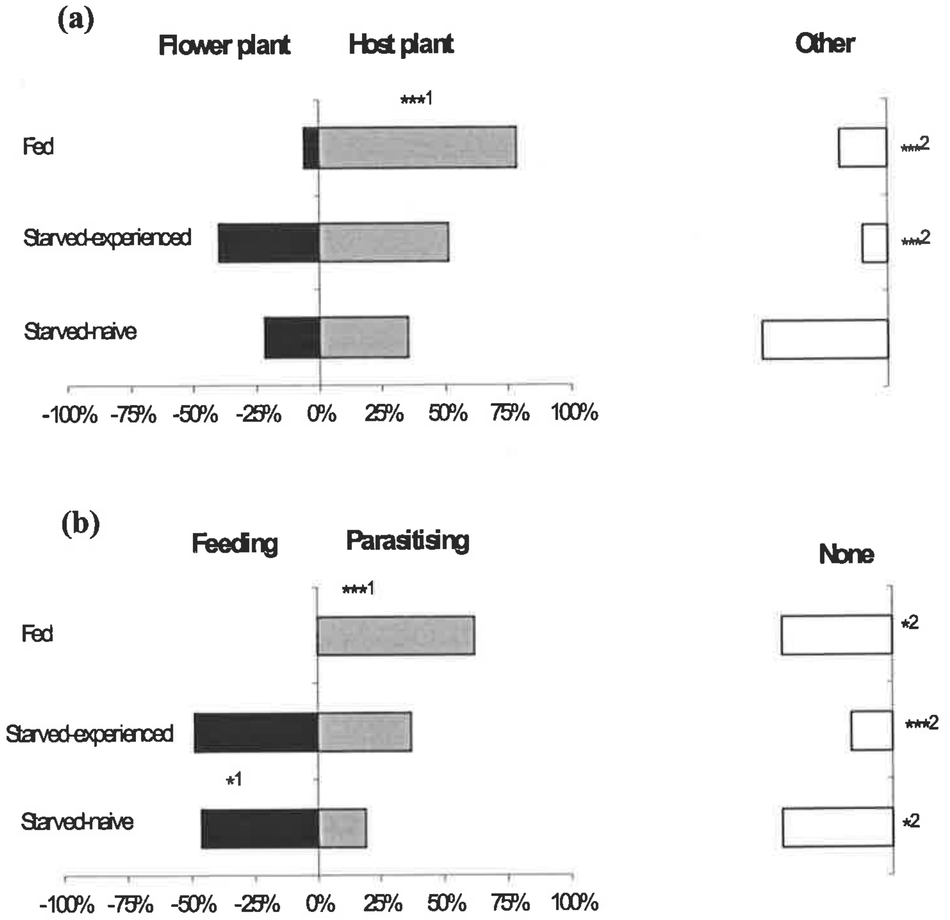


Figure 3.2 Response of 1 day-old female *C. rubecula* to a cabbage plant with larval *P. rapae* and a cabbage plant assembled with a stem of pak choi flowers in a wind tunnel arena. Wasps had either unlimited access to honey in the previous 24 hours (fed, n=37), were allowed to taste pak choi nectar on the previous day (starved-experienced, n=35) or were left unfed (starved-naïve, n=37). **(a)** Locations where wasps settled (see Materials and method for definition) and exhibited searching behaviour within 10 min observation and **(b)** number of wasps observed feeding or stinging a host.

[1] Differences in two proportions of wasps within a treatment group **(a)** choosing either hosts or flowers or **(b)** displaying either feeding or parasitising were tested with a second Binomial Test. Asterisks above bars indicate that significantly more wasps **(a)** responded to a particular location or **(b)** displayed more feeding/parasitization events.

[2] Differences between two proportions of wasps within a treatment group choosing **(a)** plants with resources (hosts or nectar) compared to another location in the wind tunnel (other) or **(b)** displaying feeding/parasitising compared to none of them, were tested with a binomial test; asterisks in figure under title **(a)** "other" and **(b)** "none" indicate that significantly less wasps chose an area somewhere else in the wind tunnel or displayed no feeding nor parasitising, respectively.

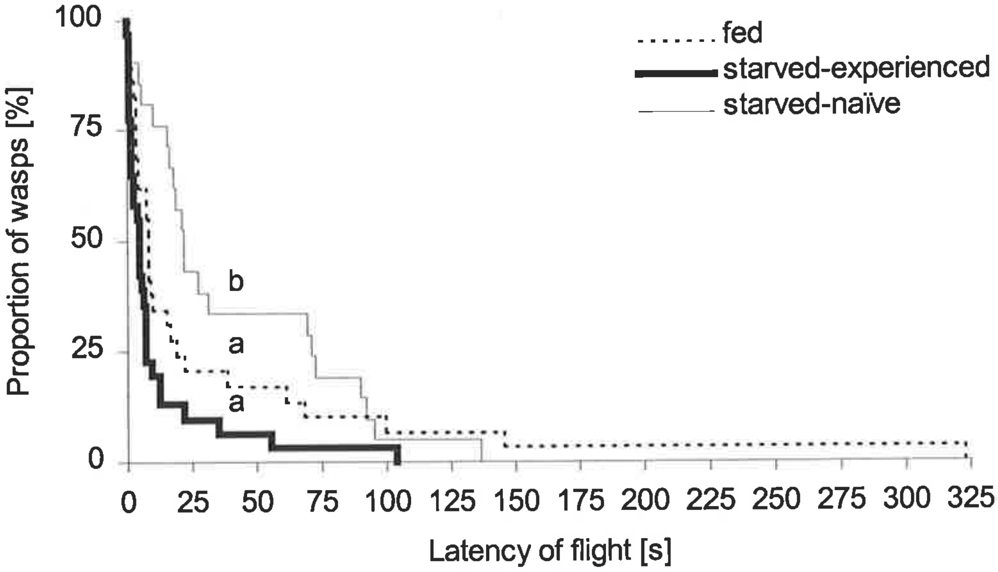


Figure 3.3 Latency of flight of one day-old female *C. rubecula* settling on either a plant with hosts or a plant with flowers in a wind tunnel arena. Wasps had either unlimited access to honey (fed, n = 31), were allowed to taste nectar (starved-experienced, n = 32) or were unfed (starved-naïve, n =21).

Different letters above curves indicate statistical difference after univariate survival analysis using Wilcoxon Test $P \leq 0.05$. Results after pairwise comparisons were adjusted using sequential Bonferroni method .

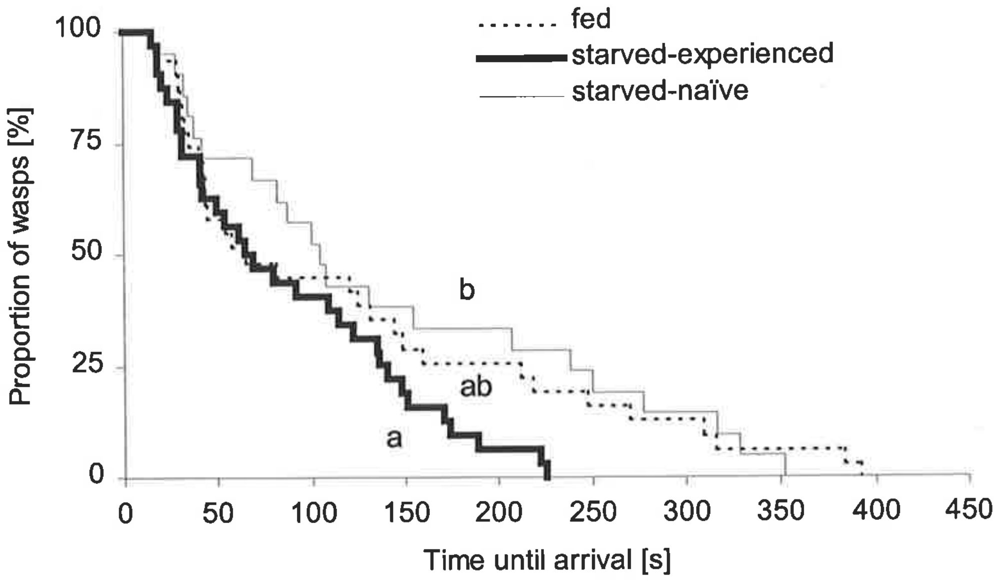


Figure 3.4 Time until arrival of one day-old female *C. rubecula* settling on either a plant with hosts or a plant with flowers in a wind tunnel arena. Wasps had either unlimited access to honey (fed,, n = 31), were allowed to taste nectar (starved-experienced, n = 32) or were unfed (starved-naïve, n =21).

Different letters above curves indicate statistical difference after univariate survival analysis using Log-rank Test, $P \leq 0.05$. Results after pairwise comparisons were adjusted using sequential Bonferroni method .

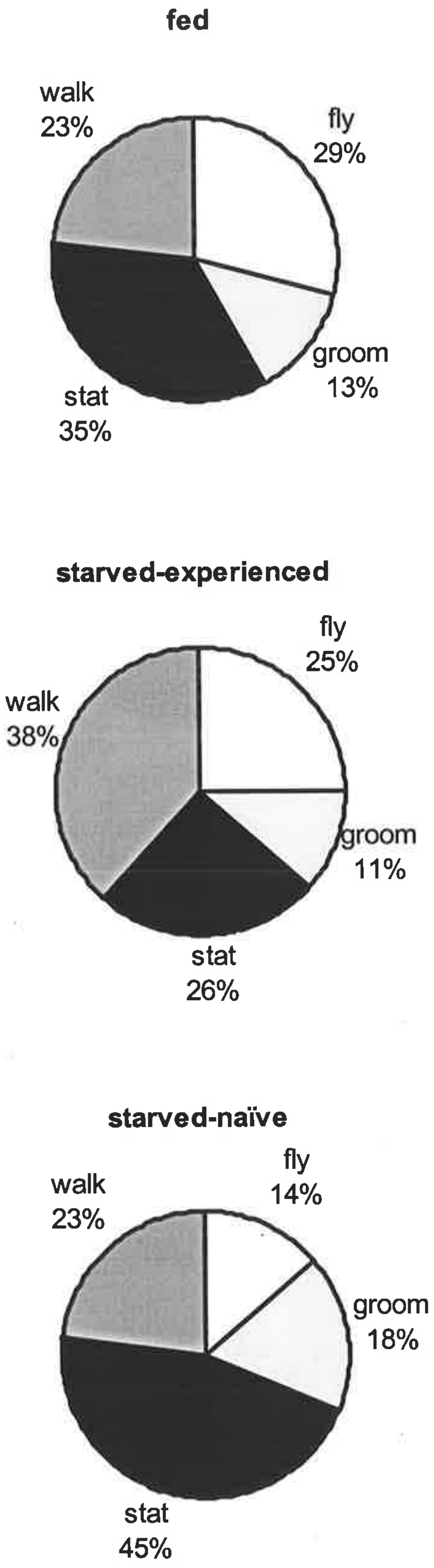


Figure 3.5 Mean proportional time spent on different behaviours in female *C. rubecula* while locating a plant with hosts or a plant with flowers. Wasps had either unlimited access to honey in the previous 24 hrs (fed, n= 31), were allowed to taste nectar (starved-experienced, n= 32) or were left unfed (starved, n= 21).

Summary statistics: Analysis of Variance on the proportions of time spent on behaviours (data underwent arcsine transformation)

Behaviour	F-test	*P*
Flying	7.131	0.0014
Grooming	0.949	0.3912
Walking	8.023	0.0007
Stationary	4.371	0.0158

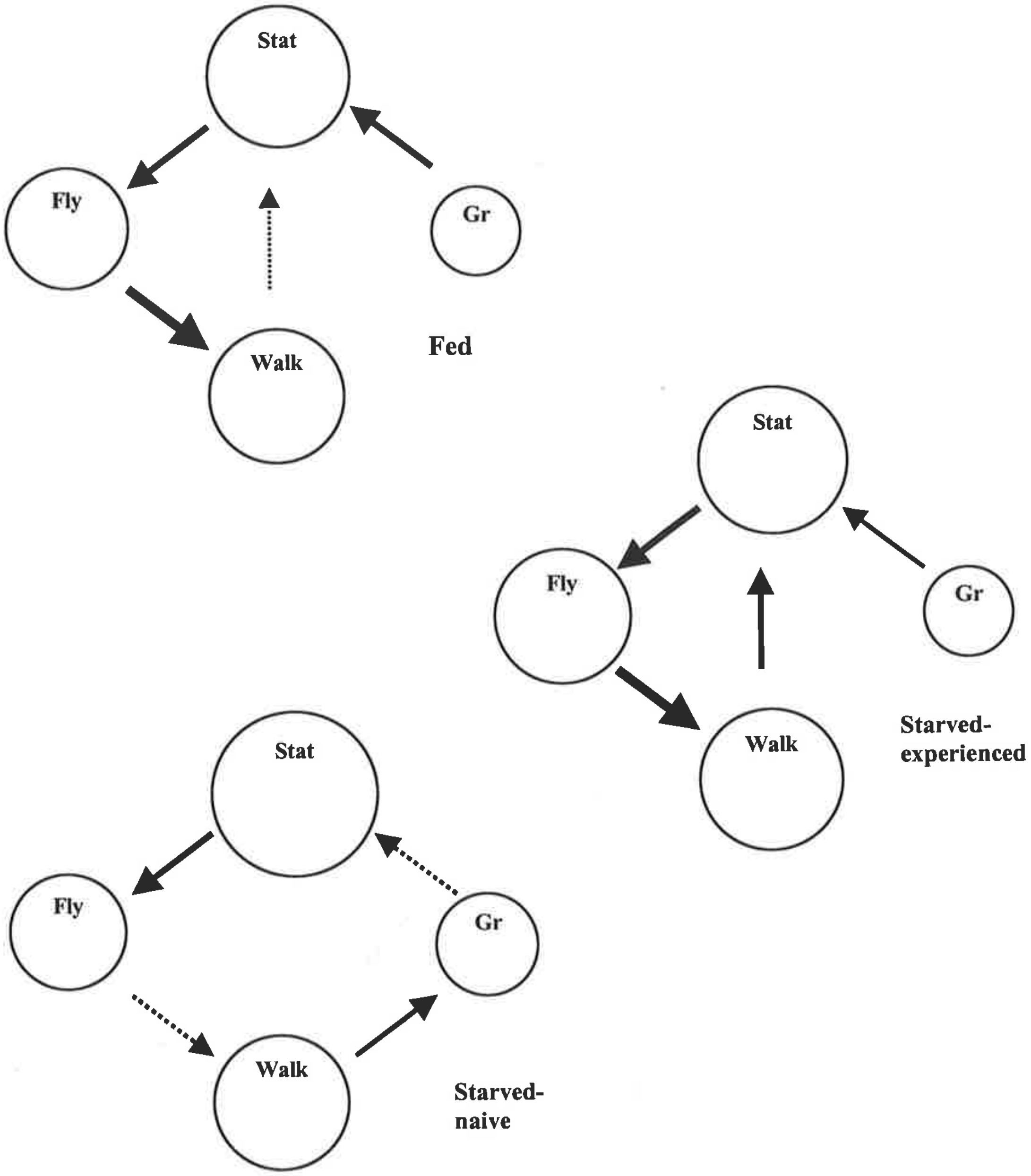


Figure 3.6 Flow diagram showing the transition of behaviour in one-day old *C. rubecula*-females while locating a plant with hosts or a plant with nectar. Wasps had either unlimited access to honey (sugar-satiated, n = 31), were allowed to taste nectar (starved-experienced, n = 32) or were unfed (starved-naïve, n =21).

For definition of area of circles and width of arrows see materials and method section. Behaviours abbreviated as stat = stationary, fly = flying, walk = walking, gr = grooming.

3.4 Discussion

Starved female *C. rubecula* wasps were tested in their ability to respond to a nectar providing plant within flying distance. At the same time hosts were available on a neighbouring plant to detect a preference for hosts or sugary food. In the remainder of this chapter, the major findings and their significance are discussed in respect to responding to flowers, perceiving odours and nectar foraging in the field.

3.4.1 Response to flowers

Unfed one-day old *C. rubecula*-females were already affected by starvation and in obvious need for food as they behaved differently than fed wasps. Only 24% of starved, nectar-naïve wasps reached the plant with flowers, whereas in Wäcker's (1994) olfactometer experiment over 60% of starved wasps preferred flowers. The responsiveness of starved-naïve *C. rubecula* females to airborne cues leading to flowers or hosts within 60 cm distance was clearly reduced. Although showing less flight activity than nectar-experienced and sugar-satiated wasps, unfed wasps were still active enough to move around in the wind tunnel arena. A full egg load, a short life expectancy and the apparent unawareness of a rich food source nearby, did not result in an increased response to hosts either. This supports the hypothesis that starvation impacts in general on the perception of distant resources.

In comparison to naïve wasps, experienced *C. rubecula*-wasps were more likely to locate both flowers and hosts. Other wind tunnel studies demonstrated a majority of hungry, food experienced *Microplitis croceiceps*-wasps flying to a food source in the presence of hosts, with food experience taking place 20 min before the observations (Lewis and Takasu 1990, Takasu and Lewis 1993). In this study nectar experience took place on the previous day approx. 19 hours before the observation. In this time the memory of food lost its strength but was still strong enough to keep *C. rubecula* females alert to their environment. Takasu and Lewis (1996) reported in another wind tunnel experiment that the memory of a scented sugar source lasted up to 6 hours when presented on the same day but decreased substantially when presented 24 and 48 hours later.

As nectar-experienced female *C. rubecula* were as active and coordinated as fed wasps, the question arises if sampling the food source on the previous day substantially increased their nutritional state. Wasps were allowed to feed for only 10 secs on pak choi nectar. This is less than 10 % of the time she needs to ingest a full nectar meal on a honey source with similar sugar concentration (Siekmann *et al.* 2001). The time to get experience is in the same order of magnitude than published elsewhere (Lewis and Takasu 1990; Takasu and Lewis 1993; Takasu and Lewis 1996; Patt *et al.* 1999). Although the exposure to food was kept to a minimum, it is possible that the combination of experience and a small ingestion of sugar resulted in a different behaviour rather than experience alone. However, nectar experienced and nectar-naïve *C. rubecula* wasps showed the same number of feeding events, indicating somewhat the same degree of hunger which would support the assumption of an equal nutritional state in both wasp groups.

3.4.2 Odour perception and decision-making

Sugar deprivation not only affects the mobility of insects but also the functioning of the neural network important for information processing (Chippendale 1978). Insects are able to change their behavioural responses to external stimuli due to complex processes in the brain and due to variation in the sensitivity of their chemoreceptors (Blaney *et al.* 1986). Internal factors such as experience, age or starvation alter the magnitude of odour perception that can be traced by electro-antennograms (Den Otter and Saini 1985; White 1989; Vet *et al.* 1990; Den Otter *et al.* 1991). Initial stages of starvation have been found to increase and late stages of starvation to decrease the sensitivity to food associated odours in beetles and tsetse flies (White 1989; Den Otter *et al.* 1991). Results in this study are consistent with these findings: Advanced starvation and nectar experience seem to alter odour sensitivity in *C. rubecula* wasps in different ways. One day old starved females are already in an advanced state of starvation and exhibited a reduced sensitivity to odours in general. In contrast, nectar experience seems to increase the sensitivity to odours from flowers. As the wasps in this experiment were in an environment with an odour mixture, presumably dominated by the presence of hosts and flowers, "conflicting" stimuli arriving at the olfactory system were processed and resulted in a choice. Research in the processing of odour mixtures in honey bees revealed inhibitory effects as single odorant responses are combined (Smith and Cobey 1994; Joerges *et al.* 1996). In this experiment the inhibitory effects of host and flower odours mixture may have had an effect on the responsive behaviour of female *C. rubecula*.

Finally, the behavioural response to an odour mixture might not only depend on the information processing capabilities of the insect, but also on the relative proportions of odour concentrations. The ratio of flower odour concentration to host odour concentration could favour a response to either of them. In this experiment starved-nectar experienced wasps did not exhibit a preference for any of the resource plants. This could point to a balanced odour concentration from both resources nectar and hosts. However a change in host or flower density might yield a different outcome. Early studies in honey bee odour perception report that 'as the proportion of a salient odorant component increases in a blend, the blend is perceived as being increasingly similar to this component' (Getz and Smith 1987). However, recent findings propose that the presence or absence of cues rather than the relative concentrations of cues seem to have the greatest importance in honey bee odour perception (Breed and Julian 1992). In regard to this hypothesis, a change of resource densities in the present experiment would not alter the outcome of the wasp's choices. The importance of qualitative versus quantitative cues in parasitoid choice studies needs to be investigated as it clarifies to which extent choices are made dependent on external stimuli rather than on the internal state.

How insects, stimulated by a given set of cues, settle on certain behavioural options rather than others (i.e., making decisions) could be explained by a dynamic and interactive model by (Miller and Strickler 1984). They were using a mechanical analogue of Dethier's (1982) model on the influence of external and internal factors on insect investment behaviour. Projecting their model on the situation of a simultaneous perception of flowers and hosts, the decision process in a parasitic wasp could happen in the following way: sensory receptors transmit the neural correlates of nectar and host odours to the central nervous system. Host odours might initially trigger a stronger potential than nectar odours as the primary aim in the life of female parasitoids is to find hosts and lay eggs. These external inputs are modulated by internal factors such as egg load and sugar deprivation that push the processing of the incoming information into different directions. The long-range sensory apparatus of starved, nectar-naïve *C. rubecula* –females is less able to detect odours, presumably due to sugar deprivation, and so random search for food is displayed. If food and host plants are in equal distance to the position of the searching wasps both locations are evenly visited.

The case in nectar-experienced wasps is different as their long range sensory apparatus is still able to process external nectar and host stimuli. Nectar odour, sugar deprivation and nectar experience are driving the process of decision making towards food search. At the same time host odour, egg load and host experience drive the process into the opposite direction towards host search. Both host and nectar stimuli seem to display the same weight and the probability of nectar search or host search in starved, nectar-experienced wasps in this scenario is therefore 50%. However, slight variations in the perception of odours or in the degree of sugar deprivation might tip the balance in favour of either food or host search.

3.4.3 Nectar-foraging of parasitic wasps in the field

The findings of this wind tunnel study suggest that nectar foraging in the field might be only successful if nectar plants occur in close proximity to plants with hosts. As long as energy reserves permit a coordinated searching behaviour host foraging may always have a higher priority than food foraging and any nectar plants are encountered opportunistically during host searching. However, after nectar experience, wasps might abandon host searching in favour of food searching. Advanced starvation seems to be linked to a decreased perception of long range odours and decreased ability of coordinated searching behaviour. With a short life span of 2- 3 days under favourable temperature conditions and a even shorter time available for coordinated searching behaviour, female *C. rubecula* seem to have little time available for host foraging until starvation sets in and limits them to random searches of their immediate environment. If a nectar plant is encountered an innate response to nectar odour will lead them to search the flower until nectar is found. Nectar-experienced wasps have a higher chance to encounter a nectar plant nearby because they are able to respond to a nectar cues over a larger distance. But at the same time the perception of host odours is not switched off but rather "competes" with nectar stimuli, attracting a large proportion of wasps to patches with hosts.

How parasitic wasps discover flowers in the field has practical implication for biocontrol-conservation projects in agricultural areas. Flowers are supposed to provide food for parasitic wasps to live longer and parasitise more hosts. The findings of this experiment suggest that parasitoids cannot be expected to search actively for flowers over long distances. The success of the propagation of flowers might critically depend on their distance to host occupied sites.

Field studies reporting effects of flowering plants on pest reduction are not conclusive with several studies reporting a positive relationship (Powell 1986; Jervis *et al.* 1993; Baggen and Gurr 1998) and others studies reporting no effect (Kloen and Altieri 1990b; Cowgill 1995; Bigger and Chaney 1998; Cappuccino *et al.* 1999; Nicholls *et al.* 2000). As several species of flowering plants beneficial for parasitoid survival have been already identified (Leius 1961a; Van Emden 1963; Maingay *et al.* 1991; Jervis *et al.* 1993; Idris and Grafius 1995; Patt *et al.* 1997), the next crucial step in understanding nectar foraging in the field would be researching distances (Topham and Beardsley 1975) and concentration of odours to which parasitic wasps respond to nectar plants.

This study suggests that nectar plants have to occur in close proximity to plants with hosts so that starved wasps that switch to random search starting from a plant with hosts have chance to encounter them. Subsequent nectar experience allows wasps to detect nectar plants at a longer distance but will result in a divided response if areas with hosts are detected as well.

Chapter 4 Suitability of sugar sources in relation to adult parasitoid survival

4.1 Introduction

Adult parasitoids feed on sugar sources to avoid starvation (Wolcott 1942; Zoebelein 1956; Leius 1961a). In the field, sugar sources vary widely in quality and quantity. Sugar can be obtained from floral and extra-floral nectar, honeydew, phloem sap, trichome exudates and even from some nectar producing fungi (DeBach and Rosen 1991; reviewed by Jervis *et al.* 1992). However, sugary foods available for parasitoids need to occur within the habitat where they search for hosts. Nectar and honeydew appear to be the most commonly exploited materials under field conditions (Jervis *et al.* 1996). When hosts are not associated with sugar sources (producing honeydew, feeding on flowering plants etc.), sugar encounters might be uncommon in the life of a parasitoid. This is often the case in agricultural areas where parasitoids forage for herbivorous hosts that feed on crops. In an attempt to increase parasitoid efficiency as biocontrol agents against insect pests, the propagation of food sources such as flowers in crop fields has been suggested (Rabb *et al.* 1976; Altieri and Whitcomb 1979; Powell 1986).

Ideal food sources for parasitoids have to be easily accessible, supply abundant sugar and occur in close proximity to host occupied sites. Flower species suitable for parasitoid feeding should have exposed or partly exposed nectaries, because the majority of parasitoids are small and do not have specialised mouthparts (Patt *et al.* 1997; Jervis 1998). Aphid honeydew is easily accessible and can be naturally abundant on leaves in the vicinity of parasitoid hosts. However, honeydew is often of lower nutritional quality than floral nectar (Leius 1961b;

Wäckers and Swaans 1993; Wäckers 1999). This study aimed to test sugar sources for parasitoids foraging in commercial crop cultures.

The following food sources were selected because they occur or could be integrated into commercial Brassica-cultures (i.e. cabbage, broccoli, Brussels sprouts).

- Pak choi was chosen as it is a commercial Brassica-crop that provides floral nectar
- Sweet Alyssum is another cruciferous plant that flowers abundantly and has been used in previous field trials to support natural enemies in cabbage cultures (Johanowicz and Mitchell 2000)
- White clover has been valued as suitable cover crop in Brassica systems (Andow *et al.* 1986; Dover 1986; Vandermeer 1989; Müller-Schärer *et al.* 1992) but has not been tested for its ability to provide nectar for parasitoids.
- Umbelliferous flowers such as dill provide accessible nectaries, are frequently visited by parasitoids, and therefore recommended for propagation in crop fields (Van Emden 1963; Patt *et al.* 1997).
- Green peach aphids occur on cruciferous plants and could provide honeydew in the vicinity of hosts of parasitoids.

An important question in parasitoid food foraging may be not how long a food source can extend survival but simply if it does. Successful food encounters in the field depend on the distribution of food sources, the wasp's ability to find and access them, seasonal flowering time and food quality. In this chapter a screening method is suggested to test the ability of sugar sources to support the longevity of parasitoids in the field. A sugar source was considered as 'suitable' when wasps caged with it simply lived longer than without food. This method also avoids problems that often occur when using a variety of natural food sources and moving them from their growing location (usually glass house or field) into the laboratory. Some flowering plants might adapt poorly to the change in light and temperature conditions than others and might cease or alter nectar production (Mohr and Jay 1990; Jakobsen and Kristjansson 1994; Boose 1997). A long-term comparison of wasps surviving on different flower-species might be therefore affected by the plant's physiological response to indoor conditions.

4.2 Materials and method

C. rubecula wasps were reared under standard conditions as described in Chapter 2.

To test the effects of naturally occurring sugar sources on longevity, female *C. rubecula* were caged with a range of flowering plants and honeydew. For comparison, the survival of wasps caged with water only, a cabbage plant and 50% honey was also assessed.

4.2.1 Cultivation of food sources

The following food sources were selected to test their ability to provide food for *C. rubecula* wasps: honeydew of the green peach aphid (*Myzus persicae*) on cabbage leaves, flowers of pak-choi (*Brassica rapa* var. chiniensis), alyssum (*Allyssum maritimum* var. Carpet of Snow), dill (*Anethum graveolens*), white clover (*Trifolium pratense,*). Plants were grown in pots (10cm in diameter) under glasshouse conditions. The onset of flowering was induced or accelerated by growing plants under a 16-hour light regime (resembling natural summer sunlight). Aphids were reared on cabbage plants under 14hr L : 10hr D light conditions and 25°C.

4.2.2 Comparing longevity on different food sources

Four newly emerged female wasps were caged with a potted plant from each food source in a rectangular cage measuring 25cm in width, 25 cm in depth and 35cm in height (made with aluminium frames covered with gauze). Light and temperature conditions were set to 14hr L: 10hr D and 25°C. The quantity of floral nectar and aphid honey available for feeding was aimed to be in excess of what wasps could deplete. In the honeydew treatment a potted plant with approx. 50 aphids distributed over two senescent leaves of a 4-6 weeks old cabbage plant was provided. Due to long growth of dill and pak choi plants, wasps were caged with a single flower stem still attached to the pot, which was outside the cage. Five replications per treatment, each with 4 wasps were conducted over a period of several months. Cages were sprayed with water each morning to provide drinking water for the wasps and to dissolve sugar that became crystallised during the pervious 24 hrs. Preliminary observations of the survival of unfed female *C. rubecula* demonstrated a maximum longevity of 3 days (≤ 72 hrs) only. Therefore the survival of more than 3 days was used as an indicator of survival for statistical comparisons. In the honey treatment, a cage was set up with a honey drop placed approx. 7cm above ground on the surface of colourless round plastic container with a diameter of 2.5cm. Water was provided on a cotton wick placed in a water container and a "resting structure" in form of a folded paper towel (approx. A4-size) as a resting place.

Two "no-food" controls were set up: one cage was set up with water only and the other one with a cabbage plant. Wasps caged with a vegetative plant might survive longer than without because it might affect the wasp's behaviour (less activity or stress when caged with a potential host habitat than an environment devoid of familiar habitat structures) or the potted plant might even provide traces of sugar. In all three control treatments survival was monitored until the last wasps had died to obtain complete survival curves. This was to document the minimum and maximum survival capability of female *C. rubecula*. Pairwise comparisons of all treatment combinations of the number of wasps surviving up to Day 3 was carried out, using Fisher's Exact Test. The results were adjusted with the sequential Bonferroni method (Rice 1989).

4.3 Results and Discussion

Minimum and maximum survival of female *C. rubecula* in the honey treatment was about 10 times higher than in the water only treatment (Fig. 4.1). In the water only-treatment, no wasp survived longer than 3 days. A few wasps caged with cabbage survived 4 days, but survival was not significantly different to the water-only control. All other tested food sources increased survival significantly up to 4 days (Fig. 4.2). The proportion of wasps surviving on alyssum, dill and pak choi was equally high. Wasps were less likely to survive on honeydew. A few wasps caged with flowering clover seem to survive marginally longer than wasps in the "no-food" treatments. The poor survival on clover flowers is the result of concealed nectaries (long tubular flowers, pers. observation). However, a small amount of nectar might have leaked through the petals or was splashed over the plant while spraying the cage. In the remainder of this study, feeding success in relation to food accessibility (landing on plant and reaching nectar), quality and quantity of the food sources is discussed.

4.3.1 Accessibility

The suitability of food sources to provide sufficient food is determined by their accessibility. Honeydew and extra floral nectaries are easier to access than floral nectar, but flowers are likely to be more conspicuous for parasitoids in terms of scent and colours (Wäckers and Lewis 1994; Lewis *et al.* 1998). Access to nectar can be restricted because of the structure of the plant, the floral architecture and the concealment of nectaries. The more difficult it is to land on a plant, the less attractive the plant may be for food-foraging wasps (Andow and Prokrym 1990). For example, single flowers of umbelliferous plants such as dill have flowers with an exposed nectary disc but the diameter of individual umbles are not only smaller than the size of *C. rubecula* but also spatially separated. The leaves do not offer a large landing area either as they are finely incised (pers. observation). Such structures might make it difficult to land and move around on the plant. These characteristics could limit access of flowers in the field where wasps have to navigate in the presence of wind (Fink and Völkl 1995; Idris and Grafius 1998). A difficult landing manoeuvre might prevent wasps from visiting those plants in spite of their exposed and abundant nectar supply.

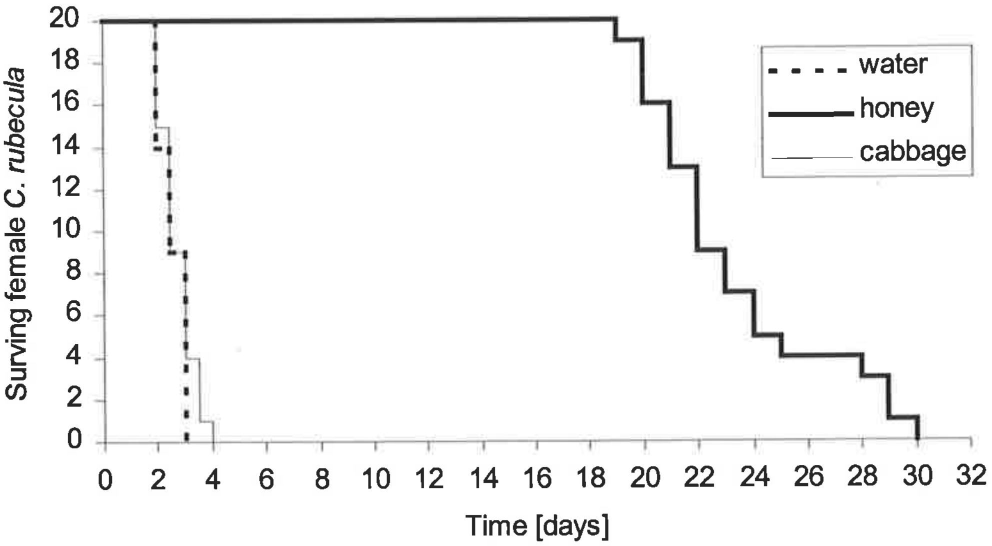


Figure 4.1 Survival of female *C. rubecula* in cages with water only, a cabbage plant or 50% honey (n = 20 wasps / treatment).

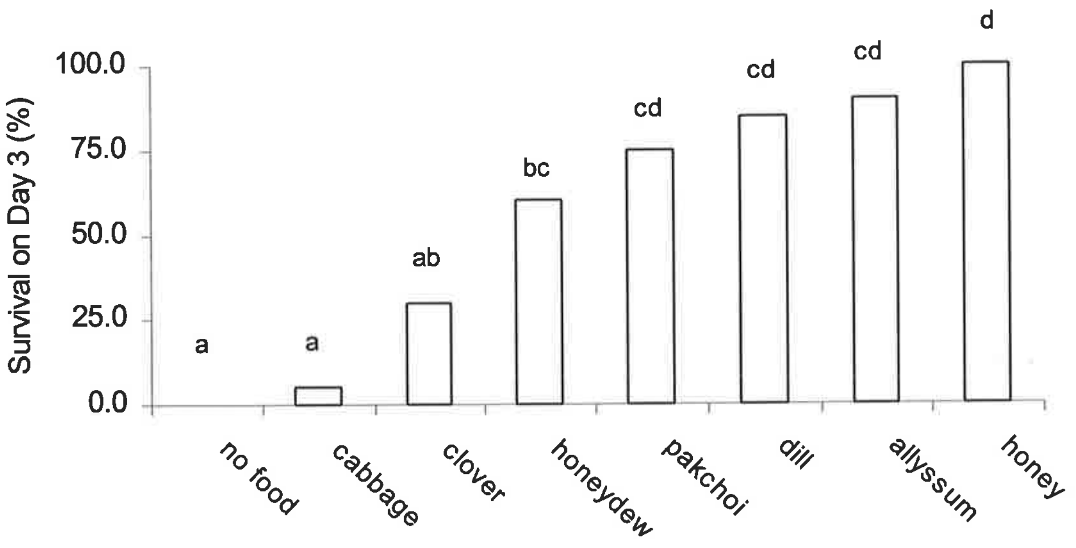


Figure 4.2 Proportion of female *C. rubecula* alive on Day 3 when caged with various sugar sources (n = 20 wasps/ treatment). Different letters indicate significant differences with $P \leq 0.05$ after Fisher's Exact Test adjusted with sequential Bonferroni method.

Flowers of the plant species Alyssum and pak choi have coloured petals and single flowers of a plant grow rather closely, which makes them easier to detect visually, land and move around. Both plants have simple leaves, offering surfaces larger in size than *C. rubecula* wasps, providing easy access as a landing point and pathway to the flowers. Honeydew is usually spread over the leaf of a cabbage plant and easy to access, but hard to locate from a distance because of the lack of odours and colours (Wäckers and Swaans 1993).

After successfully landing and reaching flowers, it can be difficult to access the nectar. Depending on the plants species, the degree of concealment of nectaries can range from completely concealed to completely exposed. Parasitoid foraging success is constrained by floral architecture and there is only a certain range of flowers parasitoids can forage on (Jervis *et al.* 1993; Idris and Grafius 1995; Lewis *et al.* 1998). Dill flowers exude nectar from a disk that is fully exposed. Flowers of pak choi and alyssum are cup-shaped and contain nectaries at the bases of the flowers, but sepals and petals are separated, thereby leaving gaps that make nectaries accessible from the side (pers. observation). Patt *et al.* (1997) investigated the accessibility of a range of flowers to small parasitic wasps (eulophid parasitoids, 1.5mm – 3.5mm) and pointed out the importance of compatibility of parasitoid morphology and foraging ability with floral architecture. Flowers with cup or tube-shaped corollas, that are narrower than the parasitoid's head width, obstruct the access to nectaries. This was obviously the case in this experiment when female *C. rubecula* were caged with white clover-flowers that have a long, tubular corolla. However, a small proportion of wasps survived marginally longer than wasps in the water only control indicating some access to sugar. Nectar traces might be available outside the flowers due to damaged flowers or water accumulating and overflowing out of flowers after watering (in the field it could be irrigation or rain). Idris and Grafius (1997) observed the parasitoid *Diadegma insulare* even chewing holes into the petals of Brassica flowers to gain access to nectar. To what extent other parasitoids are capable of this behaviour remains unknown.

4.3.2 Food quantity and quality

The quantity and type of sugars that can be consumed are important factors for parasitoid survival. The quantity of nectar and its sugar concentration can vary widely in floral nectar, depending on the species and environmental factors such as temperature and humidity (Baker and Baker 1983). Although the quantity of floral nectar available in this study was aimed to be in excess of that which wasps could deplete, the spatial distribution of nectar is also clearly important. Wasps caged with honey can feed to satiation without interruption. Nectar quantities per flower are usually much smaller and require the wasps to move within a flower and between flowers to feed until satiation. This behaviour takes much longer than feeding on one large drop. In the field, feeding on small amounts of nectar might be frequently interrupted through interference of other nectar consumers, wind or other factors alerting or disturbing the wasp trying to find sufficient nectar. Therefore, nectar feeding is prone to interruption. The nectar quantity per flower was highest in pak choi, followed by dill and alyssum (pers. observation). Another difference between the provision of honey and floral nectar is the sugar concentration. Honey usually has a much higher sugar concentration (70 %–80%) than plant nectar, which can vary considerably between 15% and 60% and higher (Maurizio 1975; Harborne 1992). This might be one of the major reasons why wasps never live as long when feeding on flowers compared to those that consume honey. Therefore, large nectar production per flower is a desirable characteristic of flowering plants, such as pak choi flowers in this study, to support nutritional needs of parasitic wasps in the field.

Food quality in terms of sugar composition and other constituents seems to have a lesser influence on longevity among nectar providing plants than among honeydews. A study of gustatory responses to single sugars revealed that common nectar sugars such as glucose, fructose and sucrose are readily accepted by *C. rubecula* wasps (Wäckers 1999). Honeydew has a different composition than floral nectar, which results in a lower energetic value as food for parasitoids (Wäckers 2000). Honeydews vary considerably in their composition depending on the homopteran species and the plant they live on (Hendrix *et al.* 1992). Food composition seems to be the major reason why honeydew of the green peach aphid, which is easily accessible, had an inferior effect on survival than floral nectars tested. As honeydew is spread over the cabbage leaf, it might mix with substances found on the surfaces of leaves such as waxy components resulting in a lower quality food. In food-choice experiments female *C. rubecula* consistently avoided honeydew of the green peach aphid (M. Fischer, pers. communication). Nevertheless, honeydew does increase the longevity of wasps to some degree and is often easily available on leaves where hosts are found, making it an important food source for many species of parasitic wasps that do not encounter flowers (Wäckers and Swaans 1993).

4.3.3 Food foraging behaviour

Although all flowers except clover tested equally well in terms of longevity, it is not known how they affect the wasp's foraging behaviour in respect to locating, landing on and moving along the plant and food intake. Differences in foraging behaviour due to differential access, nectar distribution and nectar quality (composition and sugar concentration) are not reflected in survival experiments but are important in the field. The foraging behaviour on flowers and sugar sources in general is equally important to the nutritional value of these food sources. A study on the nectar-collecting behaviour of the parasitoid *Diadegma insulare* demonstrated that some flower species were visited more frequently and longer than others (Idris and Grafius 1997).

Each food source tested, except for white clover, had some favourable and unfavourable characteristics in terms of access, food quality and quantity. Honeydew of the green peach aphid is easily accessible but has a low nutritional quality. Dill has exposed nectaries but its small flowers and finely incised leaves might make it difficult for *C. rubecula* wasps to land and move around the plant. Alyssum flowers have coloured petals (visual cues) and offer surfaces large enough to land and move on, but has small nectar quantities per flower with partly concealed nectaries. Pak-choi flowers seem to rate best in terms of detection, accessibility (strong scent, large, yellow petals, large leaves) and nectar quantity per flower.

Chapter 5 Survival gain from a single sugar meal

5.1 Introduction

Many species of parasitic wasps can increase their reproduction through feeding on supplemental sugar sources (Leius 1961a, b; Syme 1975; England and Evans 1997; Heimpel *et al.* 1997a). Sugar feeding usually results in an increase in longevity that is often positively correlated with a higher fecundity (Wolcott 1942; Leius 1961b; Sahragard *et al.* 1991; Hagley and Barber 1992; Morales-Ramos *et al.* 1996; Heimpel *et al.* 1997a). Successful sugar foraging is considered to be a critical factor that influences the lifetime reproduction of parasitic wasps in the field (Wäckers and Swaans 1993; Sirot and Bernstein 1996; England and Evans 1997; Heimpel *et al.* 1998; Jacob and Evans 1998), but little has been done on the estimation of realistic lifetimes of sugar-feeding wasps when sugar is not constantly and easily available. In many habitats foods and hosts of parasitic wasps are spatially separated or not available at the same time, resulting in rare food encounters.

Taking into account the spatial and temporal variability of sugar sources in the field this experiment addresses the specific question of how much a single sugar meal affects survival in adult *C. rubecula*. The estimation of longevity and reproduction of parasitic wasps in the field should not rely on data obtained from cage experiments with *ad libitum* sugar conditions. Considering the different qualitative, spatial and temporal scales at which sugar sources occur in the field (Jervis *et al.* 1993), this would lead to a drastic overestimation of the lifetime reproduction of wasps. The quantity and quality of sugar sources in the field vary as they can be obtained from various sources such as floral and extrafloral nectar, other plant materials rich in sugars, homopteran honeydew and even nectar-like fluids from a few fungi (DeBach and Rosen 1991; Jervis *et al.* 1992).

The sugar concentration in the majority of nectars ranges from 15% to 75% (Harborne 1992) and dried honeydew can nearly be pure sugar (Zoebelein 1956; Jervis *et al.* 1992). Sugar concentration also varies within one flower over time, even within the course of one day (Mohr and Jay 1990). Therefore, the chances of finding sufficient food on a regular basis to increase longevity might be very low.

Given a low frequency of sugar encounters in the field, the benefit from a single sugar meal becomes critical for survival. Therefore, the effect of a single sugar meal on survival was investigated in order to estimate minimum feeding frequencies in the field. In laboratory experiments wasps were allowed to feed once on a honey solution and their longevity compared with unfed wasps. As foraging wasps can encounter varying sugar concentrations in the field, honey was offered in a low, medium and high sugar concentration. The time was varied by allowing the wasp to feed on two different days to test the effect of temporal availability of food on longevity. During the experimental procedure volume consumed and feeding duration were recorded to compare the feeding behaviour between the different feeding treatments.

5.2 Materials and method

5.2.1 Feeding procedure

C. rubecula was reared using standard culturing practices as described in Chapter 2. In the treatments females were either fed on the day of emergence or one day later. Honey solutions (Kangaroo Island 'Canola' Honey) containing nectar from *Brassica* flowers were used as sugar diets because they were easy to prepare and resemble flower nectar *Cotesia rubecula* wasps would encounter in Brassica crop fields. These sugar diets were available as a drop of undiluted honey, 50% (v/v) or as 25% (v/v) honey-water solutions. Tap water was used as a control. The corresponding sugar concentrations were determined using an optical refractometer (Atago N1) and were expressed as 86%, 47% 25% (w/w) sucrose equivalents, respectively. This method gives only an estimate of the overall sugar concentration of a solution, but does not indicate about the kinds of sugar or their proportions in the honey used. For this study an estimate of a low, medium and high overall sugar concentration was sufficient and therefore no further sugar analysis was carried out. Fifteen wasps were assigned to each of the eight feeding treatments that included three different sugar concentrations and a control on two different days. An ample drop of food was given once, either 9 - 12 hours after emergence (Day 0) or on the following day, 33 - 36 hours after emergence (Day 1). As median longevity of C. *rubecula* without food was known to be 2.5 days at 25°C from preliminary experiments, these feeding times were chosen to present food to wasps early and late in life.

In the feeding procedure an individual wasp was removed from her cage and fed on a 3 µl drop of honey solution placed on a piece of parafilm. The aliquot had been dispensed from a nearly filled 10 µl micro capillary. After the wasp had fed she was returned to her cage. The end of feeding was observed as withdrawing the wasp's mouthparts from the drop to commence extensive grooming or as walking away from the feeding location. As wasps were held with water before and after the feeding procedure, water offered in control treatment was ignored. Therefore, data collection and analysis of the feeding behaviour was only carried out with diets containing sugar. Fluid consumption in the diluted sugar diets was measured by reading the difference between the filled capillary before placing the drop and after taking up the remainder. Evaporation of drops of the low and medium honey concentrations was measured at one point in time and final volume readings were adjusted accordingly assuming a linear evaporation rate. As pure honey was too viscous to be treated with a micro capillary, food uptake was measured using a microbalance (Microbalance LM 600, Beckmann). A small drop of honey was placed on a piece of aluminium foil, weighed and then offered to a wasp. After she finished feeding, the remainder was weighed again and the difference in weight recorded. The weight was then converted to volume after the weight/volume factor of honey had been obtained. Evaporation in pure honey was assumed to be negligible and was not recorded. Feeding time was recorded with a stopwatch.

After feeding once on one of the three sugar diets, wasps of the same age and feeding history were caged under the original culture conditions in groups of 3 - 4 in clear 2 l plastic cages. These contained a cotton wick soaked with water and a piece of paper towel as a resting place. The control consisted of unfed wasps. The cages were checked for dead wasps in the morning, at noon and in the evening each day until all individuals had died.

5.2.2 Data analysis

Fluid consumption and feeding duration were analysed with a two-way factorial ANOVA where sugar concentration accounted for one factor and feeding day for the other. Data were log-transformed before analysis if inhomogeneous variances occurred. After interaction between factors had been tested, a multiple comparison of the means was carried out using planned orthogonal contrasts (SAS Institute 1995).

Longevity was analysed using survival analysis. The probability of survival is estimated as a function of time from a static point such as emergence. Survival curves were generated for Day 0 and Day 1 by plotting the proportion of wasps alive against time. Differences in survival curves for each feeding day were analysed with a log-rank test (SAS Institute 1995). After that the quantitative effect of sugar concentration on survival was estimated with Cox's Proportional Hazards Analysis for each day separately (SAS Institute 1995). The quantitative effect of a variable is expressed as the risk ratio (also called hazard ratio). It characterises the risk of death in the control group in comparison with the treatment group (Lang and Secic 1997). The reverse risk ratio calculates the risk of death after feeding and is used in the following to characterise the effect of sugar feeding on survival. For example, a risk ratio of 1 indicates that a sugar diet has no influence on survival. A value greater than 1 indicates a higher risk and a value lower than 1 a reduced risk of starving to death among sugar-fed wasps.

5.3 Results

5.3.1 Food intake

Sugar concentration and feeding day affected fluid consumption, sugar intake and feeding duration significantly (Tab. 5.1). Fluid consumed (Fig. 5.1a) and time spent feeding (Fig. 5.2) were similar on the low and medium sugar diet but both were significantly different from the high sugar diet. When feeding on the high sugar diet, fluid consumption was nearly half the consumption of the lower sugar diets but the feeding duration was twice as long. Due to different fluid volumes consumed, the actual sugar intake was the same on the medium and high sugar diet and significantly lowest on the low sugar diet (Fig. 5.1b).

When fed one day after emergence (Day 1), fluid consumption (Fig. 5.1a) actual sugar intake (Fig. 5.1b) and feeding time (Fig. 5.2) increased on all three sugar diets significantly (Tab. 1). The high sugar diet showed the strongest effect of feeding day on food consumption as sugar intake (Fig. 5.1b) was nearly 2-times and feeding duration (Fig. 5.2) nearly 3-times higher on Day 1 in comparison to the previous day.

Table 5.1 Two-factorial analysis of variance of the factors sugar concentration and feeding day on feeding parameters of *C. rubecula* (n = 90).

Feeding parameter[1]	Sugar concentration		Feeding day	
	F	*P*	F	*P*
Volume consumed	25.9	*<0.001*	23.8	*<0.001*
Sugar intake	38.7	*<0.001*	23.8	*<0.001*
Feeding duration	76.4	*<0.001*	75.4	*<0.001*

[1] data log-transformed

5.3.2 Survival analysis

Median longevity (day at which 50% of the initial number of wasps are still alive) of the water-fed control group was 2.5 days and all wasp were dead after 3 days. Within both feeding days, sugar-feeding had a positive effect on survival (log-rank test, Day 0: n= 60, χ^2=31.80, $P < 0.0001$ and Day 1: n=60, χ^2=52.95, $P < 0.0001$, Fig. 5.3).

Analysis of survival data with Cox's Proportional Hazards model showed a significant reduction in the risk of starving to death for each sugar diet, except for the low sugar diet given on Day 0 (Tab. 5.2). When fed on the medium or high sugar diet on Day 0, the risk of starving to death was reduced to 0.5 on average. This means that on the day where all unfed wasps are finally dead (probability of starving to death = 1), 50% of the fed wasps will be still alive. Generally, the risk of death was further reduced when wasps fed on Day 1 instead of Day 0. The probability of death after feeding on Day 1 on the medium and high sugar diets was even reduced to 0.27 and 0.38 respectively; in other words over 60% of wasps will be still alive on the day where all unfed wasps are finally dead. Feeding on a medium and high sugar diet produced similar results within both feeding days as the 95% confidence intervals of their risk ratios overlap broadly (Tab. 5.2). Whereas feeding on the low sugar diet on Day 0 did not affect survival, the same diet given one day later (Day 1) reduced the risk of death to 0.57, similar to the risk ratios of higher concentrated sugar diets given on Day 0.

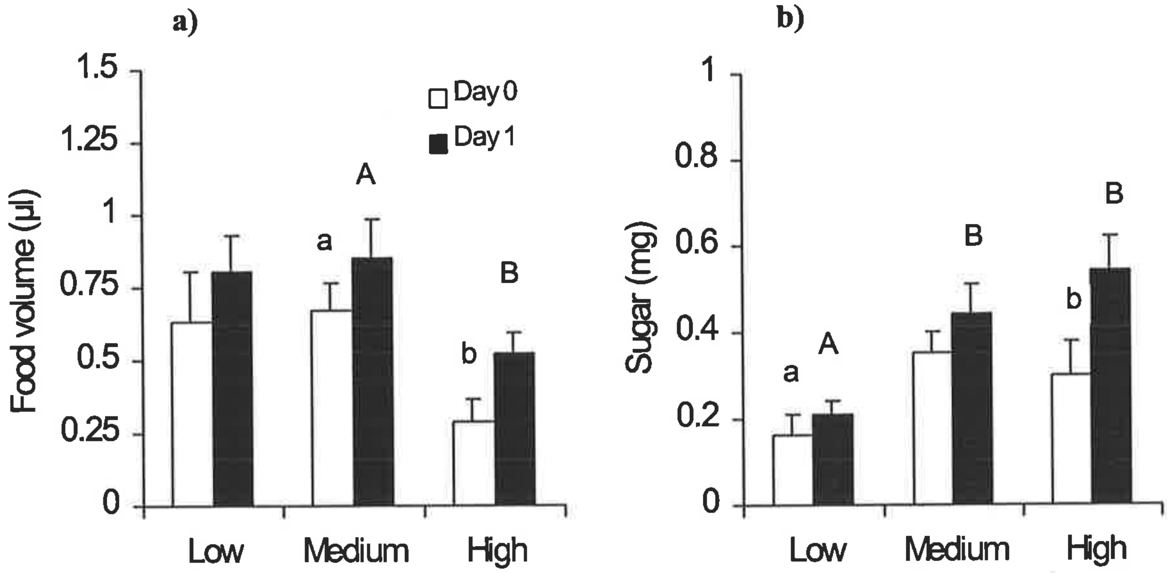


Figure 5.1 Food consumption (mean + 95% conf. interval) by *C. rubecula* in a single meal. Three different sugar diets were provided on the day of emergence (Day 0) or one day later (Day 1). **(a)** Fluid volume consumed and **(b)** sugar amount consumed.

Different letters above bars indicate statistical differences;for each sugar concentration , there was a statistical difference between Day 0 and Day 1

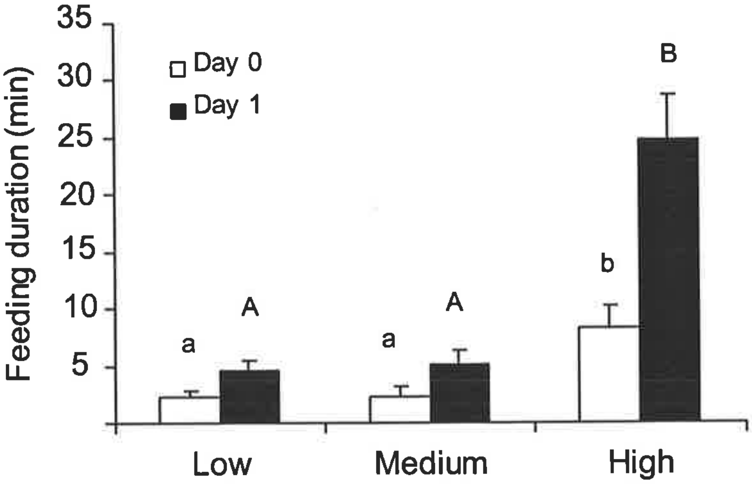


Figure 5.2 Time spent feeding (mean + 95% conf. int.) by *C. rubecula* in a single meal. Three different sugar diets were provided on the day of emergence (Day 0) or one day later (Day 1).

Different letters above bars indicate statistical differences;
for each sugar concentration , there was a statistical difference between Day 0 and Day 1

Table 5.2 Cox's Proportional Hazards Analysis for each feeding day (n = 60). Each sugar diet (low medium high) is an explanatory variable on the survival time of *C. rubecula*. Risk–ratios were obtained from each sugar diet / control - pair and characterise the risk of starving to death after feeding on a particular sugar diet in comparison to the control (risk-ratios <1 indicate a lower risk and ratios > 1 a higher risk of death).

	Day 0			Day 1		
	Sugar concentration					
	low	medium	high	low	medium	high
risk-ratio	0.95	0.56	0.50	0.57	0.27	0.38
95% conf. int.	0.68-1.32	0.40-0.78	0.34-0.73	0.38-0.83	0.17-0.42	0.24-0.60
P	0.764	0.0006	0.0003	0.0025	<0.0001	<0.0001

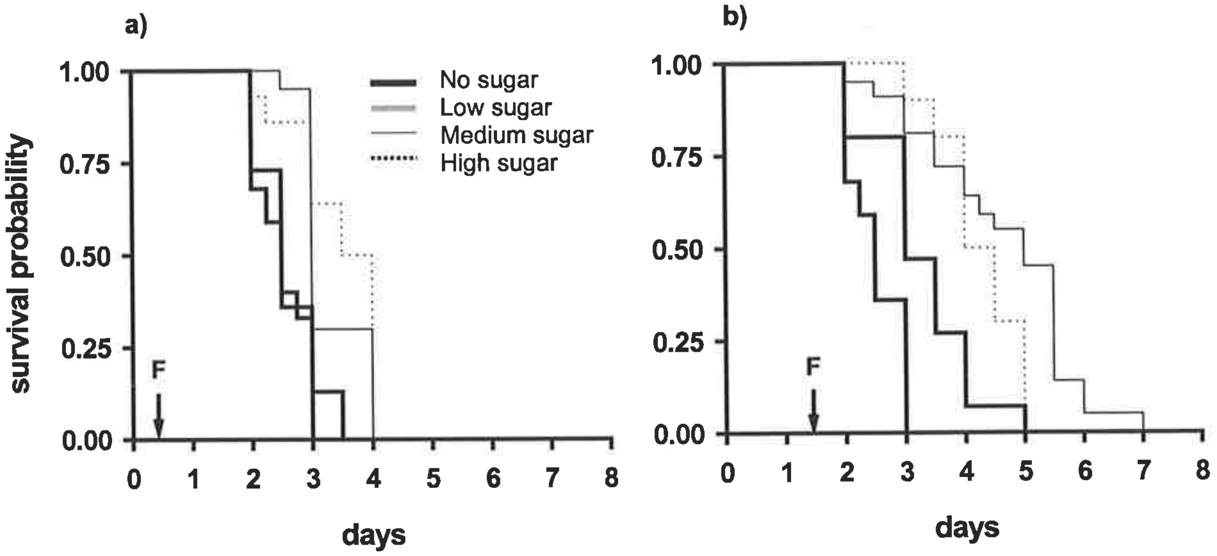


Figure 5.3 Survival probabilities of *C. rubecula* after a single meal on three different sugar diets. Survival after meal (**F**) given **a)** on the day of emergence (Day 0) and **b)** one day after emergence (Day 1).

5.4 Discussion

If food patches are spatially or temporarily separated from host patches, sugar encounters might be a rare event in the life of parasitic wasps. In this study the effect of a single sugar meal on the survival of female *Cotesia rubecula* wasps was measured. The results showed that sugar concentration as well as timing of the food encounter play an important role in extending longevity.

The influence of feeding on survival was linked to the amount of sugar consumed, which varied between feeding days, but only partly between different sugar diets. By providing an ample drop of honey solution, the quantity of sugar consumed was limited by the feeding capacity of the wasp and the physical properties of the sugar diets. The amount of food consumed was higher on the day after than on the day of emergence, suggesting a higher feeding capacity in the wasps that have depleted carbohydrate reserves. On the low and medium sugar diet wasps were observed to feed until their gut was completely filled. While feeding, the abdomen increased in size balloon-like and the membranes between the abdominal segments became visible. Fluid consumption in the low and moderate sugar diets was probably limited by the stretching ability of the abdomen. Studies in blowflies have shown that abdominal stretch receptors are likely to be involved in the termination of feeding (Stoffolano Jr. 1995). However, when offered pure honey, *C. rubecula* consumed only a fraction of the volume that was consumed on the diluted diets. Similar results were observed in the food intake of ants (*Camponotus mus*), filling their crops only partially on sucrose solutions when sugar concentrations were over 70% (Josens *et al.* 1998). As *C. rubecula* consumed a highly concentrated amount of sugar on pure honey, the termination of feeding on honey could be explained through the osmotic pressure of sugar on the insect fluid system. A negative feedback between the osmotic pressure of the hemolymph and the rate of sugar passage through the intestine was found to limit sugar consumption in *Drosophila*-flies (Chippendale 1978).

Feeding on pure honey differed from the diluted diets not only in the volume consumed but also in time spent feeding. Feeding duration on pure honey was relatively long, suggesting that similarly high sugar concentrations in the field (*ie* 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; Maingay *et al.* 1991) and delays her return to host searching and egg laying. The long feeding duration on pure honey suggests a reduced rate of fluid intake due to high viscosity. An exponential increase in viscosity is a major characteristic of increasing sugar concentration (Kingsolver and Daniel 1995), a factor that affects the handling of food in insects (Heyneman 1983). For example, (Josens *et al.* 1998) reported an exponential increase in feeding time with increasing sugar concentration in ants. Optimal sugar concentrations for parasitic wasps such as *C. rubecula* seem to be just below 50% sugar, as a high amount of sugar can be consumed in a relatively short feeding time.

According to the survival data collected in this study, the majority of wasps feeding on the medium sugar diet were still alive two or more days after the feeding event , but a few wasps did not survive the following day. These data suggest that food should be available once every day to prevent starvation in all wasps. As energy reserves and consequently life expectancy can decline at a different rate depending on factors such as temperature and locomotory activities, food requirements may vary accordingly. For example, on a warm day the energy metabolism of a wasp will increase (Downer 1981; Woodring 1985) and consequently a higher feeding frequency is needed to replace energy reserves. Flying is an energy demanding process in hymenopteran insects and uses up carbohydrate reserves (Nayar and Van Handel 1971; Wigglesworth 1972; Steele 1981; Neukirch 1982; Friedman 1985), so wasps flying a lot during the day need to feed more often than wasps resting most of the time.

The extend to which sugar-feeding contributes to survival in the field will depend finally on the impact of other mortality factors such as predators (Völkl *et al.* 1996; Heimpel *et al.* 1997b; Völkl and Kroupa 1997) or harsh weather-conditions (Weisser et al. 1997). For example, Heimpel *et al.* (1997b) studied the predation on two parasitoid species in the field and concluded that during three months in autumn the medium longevity of *Aphytis*-wasps will be less than a day due to predator encounters. For a large proportion of a wasp population feeding can be irrelevant if they get killed early by extrinsic mortality conditions. However, assuming a low impact of mortality factors other than starvation sugar feeding becomes beneficial for survival and fecundity of parasitic wasps such as *Cotesia rubecula* in the field. This study demonstrated that the effect of sugar feeding on survival should not be estimated from cage experiments with ad libitum-sugar conditions but instead with the minimum feeding frequency needed to avoid starvation. Combining the minimum feeding frequency with the chances to find sugar sources in a particular habitat can lead to a realistic estimation of the expected lifetime of parasitic wasps in the field if extrinsic mortality factors are not important.

Chapter 6 Survival and reproduction under semi-field conditions

6.1 Introduction

It is often not easy for parasitoids to find hosts as they are distributed in a patchy and unpredictable way throughout space and time (Bailey *et al.* 1962; Godfray 1994). Available field data suggest that the majority of parasitoids die before their egg supply is exhausted (Driessen and Hemerik 1992; Weisser *et al.* 1997; Ellers *et al.* 1998). Insect mortality rarely occurs due to old age but due to adverse climate conditions, predation, diseases or starvation (McNamara and Houston 1987; Weisser *et al.* 1997; Heimpel *et al.* 1997b).

Many laboratory studies have demonstrated that the availability of sugary substrates promotes the lifetime of parasitoids and their reproductive success manyfold because they have more time available to find hosts (reviewed in Jervis *et al.* 1992; Jervis *et al.* 1996). There is considerable evidence in literature that parasitoids respond to flowers and readily feed on accessible nectaries (Leius 1961a, b; Van Emden 1963; Shahjahan 1974; Syme 1975; Foster and Ruesink 1984; Jervis *et al.* 1993; Wäckers and Swaans 1993; Idris and Grafius 1995; Patt *et al.* 1999). Naturally occurring sugar sources readily available for insects in the field are mainly floral and extra-floral nectar and homopteran honeydew (DeBach and Rosen 1991; Jervis *et al.* 1992). In regard to lifetime extension, floral nectar seems to be of higher quality than honeydew (Leius 1961b; Wäckers and Swaans 1993; Wäckers 1999; Hougardy and Gregoire 2000). Flowers with olfactory and visual cues are also easier to find than honeydew (Wäckers and Swaans 1993; Wäckers 1994). Therefore, while foraging for hosts, parasitoids are expected to intermittently search for flowers to avoid starvation (Sirot and Bernstein 1996).

Understanding flower foraging is important in the biological control of insect pests. It has been suggested to plant flowers along or within crop fields and orchards to enhance parasitoid reproduction (Rabb *et al.* 1976; Altieri and Whitcomb 1979; Van Emden 1990; DeBach and Rosen 1991). The effectiveness of this method in controlling pest populations is unclear: some field studies found that the presence of flowers increases parasitism rates of a pest population (reviewed by Powell 1986; Jervis *et al.* 1993) while other studies found no effect (Kloen and Altieri 1990; Cowgill 1995; Bigger and Chaney 1998; Cappuccino *et al.* 1999; Nicholls *et al.* 2000). Information on the underlying mechanisms of parasitoid flower foraging in the field could help to explain these contradictory results. For example, parasitoid numbers could increase in a crop field because flowers might cause an immigration from adjacent areas, thereby increasing the number of parasitised hosts. Alternatively, individual parasitoids that feed on flowers either live longer or are more active, thereby increasing their reproduction. It is also possible that pest populations such as moths and butterflies also respond positively to flowers, increase in numbers and cause a density dependent response from parasitoids.

The aim of this study is to identify to what extent the presence of flowers increases the lifetime reproduction of a parasitoid in the field. A semi-field approach was adopted where the lifetime of individual wasps and their reproduction (number of eggs laid) was monitored in the presence of flowers. Several releases during the two Australian summer and fall seasons were conducted. A semi-field set up such as large field cages provide a valuable link between laboratory findings and open field phenomena. The advantages are that a controlled number of hosts and parasitoids can be released into spacious outdoor arenas and monitored on a daily basis under natural climate conditions.

6.2 Materials and method

Insects and plants were obtained by standard culturing practice as described in Chapter 3, Materials and method section. The study was conducted during two Australian summer and autumn seasons between 1999 and 2001 at the Waite Campus, Adelaide University, South Australia. The experimental set up and procedure in both seasons was slightly different and are described separately in this section. The first set up in 1999/2000 functioned as a pilot study and lead to the main experiment in 2000/2001.

6.2.1 Pilot study (Season 1999/2000)

Two rectangular field cages, measuring 3m x 3m at the base and 1.70m height, were erected 1m apart on the Waite Campus, Adelaide University. Cages was made out of grey fibreglass micro mesh with 18 x 30 strands to 25 mm. This material was sown into shape, fitted with a zipper and pulled over a supporting frame made from pvc-pipes. The ground was covered with horticultural sand to provide a bright background to the dark coloured wasps.

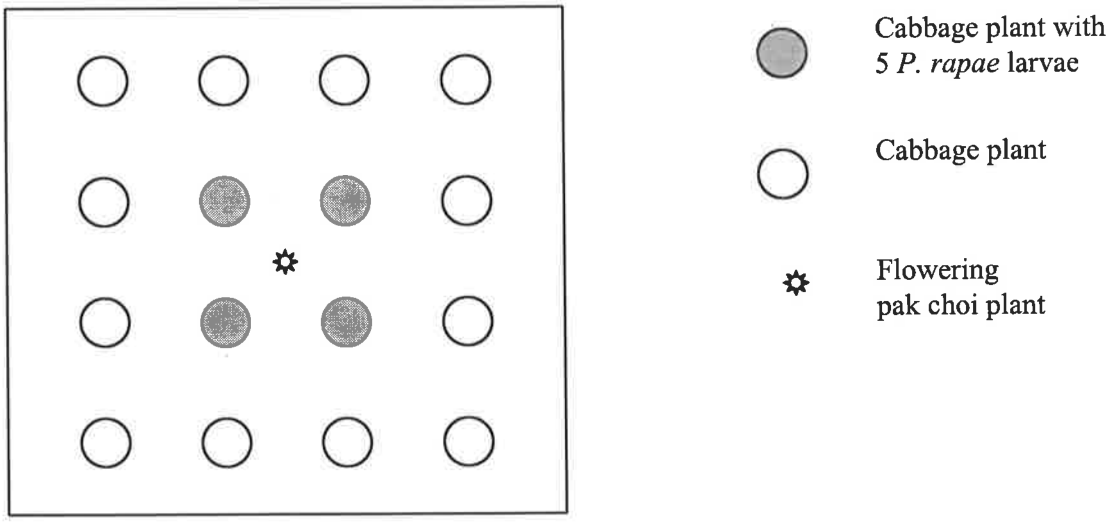


Figure 6.1 Positioning of cabbage plants and flowering pak choi. Two cages were simultaneously used in experiments, one cage contained a flowering pak choi plant and the other cage was set up as a flower-free 'control'.

In each cage sixteen potted cabbage plants carrying 6-8 leaves were inserted into the sandy ground 0.7 m apart (Fig. 6.1). The 12 cabbage plants comprising the outer square stayed permanently in the cage during the experiment. Each of the four inner plants were infested with 5 second instar *P. rapae* on 5 different leaves and were replaced daily with a new set of plants and *P. rapae*. At the beginning of the experiment four wasps were released into each cage. As female *C. rubecula* do not discriminate between unparasitised and parasitised hosts (M. Keller, pers. communication), each wasp had potentially each day 20 new hosts available to parasitise. Individual *C. rubecula* have been found to parasitise a maximum of 10 hosts per day when foraging in the field at high host densities (Nealis 1990). To compare the effect of flowers on survival and oviposition a flowering pak choi plant was added to one cage and the second cage was kept flower free. All plants were watered each morning. Nine releases were conducted for each treatment. Five releases were paired, the other four releases were carried out unpaired due insufficient numbers of newly emerged wasps. Average day temperatures were obtained daily from a weather station on campus and included in the analysis of parasitoid survival and reproduction.

Monitoring survival of parasitoids Four newly emerged female *C. rubecula* were released around 9am (~2-3 hours after hatching) into each cage. Females were recaptured each following morning for a period of three days. Under warm temperature conditions (25° C) the maximum life span of female *C. rubecula* without sugar access does not exceed 3 days and any effects of the presence of flowers were expected to appear within this time. During the release single wasps were transferred with an aspirator onto a leaf of one of the outer cabbage plants downwind to the inner cabbage plants. To overcome the tendency of wasps to fly straight up to the ceiling, the lip of the aspirator was gently rubbed against a cabbage leaf to induce the release of volatile chemicals. All wasps found alive on the next morning (Day 1) were collected and marked with differently shaped dots of a red enamel paint to track individual wasps during the observation. Each wasp was gently pressed between two sheets of cloth material with a square mesh size of 1.5 mm. This way the dorsal thorax was exposed in one square to place a colour dot on it with a fine paint brush. The paint usually dried within a few seconds. Each wasp received a dot with the same colour in a slightly different position to distinguish individuals. To allow wasps to calm down and groom they were placed temporarily into a clear, air ventilated plastic container.

Each morning the ground, the plants and the cage walls were searched for wasps and their presence recorded. During the experiment all but one dead wasp disappeared. It is assumed that dead wasps were picked up and carried away by ants that were frequently patrolling the ground of the cages. Living wasps were usually discovered either on the cabbage plants or on the wall of the cage. They were taken up with an aspirator and kept for about 10–15 minutes in a clear, air-ventilated plastic container. During this time cabbage plants from the previous day were removed and replaced with a new set of plants carrying new hosts.

The daily number of oviposited parasitoid eggs were estimated by counting parasitoid larvae found after dissecting *P. rapae* larvae. After removing plants with larvae from the field cage, they were stored in the laboratory for 48 hrs in 0.7 l clear, air ventilated plastic containers with ample cabbage leaves at 25° C before dissection. Within this time parasitoid larvae hatch, which are easier to detect than eggs. After the incubational period, larval *P. rapae* were killed in a sodium chloride solution (9g NaCl / l) to obtain motionless insects for dissection. Dead larvae were placed in a watch glass containing the same salt solution. Their skin was sliced open along the body using fine forceps and a scalpel under a stereo microscope with 10x magnification. If parasitoid larvae did not fall out at this stage, the gut of the host larva was removed and the skin with attached tissue was systematically and carefully torn into pieces to detect any attached larval parasitoids. The number of first instar parasitoid larvae would be counted as successfully developed eggs in the body of the host. A small number of eggs could have been missed due to an adverse immune reaction of the host and subsequent disintegration of the parasitoid egg (Asgari and Schmidt 1994).

6.2.2 Main study (Season 2000/2001)

Due to the destruction of the previous field cages by adverse weather conditions, different cages were used. These cages consisted of an aluminium frame, a rectangular base which measured 6m by 4m and a dome -shaped roof 2m to 2.30m high, covered with white thrips safe mesh. The following modifications were adopted

- Inside the cages a sand coloured shade cloth covered the ground to suppress weeds and provide a bright background to the dark coloured wasps.
- Both cages were fitted with temperature data loggers (TinyTalk, Gemini Data Loggers) recording data every 15 min to obtain a temperature profile inside each cage.
- Pak choi plants grow considerably higher than cabbage plants and were therefore, inserted into a hole in the centre of the cage to position the flowers at the same height as the feeding damage on the cabbage plants.
- Wasp survival and parasitism was now monitored until no wasps could be found for a period of 2 days and they were presumed dead. Preliminary observations in the previous season revealed no effect of flowers on survival within 3 days, therefore the observational period was extended to detect potential differences at a later stage. Otherwise the techniques of wasp release, recapture, marking, host larvae collection and dissection were identical to those employed in the previous season. During this season ten paired releases with 4 female parasitoids per cage were conducted.

6.2.3 Data analysis

Survival curves of parasitoid lifespan observed in the cage with and the cage without flowers were compared with a univariate survival analysis. Additionally, logistic regression analysis was used to test the effect of the presence of flowers, together with temperature, on the survival of *C. rubecula* females for each day separately (JMP version 3.1, SAS Institute 1995). This was done to account for the daily variation in temperatures and to detect wether potential differences in survival would occur evenly over time or at a certain point in time. Parasitoid offspring data could be only analysed per observational period (n = 18-20), not per individual wasp (as four wasps were simultaneously released per cage). Daily number of offspring produced per female was estimated by dividing the total number of parasitoid larvae per day by the number of wasps alive. Due to low number of replications and unequal variances, mean values of reproductive activity were compared with Mann-Whitney test.

6.3 Results

6.3.1 The effect of flowers on survival

Season 1999/2000 There was no difference in the survival of female *C. rubecula*-wasps within a 3 day observational period when caged with flowers (univariate survival analysis, log rank test, $\chi 2 = 2.647$, $P = 0.103$). Median longevity (50% survival) was only 1.5 days (Fig. 6.2 a). Logistic regression analysis for each observational day confirmed that flowers had no effect on survival but temperature limited survival significantly (Tab. 6.1). A temperature history characterised by an average day temperature around 30 °C usually resulted in longevity no longer than 1 day (Fig. 6.3a). Maximum day temperatures over 35°C were not uncommon during summer release periods.

Table 6.1 Effect of presence of flowers and temperature on survival of female *C. rubecula* in field cages in 1999/2000 using logistic regression analysis with Likelihood-Ratio Tests (n=67). Each day of a three day-observational period was analysed separately.

Source of variance	L-R Chi Square	*P*
	After 1 day	
Flowers	0.061	0.803
Temperature	42.86	<0.0001
Flowers x Temperature	0.117	0.731
	After 2 days	
Flowers	1.917	0.166
Temperature	46.67	<0.0001
Flowers x Temperature	1.547	0.213
	After 3 days	
Flowers	1.586	0.207
Temperature	25.15	<0.0001
Flowers x Temperature	0.999	0.317

Season 2000/2001 Similar results occurred in the following year although there was a tendency of a marginally higher survival of wasps in the presence of flowers (univariate survival analysis, log rank test, $\chi 2 = 3.118$, $P = 0.077$). Median longevity in the presence of flowers was estimated to be 2.5 days and 1.5 days in the flower-free cage (Fig. 6.2 b). Logistic regression analysis confirmed the strong effect of temperature on survival and revealed that significantly more wasps survived 3 and 4 days after release in the presence of flowers (Tab. 6.2). However, at the same time temperature interacts with the flower treatment, indicating that differences in survival are temperature dependent.

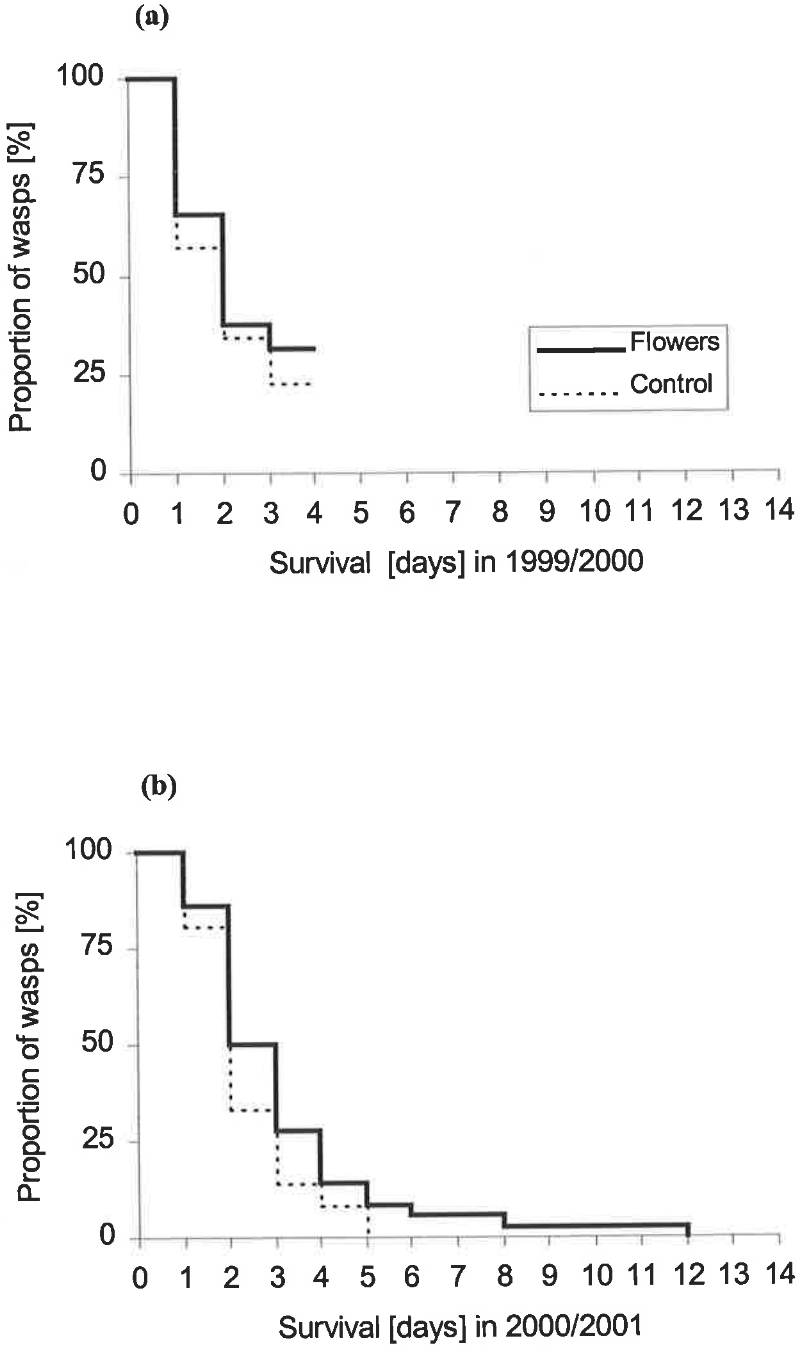
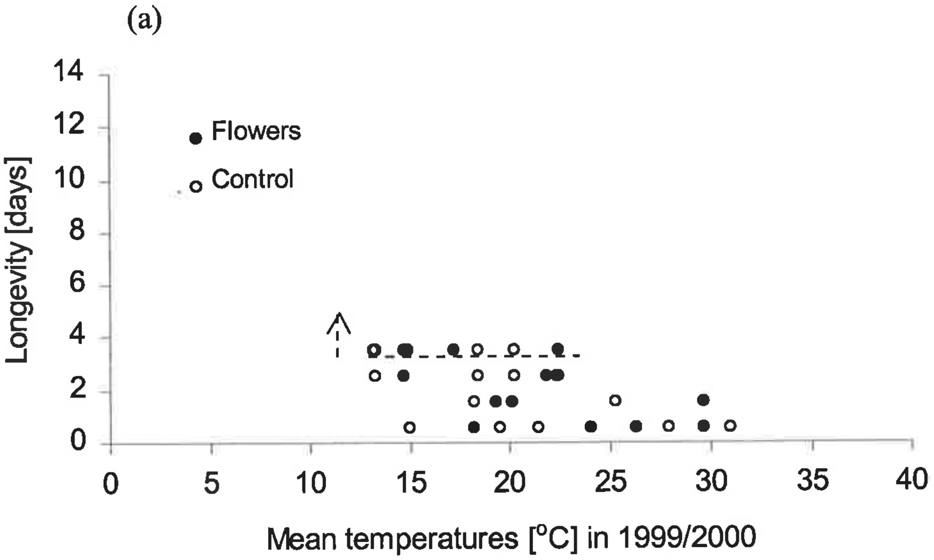


Figure 6.2 Survival of female *C. rubecula* in a field cage with flowering pak choi (= Flowers) or without (= Control) during **(a)** season 1999/2000 where observational period was restricted to 3 days and **(b)** season 2000/2001 with unrestricted observations. Data are summed across 9-10 independent releases with four wasps each.



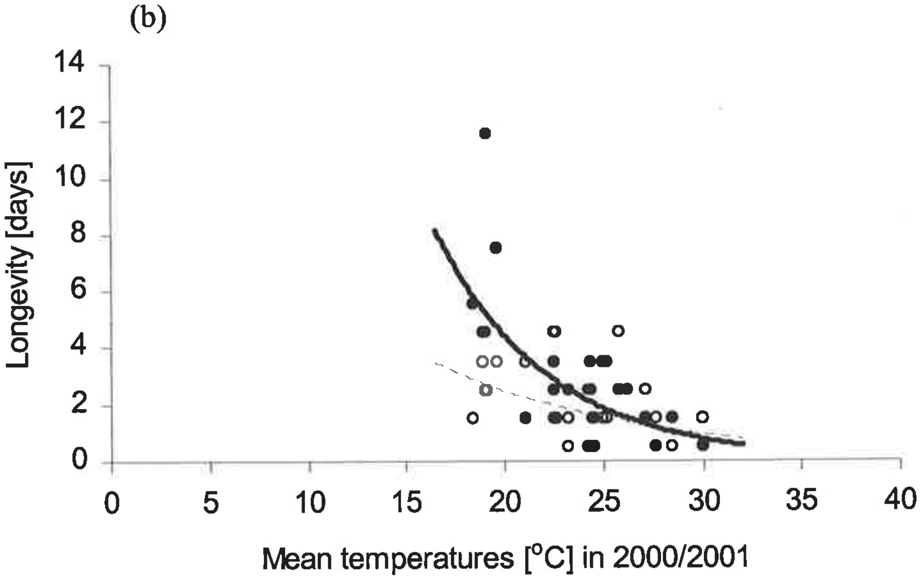


Figure 6.3 Longevity of individual female *C. rubecula* plotted against average of daily mean temperatures experienced during life **(a)** in season 1999/2000 observed within 3 days period (highlighted data points at 3.5 days indicate censored observations and unknown higher longevity, $n_{wasps} = 67$) and **(b)** in season 2000/2001 uncensored ($n_{wasps} = 80$).

Curves in second graph are fitted via exponential function to indicate trend of relationship between longevity and temperature.

As a rule of thumb, a temperature history of 25°C and higher seems to limit survival generally to 1 –3 days. At lower temperatures wasps were able to survival longer in the presence of flowers than wasps without food sources (Fig. 6.3 b).

Table 6.2 Effect of presence of flowers and temperature on survival of female *C. rubecula* in field cages in 2000/2001 using logistic regression analysis with Likelihood-Ratio Tests (n=80). Each day of a four day-observational period was analysed separately.

Source of variance	L-R Chi Square	P
	After 1 day	
Flowers	0.638	0.424
Temperature	9.108	0.0025
Flowers x Temperature	0.564	0.452
	After 2 days	
Flowers	1.451	0.228
Temperature	15.05	0.0001
Flowers x Temperature	1.040	0.307
	After 3 days	
Flowers	2.144	0.143
Temperature	14.68	0.0001
Flowers x Temperature	3.779	0.051
	After 4 days	
Flowers	6.713	0.0096
Temperature	11.96	0.0005
Flowers x Temperature	5.954	0.0147

6.3.2 The effect of flowers on reproduction

Season 1999/2000 There was no difference of the total number of offspring produced by 4 female *C. rubecula* at the end of a three-day period when caged with flowers or without (Fig 6.4 a).

Average reproduction per female during a 3-day observational period is estimated to be approximately 3 larvae only (total number of parasitoid larvae counted divided by 4 females released). However, wasps that survived at least 3 days could produce as many as 5 to 8 larvae. An examination of the average daily reproduction indicated that the reproduction of wasps in the flower cage was higher on the third day after release than at the same time in the flower-free cage (Fig. 6.5 a). Because it affected only a small number of observations (n=3) it did not amount to a significant difference.

Season 2000/2001 The number of offspring produced in the following season was significantly higher in the presence of flowers (Fig. 6.4 b) when reproduction was observed until the last wasps had died (in contrast to the previous season). Average lifetime reproduction per female in the presence of flowers was approximately 10 larvae. Although this was twice as much as in the control treatment, it is still only a tenth of the reproductive potential of *C. rubecula* (approx 100 eggs). A difference in daily reproduction between wasps with the opportunity to nectar feed and those without, appeared on the third and forth day after release (Day 2 and Day 3, Fig. 6.5b). This indicates that wasps, surviving less than 2 days because of high temperatures or other mortality factors, do not benefit from flowers in respect to reproduction. In this study, this is the case for approximately 50% of the release population (see survival curve in Fig. 6.2 b).

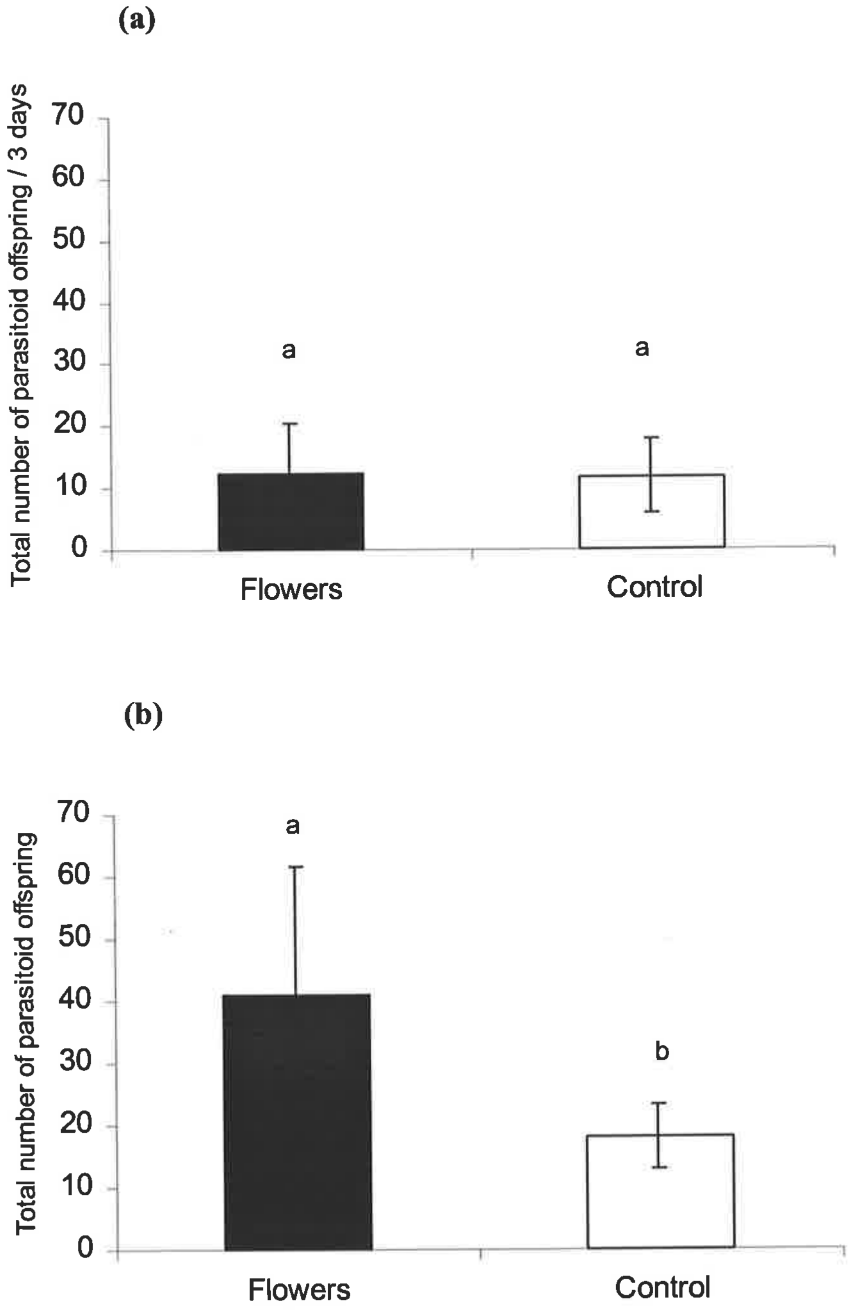


Figure 6.4 Total number of parasitoid larvae (mean ± 95% conf. interval) counted after the release of four female *C. rubecula* into a field cage with flowering pak choi (=flowers) and one without (=control) in **(a)** season 1999/2000 (observational period restricted to 3 days, $n_{periods} = 9$) and in **(b)** season 2000/2001 ($n_{periods} = 10$).

Different letters above columns indicate a statistical difference after Mann-Whitney test, $P \leq 0.05$.

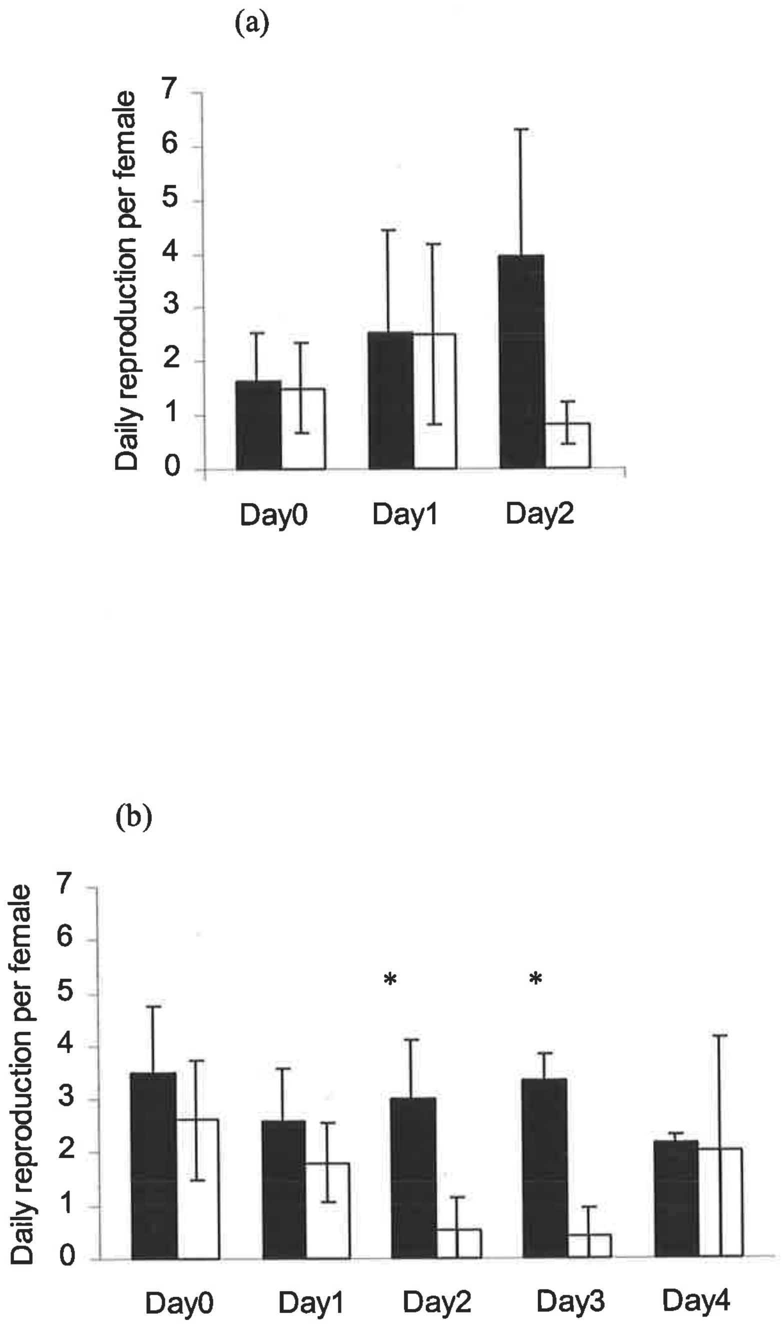


Figure 6.5 Daily number of larval *C. rubecula* (mean ± 95% conf. interval) produced per female wasp (total number of larval parasitoids divided by total number of female counted per day) in **(a)** season 1999/2000 and **(b)** season 2000/20001.

Asterisk above columns indicate significant difference between different treatments on a particular day at $p \leq 0.05$ after Mann-Whitney test.

6.4 Discussion

The marginal effect of flowers on the lifespan of female *C. rubecula* point to a lack of flower visits or nectar intake. In the laboratory, female *C. rub*ecula can extend her life expectancy by 1-2 days by a single honey-water meal (Siekmann *et al.* 2001). The increase in lifespan by approximately 1-2 days of some wasps in the field cage with flowers indicate that they had only a single "proper" meal or a few meals with little food intake during the experiment. There should have been ample food supply during the whole experiment because pak choi flowers were observed to flower successively from top to bottom, opening new flowers and supplying fresh nectar each day. However, some flowers of the inflorescence might supply little nectar depending on the time of the day, humidity or age of the flower. Perhaps, if wasps do not find any or little nectar in a certain time interval (giving up-time, Pleasants 1989; Stephens and Krebs 1986) they might leave the plant before finishing the examination of the whole inflorescence. Unrewarded or poorly rewarded visits could even result in "negative conditioning" (Takasu and Lewis 1993; Vet 1995; Takasu and Lewis 1996). A visit might be "remembered" as not rewarding and consequently flowers will be not revisited although fresh nectar is supplied at a later stage.

Survival of female *C. rubecula* was only marginally higher in the presence of flowers but reproduction was on average about 2 times higher in the presence of flowers. However, the estimated average lifetime reproduction was estimated to be 10 parasitoid larvae per wasp. This was surprisingly low as female *C. rubecula* wasps have an egg complement of about 100 eggs and have been observed in a field experiments to sting 10 hosts per day under abundant host conditions. The low number of parasitoid offspring could be due to the experimental design such as confining wasps within the cage and providing hosts each day at the same location. Unrestrained, they would not forage their whole lifetime in the same location.

Although the cages were covering an area of 4m by 6m, adult *C. rubecula* in the field disperse over greater distances during their lifetimes. As mentioned earlier, larval *P. rapae* are solitary hosts and are rather randomly distributed through space, hence, females are adapted to cover a large area within their lifetime. In wind tunnel flight exercises, newly emerged and unfed female *C. rubecula* were able to fly 50 m against a high windspeed (70 cm/s) within a day which indicates good dispersal capabilities (Chapter 3). During counts of wasps in the field cages each morning, a few wasps were usually found on the walls or in the upper corners of the cages. This suggests, that wasps may have dispersed from the experimental arena if the cage had not been there. As parasitism occurred continuously throughout the observational period, wasps did not avoid to forage in the same location but were likely to be restrained in their natural foraging behaviour.

The increase in reproduction in the presence of flowers was characterised by a high variation, indicating that the effect of sugar feeding on reproduction originates from only a few wasps. This could be due to only a few wasps feeding on nectar or, if the majority of wasps could feed successfully, only a few of them were able to express a higher searching activity (other nectar consuming wasps might have died early, i.e. because of high temperatures). This variation may also be affected by an interaction between temperature and the energetic state of the forager. For example, wasps after nectar intake might react differently to climatic conditions, such as temperature, than unfed wasps. This could be tested in further research by determining the influence of temperature on the host foraging behaviour of fed and unfed wasps. Fed *Microplitis croceipes* do display a higher searching activity (higher rate of host encounters) (Takasu and Lewis 1995; Stapel *et al.* 1997), but this has not been tested under different temperature conditions.

Ambient temperature had a dominant effect on the survival of female *C. rubecula*. Approximately 50% of the wasps released had died within 2 days, presumably due to warm to hot temperature conditions. Flower foraging might not have occurred within this time. The rate of metabolic oxygenic processes is strongly temperature dependent and a temperature increase of 10°C usually increases metabolic oxygenic processes by 2-3 times ("Q10 rule", see refs. in Bursell (1974)). Many days during the observational period had maximum temperatures up to 35 °C and sometimes higher. Although the maximum temperatures were not lethal (upper lethal limit for most insects = 40°C - 50°C lasting 1 hour, Bursell (1974)), the acceleration of metabolic rates during this time contributed to rapid ageing or other sublethal effects. Parasitic wasps seem to be able to perceive a short life expectancy and might invest this time exclusively to host foraging. For example, Roitberg *et al.* (1993) demonstrated that a *Drosophila*-parasitoid increased its foraging activity when thunderstorms were approaching and a fall in the barometric pressure occurred.

The way ambient temperature was incorporated into the statistical analyses and graphical plots in this study can only account as an approximation. Average daily temperatures are a quick and easy way to characterise the temperature profile of a time period, but poses the following problems, a) temperature variation is not taken into account as different temperature profiles can yield the same mean (i.e., an average of 25 can result from 25/25 or 20/30), b) by taking the mean of several days the order of temperature occurrence is lost. For example, with an average daily mean of 20°C obtained from a three day period, the temperature history could have been 30, 20, 10 or 10, 20, 30 °C which will lead to different physiological and behavioural outcomes. Ideally, temperature ought to be included in such way that sequence and variation within a day and over the whole lifespan of an individual is captured in an analysis, to untangle the effect of parasitoid feeding and temperature history on the outcome of survival.

C. rubecula is a solitary parasitoid of larval *Pieris rapae*. These occur usually with 1 or 2 individuals (1983) and sometimes up to 5 individuals per plant (Harcourt 1961; Kobayashi 1966; Jones 1977). In a crop field, infested plants are randomly distributed in a poisson or negative binomial fashion (Kobayashi 1966; Vos 1999) and usually spread over a large area. Therefore, these wasps are adapted to habitats where hosts are not easy to find. Given that *C. rubecula* in the present study design were exposed to a relatively "host-rich" area (4 plants close together with five hosts each), foraging for hosts could have taken a higher priority than nectar foraging. The concentration or continuous presence of host cues might have been "overriding" nectar cues from flowers and attracted the wasps to the host bearing plants. A set up without hosts might have lead to a higher incidence of nectar foraging. The spatial arrangement of host and flowers and their densities might be important in regard to detection and foraging preferences.

This study predicts that the presence of flowers increases parasitoid reproduction (and host mortality) under mild temperature conditions. However, even under optimal foraging temperature conditions, survival will be only marginally higher due to limited flower visits and inadequate sugar intake. The magnitude of reproductive success during the time gained through feeding depends on the availability of hosts. As predation on parasitoids and interspecific competition through other nectar foragers such as bees will also impact on wild parasitoids foraging in the field, the likelihood of flowers contributing to lifetime reproduction of parasitic wasps might be even further reduced.

Chapter 7 Searching for hosts or for food: optimality modelling of state -dependent strategies

7.1 Introduction

Optimal foraging theory aims to predict strategies a forager should adopt in order to gain maximum fitness when exploiting resources. Parasitic wasps are often used to study optimal foraging behaviour because of the direct link between searching behaviour and fitness (Van Alphen and Vet 1986). Most of the theoretical work is concerned with how host density and host distribution affect parasitoid foraging decisions (Cook and Hubbard 1977; Waage 1979; Bernstein *et al.* 1988; Visser *et al.* 1992; Tenhumberg *et al.* 2001). In the past decade the influence of the parasitoids' internal state such as egg load and energy reserves on foraging decisions have been recognised (Mangel 1989; Collier *et al.* 1994; Briggs *et al.* 1995 Collier 1995); Jervis and Kidd 1995; Heimpel *et al.* 1996; Sirot and Bernstein 1996; Murdoch *et al.* 1997; Sirot *et al.* 1997). Food intake improves the physiological state of the forager and can potentially contribute to fitness (Jervis and Kidd 1995). As a consequence of feeding a female parasitoid might develop more eggs or live longer, both increasing her chances to lay more eggs during her lifetime. This study investigates the role of food searching in the optimal foraging strategy of parasitoids. It is concerned with sugar foraging as sugar intake can substantially increase parasitoid lifetime and consequently future reproductive success in non host feeding as well as in host feeding species (Jervis and Kidd 1986; Heimpel *et al.* 1997a).

Apart from extrinsic mortality factors such as harsh climate and predation, a substantial part of a parasitoid population in the field is believed to die prematurely due to starvation (Van Emden 1990; Heimpel *et al.* 1997b). In many parasitoid species the supply of sugary substrates increases adult lifespan manyfold (Jervis *et al.* 1992; Jervis *et al.* 1996). It is also known that hungry parasitoids respond to food cues such as odours or colours (Lewis and Takasu 1990; Wäckers 1994; Chapter 3). As parasitoids in the field have been found on flowers (Van Emden 1963; Maingay *et al.* 1991; Jervis *et al.* 1993), it is fair to assume that parasitoids forage for nectar.

Natural sugar sources available for parasitoids in the field are mainly floral, extra floral nectar and homopteran honeydew (Jervis *et al.* 1992) and are usually found at locations other than host sites. As host foraging and sugar foraging may be mutually exclusive, a parasitoid faces the following trade-off: Host foraging increases the likelihood of finding hosts quickly, but continuously decreases the forager's energy reserves and consequently life expectancy. On the other hand, food foraging postpones oviposition opportunities but increases the time available to find hosts in the future through replenishment of energy reserves. However, food foraging can be risky as the sugar reward might be sparse or lacking, attacks by predators attracted by sugar sources might happen or diversions from host patches might occur. Risky food foraging would result in a low lifetime reproductive success. Under these circumstances natural selection would favour parasitoids that exclusively search for hosts and invest little into food foraging.

Sirot and Bernstein (1996) developed a model that examined the trade-off between food foraging and host foraging. Their model predicts when it is optimal for a parasitoid to search for hosts instead of sugar sources and vice versa. In their model energy state, food availability and mortality risk are important factors in the "decision making process". Sirot and Bernstein (1996) demonstrated that 1) parasitoids should never search for food at a low food availability, 2) if food is moderate they should search for food before their energy reserves are exhausted, 3) if food is abundant and mortality is independent of food consumption they should search for food when their energy reserves are nearly exhausted, 4) but if mortality depends on food consumption parasitoids should not wait so long. The parameters in this model are arbitrarily chosen and do not relate to any parasitoid species. Therefore, predictions such as "before energy reserves are exhausted" are still vague. The question arises if experimentally derived energy parameters would change the aforementioned predictions. This study also did not account for different energy expenditure at different food availabilities, variation in food reward and the availability of hosts. These factors might have a profound impact on the decision to search for food or for hosts.

A dynamic state-variable model was constructed to explore the behavioural choice between food and host searching, taking into account the stochastic nature of the environment. The model in this study was parameterised with experimentally obtained estimates on energy states, energetic values of food and foraging costs of the parasitic wasp *C. rubecula*. Scenarios were introduced that varied not only in food but also in host availability. If hosts are abundant and easy to find, the pressure of "time limited" reproduction should be relaxed; consequently, finding food to increase lifespan would become less important. In contrast, if hosts are hard to find, the significance of food foraging should increase as a means to extend lifespan in order to find more hosts. In addition to the effect of host availability, the effect of the energy value of food on the onset of food foraging was explored. The energy value of a food source can be limited by quantity or quality. For example, honeydew has been found to be of lesser quality than floral nectar in regard to lifetime extension (Leius 1961b; Wäckers and Swaans 1993; Wäckers 1999; Hougardy and Gregoire 2000). Floral nectar, on the other hand, is often high in quality but can vary considerably in quantity, depending on the time of the day, climate and competition with other nectar foragers (Baker and Baker 1983; Kevan and Baker 1999).

7.2 The Model

7.2.1 Physiological and ecological variables

In dynamic state-variable models, fitness is identified as a function of an organism's physiological state, the time an organism has left to live and ecological parameters (Mangel and Clark 1988). The dynamic model that is considered for this study generates predictions for two behavioural tactics faced by many species of insect parasitoids: host foraging and food foraging. The present foraging model includes a physiological state variable (carbohydrate energy reserves), a time variable, probabilities of finding resources (nectar or hosts) and a mortality risks variable. Time is measured in discrete intervals and only a single resource encounter is allowed to occur in one time interval. The maximum life expectancy of the parasitoid is set to T. During each time interval t, the female parasitoid survives with probability β and encounters hosts or nectar with a probability of λ. The energy state is characterised by a level of carbohydrate energy reserves x. The parasitoid dies if the energy state drops below the minimum threshold, c. The energy state has an upper boundary not exceeding capacity C.

$$\text{If } x \leq c \text{ then } x = 0$$

$$\text{If } x \geq C \text{ then } x = C$$

Foraging costs a are dependent on the density of resources in such way that a high resource density is linked with low energy costs and a low resource density with high costs. For example, if hosts are available on almost every leaf of a plant, the parasitoid only needs to walk or fly a short distances to encounter them all. Hosts sparsely scattered throughout a habitat require relatively longer travel distances and higher energy expenditure. For the sake of simplicity, the time interval for moving long or short distances in this model is the same.

Probabilities of finding resources $P(R)$ are estimated as a function of resource density D_R and foraging costs a_R. Foraging costs are measured in the smallest discrete units 1, 2, and 3 and correspond to spending foraging energy at a high, medium or low resource density, respectively. The behaviours available to the parasitoid are host foraging, food foraging and resting and are specified by the subscripts, *h, f, r,* respectively. Finding a host yields an immediate fitness gain of *R*. As *Cotesia rubecula* usually lays a single egg into a host, R equals 1 egg. The total number of eggs oviposited by a female during her life is used as fitness currency. A potential feeding reward *E* yields an increase in carbohydrate reserves and foraging costs *c* decrease energy reserves.

If feeding occurs then $x_{t+1} = x_t - a + E$

If no feeding occurs then $x_{t+1} = x_t - a$

Fitness values are calculated using backward iteration, beginning at time T, for which the expected future fitness is set to 0 (no fitness can be accrued after death).

$$F(x, T, T) = 0$$

For each combination of states, the behaviour is calculated that results in the highest fitness. Using this approach an individual can be followed forward in time; at each time interval its current state determines what action it takes, and thus the probability of being in a particular state during the following time interval.The model consists of three parts: searching for hosts, searching for food or resting. The parasitoid's choices depend on the state variables energy state and life expectancy, the probability to find hosts or food and the probability to die. It is assumed that a wasp has perfect knowledge of all these variables. A wasp has to decide at each time interval whether to search for hosts, for food or to rest. A wasp survives with a probability of (1-β) in each time interval. If she decides to search for hosts then there is a probability λ_{Rh} to either find a host and lay an egg or to fail in finding a host (1-λ_{Rh}). In both cases her energy state decreases by the same foraging cost a_R. If she decides to search for food, then there is a probability to find food λ_{Rf} and to increase her energy state by sugar intake (E) or to fail in finding food (1- λ_{Rf}). In both cases the same foraging costs occur but in the former case, the energy gain through sugar intake is higher than energy expenditure. As a third option a wasp can remain where she is and rest. This is characterised by no foraging costs and very low mortality risk. No gain in respect to energy or reproduction is possible.

7.2.2 Parameterisation of the model

All values affecting the energy state of the forager were derived from previous results on female *C. rubecula* carbohydrate energetics (Chapter 2). The energy state and food reward in this model were expressed in similar proportions with smaller values for easier computation (Tab. 7.1).

Table 7.1 Approximation of energy state used in model derived from experimental carbohydrate measurements in female *C. rubecula*

Energy state	Original values [µg]	Units in model
At emergence	52	24
Starved near death	14	6
After unlimited access to honey or access to lower conc. sugar source[1]	170 110	48
Costs of flight/day	18	
[2]Cost of flight/hr	1.8	1-3

[1] sugar source encountered in field to be assumed of lower value than honey

[2] assuming a 10 hrs foraging day

Results are presented as graphical figures in percentage energy reserves for clarity. To account for increasing searching costs through decreasing resource density, foraging costs were set to the smallest increasing set of integers (1,2,3). In comparison to the costs of flying when searching for resources, metabolic costs during resting were assumed to be very small and were set to 0. Three "benchmark" resource availabilities, or the probability of encountering a resource D_R, such as hosts or sugar within a given time interval, were estimated from a high, medium and low resource density: resource densities were characterised as probabilities of each potential patch, i.e. plant, containing a resource. Densities were characterised by the values 1, 0.5 and 0.1 respectively. Probabilities of finding resources P{R} were then calculated as a function of resource density (D_R) and foraging costs (c_R). A constant k characterises the slope of the curve (Fig. 7.1)

$$P(R) = \frac{D_R * c_R}{c_R * k}$$

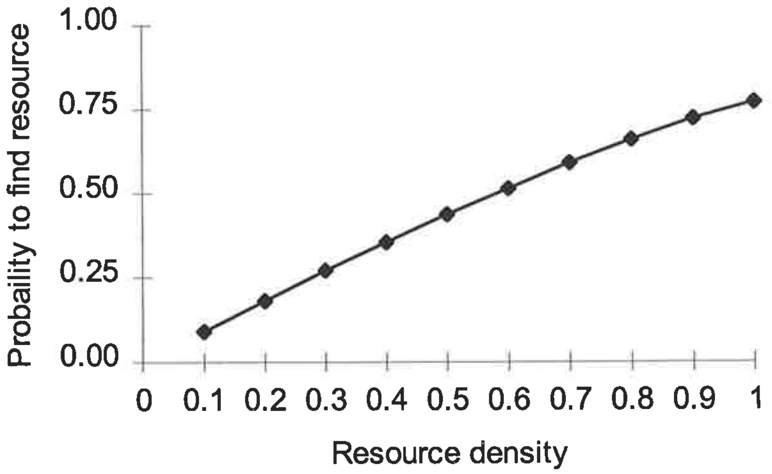


Figure 7.1 Probability to find a resource as a function of its density. Constant k = 0.3 characterising the slope of the curve was arbitrarily chosen

Based on these calculations the probability values 0.09, 0.43 and 0.77 were used in the model. A low, medium and high mortality during foraging was estimated from literature. Predation is likely to be the dominant mortality risk, apart from adverse weather conditions, for small insects in the field (Heimpel *et al.* 1997b). Field observations on the parasitoid *Aphytis aonidiae* foraging for scale insects calculated a predation risk of 0.06 per hour (Heimpel *et al.* 1997b). Adult parasitoid mortality due to adverse weather conditions is currently unknown. The mortality risk in this model was set to $p = 0.001$, 0.01 and 0.1 to test the effect of mortality on foraging decisions. For all other model computations a fixed mortality risk of $p = 0.01$ for host foraging and $p = 0.1$ for food foraging was selected. Feeding on a nectar source poses a higher mortality risk than ovipositing into a host as for example, nectar or honeydew attract frequently insect predators (Morse 1986; Maingay *et al.* 1991; pers. observation). A wasp is assumed to encounter a maximum number of 10 hosts per day (Nealis 1990) if hosts are abundant. For the sake of simplicity, a foraging day consisted of 10 hours foraging time. One time interval in the model was one hour. Female *C. rubecula* in large field cages had a maximum longevity of 12 days (Chapter 6). Therefore, the time horizon was set to 120 day light hours (1 day = 10 foraging hours). The model was written and executed in MS Excel using Visual Basic programming code (see Appendix). Parameters used in the model are summarised in the following table (Tab.7.2):

Table 7.2 Description of parameters used in the stochastic state dynamic model

Parameter	Description	Units
t	Single time unit	1 hr
T	Number of total time intervals	120 hrs
C	Initial and maximum carbohydrate capacity	24 48
c	Minimum carbohydrate reserves	6
E	Carbohydrate food reward	12
a_R	Foraging costs per time interval depending on resource density	$a_{low} = 1$; $a_{medium} = 2$; $a_{high} = 3$
a'	Foraging costs at rest	0
R	Immediate fitness reward after oviposition	1 egg
λ_R	Probability of encountering a resource at a given density (applies to both host and food densities)	$\lambda_{low} = 0.01$; $\lambda_{medium} = 0.43$; $\lambda_{high} = 0.77$
β	Mortality per time interval;	$\beta_{low} = 0.001$ $\beta_{medium} = 0.01$ $\beta_{high} = 0.1$
	fixed mortality rate per time interval for host and food foraging in basic model	$\beta_{host} = 0.01$; $\beta_{food} = 0.1$

7.2.3 Programming equation

The dynamic programming equation of fitness gain during a time interval t is as follows:

$$
\begin{aligned}
F(x, t, T) = \max \big[& (1 - \beta_h) * \lambda_{Rh} * (F(x - a_R, t + 1, T) + R) && \text{'host foraging successful'} \\
+ & (1 - \beta_h) * (1 - \lambda_{Rh}) * F(x - a_R, t + 1, T), && \text{'host foraging unsuccessful'} \\
& (1 - \beta_f) * \lambda_{Rf} * F(x - a_R + E, t + 1, T) && \text{'food foraging successful'} \\
+ & (1 - \beta_f) * (1 - \lambda_{Rf}) * F(x - a_R, t + 1, T), && \text{'food foraging unsuccessful'} \\
& (1 - \beta_r) * F(x - a', t + 1, T) \big]. && \text{'resting'}
\end{aligned}
$$

The first two terms on the right hand side of the equation are concerned with host foraging, the following two terms with food foraging and the last term represents resting as an alternative. The first term in the host foraging equation represents a fitness value associated with encountering a host and the second term a fitness value associated with not encountering a host. The food foraging equation is structured in the same way: the first term represents fitness associated with encountering food and the second, fitness after not encountering food. An equation for resting is given as a third "choice".

7.3 Results

Results were presented as graphical plots in which the optimal behaviour is a function of energy reserves, extrinsic mortality risks, energetic values of the food and parasitoid life expectancy. Three "benchmark" probabilities to find resources such as hosts and food were set to a low, medium and high probability and its combinations resulted in nine different habitat scenarios (ranging from a pessimistic scenario where both resources, hosts and food are scarce to an optimistic scenario where both resources are abundant). The behavioural strategy 'resting' never appeared as an optimal choice and was not further described in this section.

7.3.1 The impact of extrinsic mortality risk on food foraging

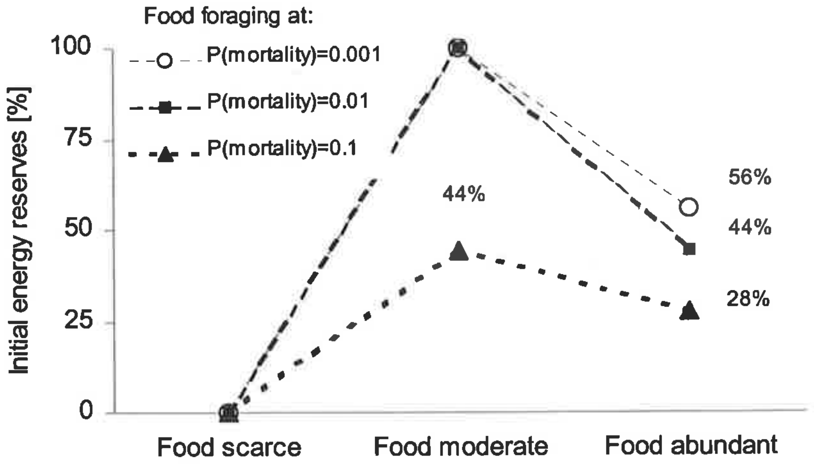
Generally, an increase in the risk of death leads to a switch to food foraging at lower energy reserves (Fig. 7.2 a-c). Variation in the mortality risk has little impact on the switch to food foraging if food is abundant but a large impact if food is moderately available. Parasitoids never forage for food if the availability of food was low regardless of mortality risk. If the availability of food is moderate and the mortality risk is relatively low (prob. = 0.001- 0.01), parasitoids forage immediately after emergence (energy reserves ≤ 100%). At a relatively high mortality risk (prob. = 0.1) parasitoids start to forage for food after their reserves drop below approx. 44%.

The impact of extrinsic mortality on foraging behaviour changes little if the availability of hosts is considered. Generally, an increase in host abundance enhanced the impact of mortality risk leading to a start of food foraging at slightly lower energy reserves. If the mortality risk is relatively low and food is moderately available, the availability of hosts has no impact on foraging decisions (Fig.7.2 a-b). If hosts are abundant and the mortality risk is high, parasitoids wait longest before starting to search for food (Fig. 7.2c). Under these conditions the availability of food has less impact on foraging decisions.

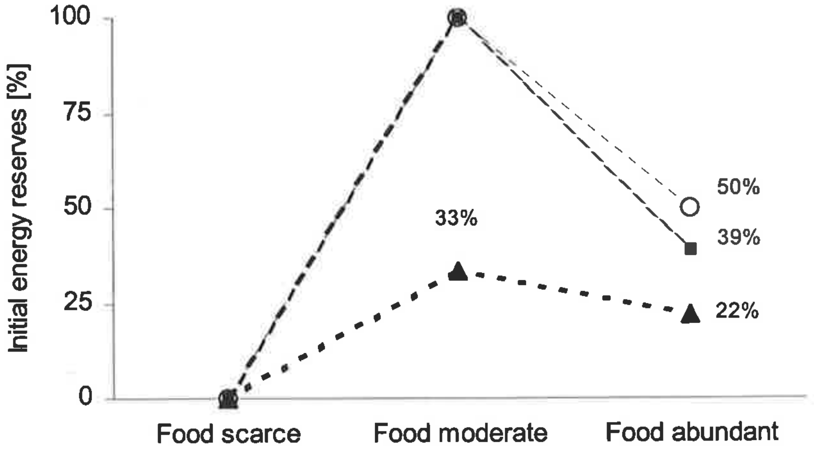
7.3.2 The effect of resource availability on food foraging

Food availability had a stronger effect on the switch to food foraging than host availability (Fig. 7.3). Generally, if hosts are becoming scarce, food foraging starts at slightly higher energy reserves. If food is scarce, the ‘optimal forager’ devotes its time exclusively to host foraging (Fig.7.3 a-c). The model predicts that the frequency of food foraging is highest (in the sense that the switch to food foraging starts at relatively high energy reserves) in habitats where hosts are scarce and food is moderately available (Fig. 7.3a). The lowest frequency in food foraging occurs when both, hosts and food, are abundant (Fig. 7.3c).

(a) Hosts scarce



(b) Hosts moderate



(c) Hosts abundant

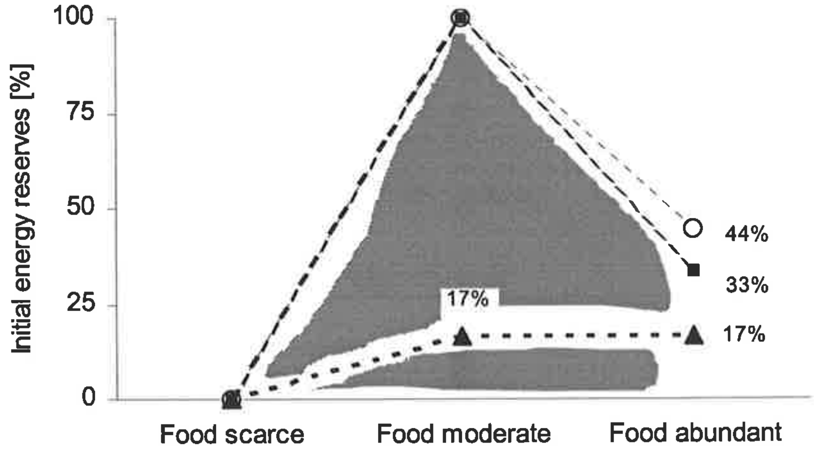


Figure 7.2 Impact of extrinsic mortality on the switch to food foraging as a function of carbohydrate energy reserves and availability of food. Scenarios are modelled for **(a)** low host availability, **(b)** medium host availability and **(c)** high host availability.

Symbols mark the state of energy reserves that initiate food foraging (at higher reserves the parasitoid searches for hosts, at lower reserves for food). Same symbols are connected with dotted lines to show trends within same mortality risk (p=0.001, 0.01 or 0.1) and do not present calculated curves. Area with stripes in **(c)** indicates that at high host availability and moderate food availability, an alternating food/host foraging pattern occurs (for explanation see text).

An alternating foraging pattern with each decrease per unit energy reserves is suggested in a habitat where hosts are abundant and the availability of food is moderate (Fig. 7.3c). Two alternative interpretations on this peculiar output are offered: a) If the forager's energy reserves fall below 50%, a host foraging bout should be followed by a food foraging bout and that in turn, followed by host foraging. If food foraging was successful and the forager increases its reserves to more than 50 %, it should continue to forage for hosts until it reaches the 50% reserve threshold again. In case the forager did not find food, it keeps searching alternately for hosts and food until food is found or until it dies; b) at energy levels below 50%, host and food foraging yield approximately the same fitness and the forager can either continue to forage for hosts or start to forage for food. In respect to overall maximum fitness, it does not matter if the parasitoid searches for food or hosts below 50% energy reserves. The critical energy threshold for food foraging is around 6%. Below this threshold only food foraging is optimal.

7.3.3 Optimal behaviour as function of lifetime

The plots in Figure 7.4 show the same information as described in the previous paragraph, additionally showing optimal behaviour as a function of expected lifetime. The optimal behavioural strategy is fairly constant at high to moderate life expectancies. At short life expectancies the switch to food foraging drops quickly to low energy reserves until shortly before death no food foraging should occur anymore.

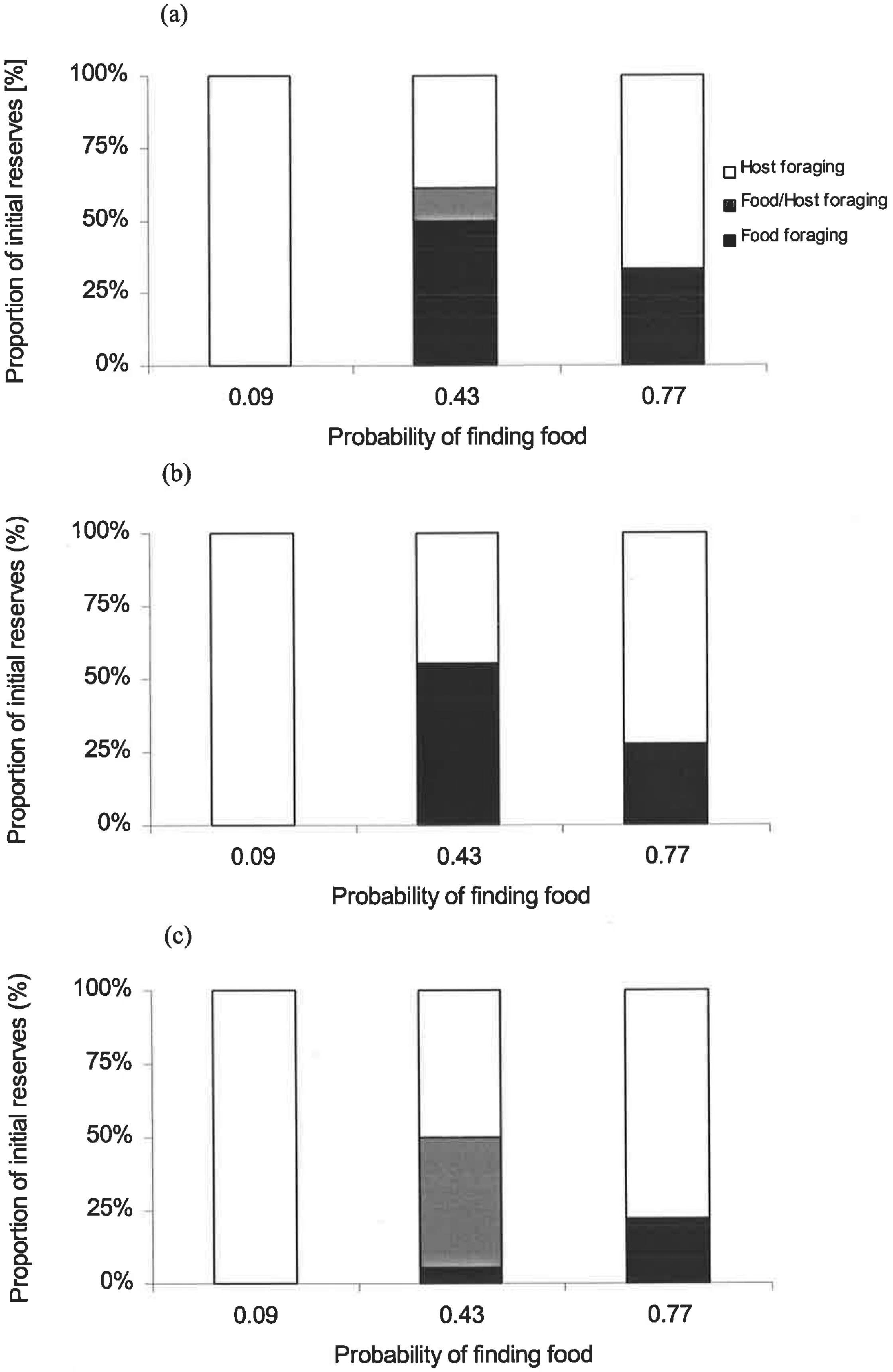
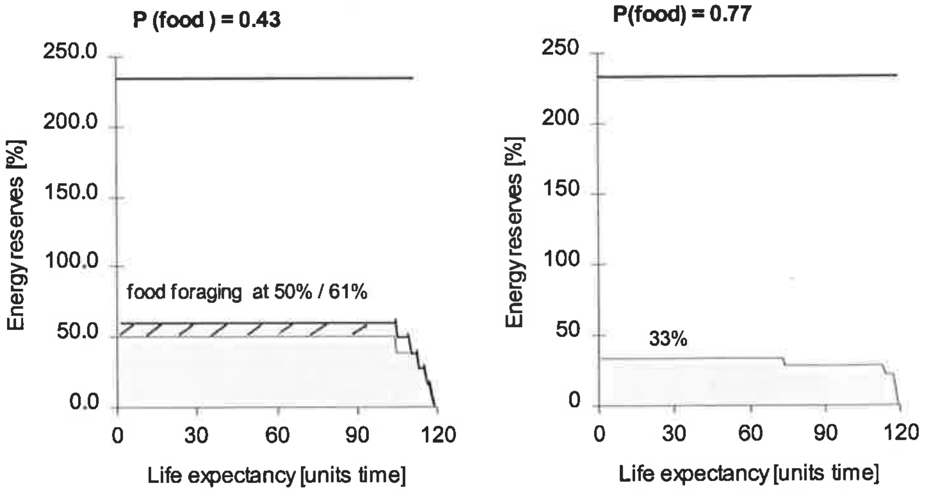
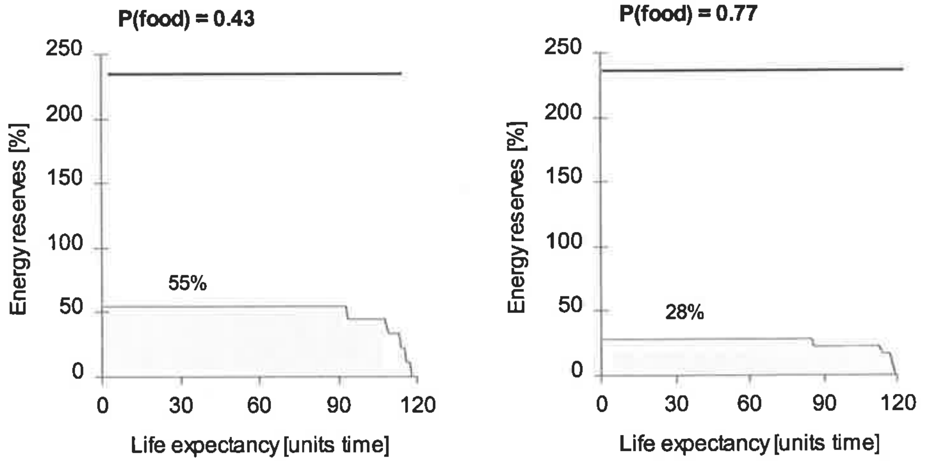


Figure 7.3 Foraging strategy as a function of carbohydrate energy reserves and food availability under **(a)** low, **(b)** moderate and **(c)** high availability of hosts. Energy state at which food foraging occurs is indicated by dark areas and host foraging by white areas. Striped area indicates alternate foraging pattern (for explanation see text).

(a) Hosts scarce



(b) Hosts moderate



(c) Hosts abundant

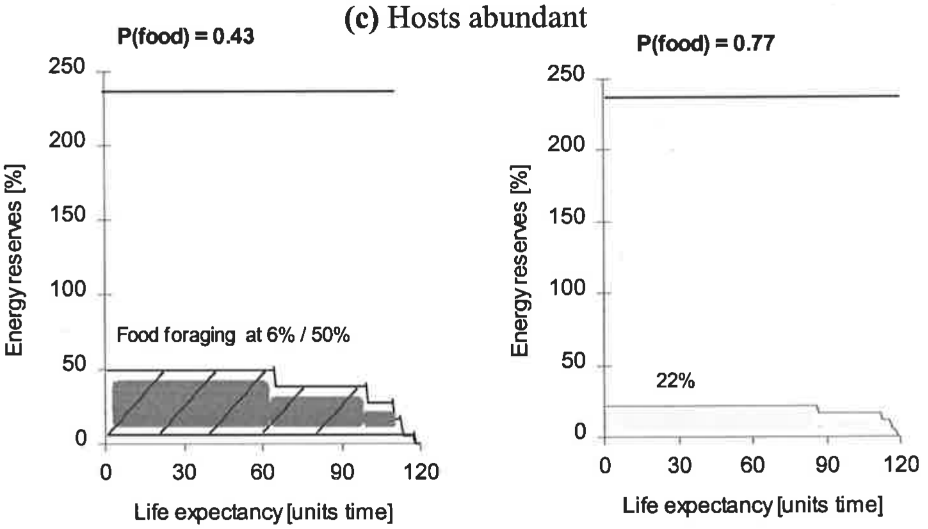


Figure 7.4 Switch to food foraging as a function of carbohydrate energy reserves, food availability and lifetime given **(a)** low, **(b)** moderate and **(c)** high availability of hosts. Solid curve within plot indicates the energy threshold at which food foraging starts. Below the line, parasitoids search for food and above they search for hosts (grey areas indicate food foraging and white areas host foraging). Area with stripes indicates alternate foraging pattern (see explanation in text). Energy reserves at emergence are 100% and 233% at capacity (= upper solid line).

7.3.4 Variations in food reward

To test the effect of the energetic value of food on the onset of food foraging, two scenarios were chosen that *C. rubecula* wasps might encounter in the "real" world: hosts are moderately available, and in those habitats, the probability to find food is either moderate or high ('low' was omitted as a low food availability never results in food foraging in this model). The first scenario could resemble a habitat with nectar producing flowers within some distance to host plants. The other scenario simulates a habitat where honeydew or flowers occur on the same plant that provides the host(s).

The effect of the energetic value of food on the onset of food foraging depended on food availability. The minimum energetic value of a food source that makes it worthwhile to forage for it, is two times higher if food availability is moderate instead of high (Fig. 7.5). If food is moderately available, an increase in the energetic value of food results in the onset of food foraging at increasingly higher energy reserves until it reaches an upper threshold (Fig. 7.5a). If food is abundant, the switch to food foraging above a minimum energy value is independent of the energetic value of the food source (Fig.7.5b).

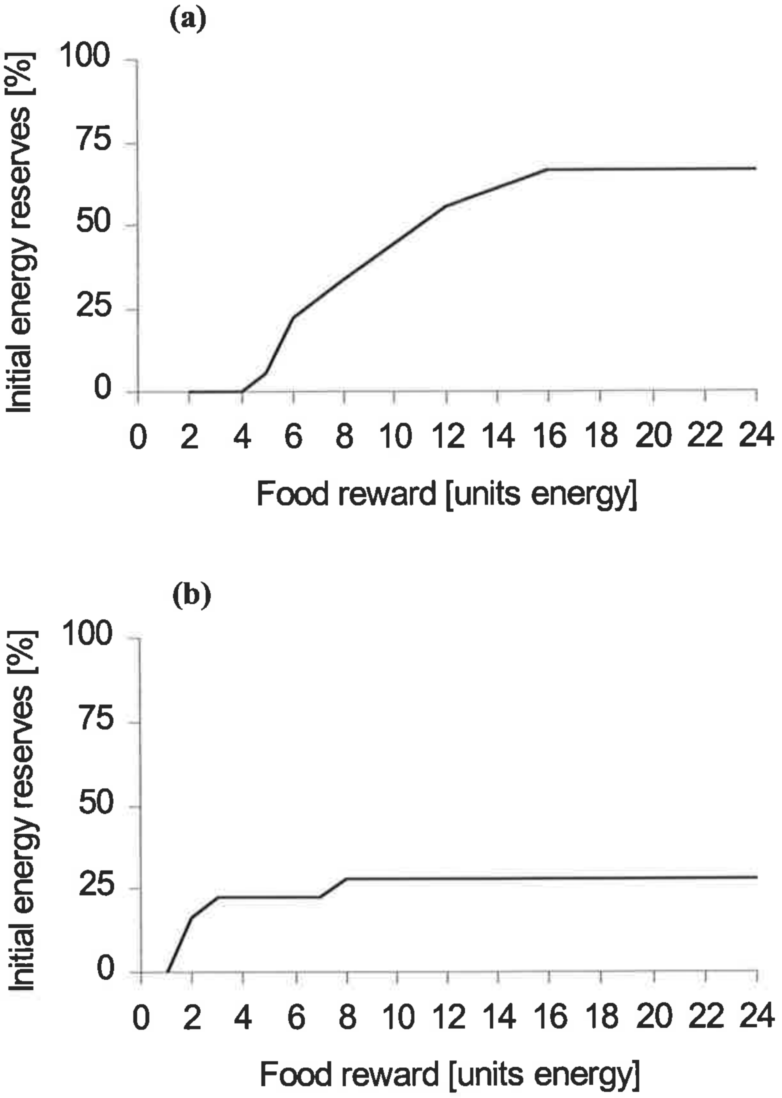


Figure 7.5 The switch to food foraging as a function of energy reserves and expected food reward. Food reward functions are calculated for **(a)** moderate and **(b)** high availability of finding food in an environment where host availability is moderate. Below the solid line parasitoids search for food and above they search for hosts.

Please note that a food reward of 18 units is equivalent to the wasp's initial carbohydrate energy reserves (18 units = 100%)

7.4 Discussion

Insect parasitoids are likely to forage not only for hosts but also for food. The probability of finding hosts and food, along with extrinsic mortality risks and the energetic value of food are major factors influencing the switch from host foraging to food foraging.

Food foraging is a means to extend time available for host foraging (Sirot and Bernstein 1996). The importance of food foraging is therefore tightly linked with the number of hosts a parasitoid can encounter in her lifetime. If it takes a long time to find hosts (= low availability), extending lifespan becomes increasingly important. A low host availability can be characterised by long distances between low density - host patches or effective hide and defence-mechanisms of the host or any other circumstances that result in a low host encounter rate (Godfray 1994). In spite of the advantages of sugar foraging, parasitoids might seldom forage for food if they "expect" a short life due to extrinsic mortality factors. The model predicts that food foraging activities in parasitoids at the end of their life should cease, as the little time remaining must be spent on host foraging to achieve maximum fitness. This applies not only to foragers at the end of their natural maximum lifespan but also to "young" parasitoids with a short life expectancy due to high temperatures or approaching harsh weather conditions. Sugar intake becomes useless shortly before death. It has been shown in experiments that parasitoids are able to perceive short life expectancies and subsequently change their host foraging behaviour (Roitberg *et al.* 1993; Fletcher *et al.* 1994).

The present model predicts that an increase in extrinsic morality (independent of the forager's behaviour) leads to a decrease in the frequency of food foraging. These results confirm the findings of Sirot and Bernstein's parasitoid food foraging model (1996), although they used different mortality values per time period (high risk = 0.05 and low risk =0.00005). Interestingly, the model in this study predicts that variation in the mortality risk hardly affects the switch to food foraging if food is abundant. This is due to the fact that food availability influences the frequency of food foraging. If food is abundant the optimal forager switches from host to food foraging at low energy reserves, which means foraging for food does not occupy a lot of time. Therefore, extrinsic mortality factors such as predation or adverse weather conditions are less likely to hit the parasitoid during food foraging activities but more likely during host foraging activities.

The present model predicts a switch to food foraging at higher energy reserves when food is moderately available than when is abundant. This is also consistent with the findings of Sirot and Bernstein (1996). Additionally, in this study it was possible to quantify at which state of energy reserves female *C. rubecula* start to search for food. If food is not easy to find the forager has to work harder to find it, therefore, food foraging starts when the forager has still substantial carbohydrate reserves left. If food is easy to find, food foraging can wait until around a ¼ or less of the initial energy reserves are reached. This is due to the low cost of energy and time invested into finding food when it is abundant. No food foraging activity should be displayed if food is scarce. The risk of spending time and energy on an unsuccessful food search is too high. However, observations on starved *C. rubecula*-females have shown, that advanced starvation always results in searching the immediate environment for food (Chapter 3).

Given that the forager has some kind of information about food in its environment, the present model predicts that food availability impacts strongly on the onset of food foraging. Such information is likely to be characterised by an innate expectation of food availability and feeding experience. Wäckers (1994) has shown, that starved *C. rubecula* wasps display an innate response to flowers within parasitoid walking distance. Responsiveness to foods that are associated with odours at a longer distance can be stimulated through a previous feeding experience (Lewis and Takasu 1990; Takasu and Lewis 1993; Chapter 3). During the course of life, food foraging can become more efficient because the forager modifies its information on food availability.

Not only the availability of food but its energetic value seems to be of major importance on the switch to food foraging. The present model suggests if the expectation of encountering food is low, the switch to food foraging is highly dependent on the energetic value of the food source. It predicts also that at a high food availability, food foraging starts when energy reserves are low. In this case the expected energy value of food bears no influence on the onset of food foraging because little time and energy has to be invested into finding food. As sugar sources may be abundant but variable in their quality and quantity, many parasitoid species have probably evolved with the expectation that food is "around" but unreliable in its reward. The scenario of always finding some kind of food but it being unreliable in its reward, probably characterises the food foraging behaviour of *C. rubecula* best. As natural sugar sources are tightly linked with plants, one can assume that parasitoids of herbivorous hosts have evolved with sugar sources reliably available. What is not reliable is the quality and quantity of sugar of the food source. Small traces of sugars such as from injured plant tissue, splashed nectar underneath flowers and homopteran honeydew are likely to occur frequently on wild Brassica plants. Many of those, if not all, flower annually (Tsunoda *et al.* 1980) and for a limited time in a season, hosts can be found on plants that are also flowering. Depending on the exposure of nectaries and competition for this food source, food can be highly rewarding when consumed directly from freshly produced nectar or insignificant if nectaries are exploited or concealed.

It seems unlikely that parasitoids such as *C. rubecula* have evolved to actively search for distant sugar sources. It follows from previous observations and this model that food naïve *C. rubecula* will start to forage for food only when its reserves are low (i.e. 25% of its initial carbohydrate reserves) and examine its immediate environment in random search for food. It will not display a long range - food searching behaviour because of an innate short range searching mode and because at this state, has not enough energy left for coordinated flight (see Chapter 3). Generally, if food is not abundant and near host occupied sites, food foraging becomes too risky as an alternative to host foraging. This study has illustrated how the energy state and environmental factors can interact in a female parasitoid and thus provides new directions for future experiments investigating parasitoid (sugar-) foraging behaviour.

Chapter 8 General discussion

The main objective of this thesis was to evaluate the role of sugar foraging in parasitic wasps. A methodological approach was adopted that combined elements from insect physiology, life-history theory and behavioural ecology. This concluding chapter summarises the major research findings and their significance. At the end of this section a brief overview of possibilities for future research is given.

8.1 Sugar foraging in parasitic wasps

The process of sugar foraging has to be placed into its ecological context: sugar foraging and its ultimate effect on fitness (number of offspring produced) can be presented as a chain that is as strong as its weakest link as depicted in the following diagram (Fig. 8.1):

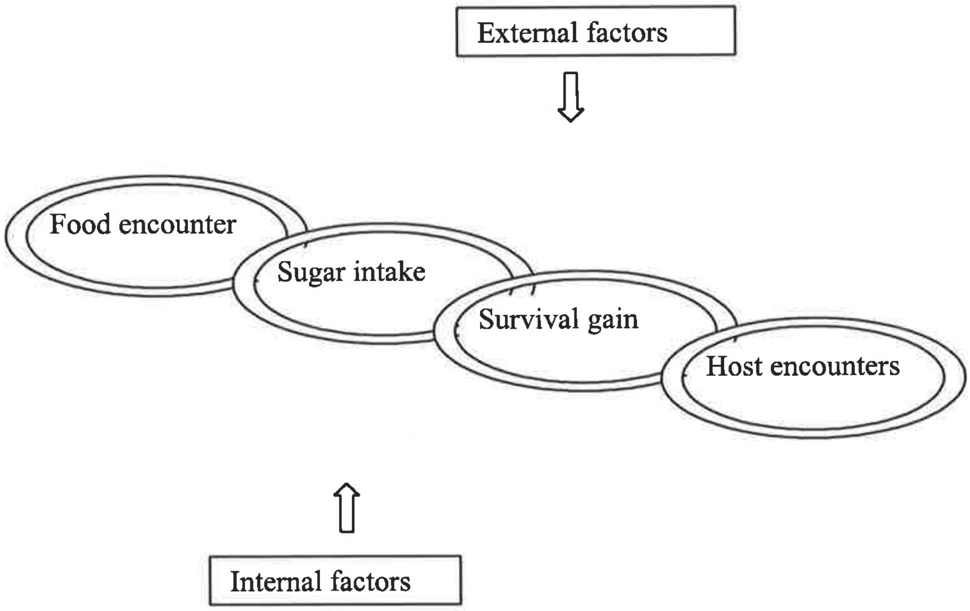


Figure 8.1 Fitness gain from sugar foraging in relation to parasitoid physiology, behaviour and environment.

First of all, a wasp has to succeed in finding or encountering a sugar source, and then it has to consume an amount of sugar that leads to a biologically significant gain in energy reserves. After that, the gain in fitness depends on the availability of hosts that can be encountered during the time gained by sugar intake. To a different degree these steps are affected by environmental (external) factors such as predation, inter- and intraspecific competition and climate. At the same time internal factors in the wasps such as the rate of energy depletion, response to food stimuli, egg load and age act upon steps involved in foraging for sugar as well. Because of all the aforementioned steps involved in this process, the success of sugar foraging depends strongly on the environment, the physiology and behaviour of the forager. Therefore, the rather substantial increases in parasitic wasp survival and fecundity when confined with a sugar source in the laboratory or under similar conditions must be treated with caution when estimating parasitic wasp fitness in the field.

In the remainder of this chapter, findings of this study relevant to the process of sugar foraging are discussed. No biological problem is solved until both proximate and ultimate (= evolutionary) causations are determined (Mayr 1982). Applied to sugar foraging in parasitic wasps, hunger or energy depletion is the proximate causation in parasitic wasps to forage for food. Ultimately, sugar foraging is assumed to increase reproduction by increasing the lifespan to find more hosts (reviewed by Jervis *et al.* 1996). This discussion will reflect on the proximate cause of sugar foraging, followed by an evaluation of the steps involved in the process of food foraging within the lifetime of a parasitic wasp. This will provide a foundation to shed light on the ultimate cause of sugar foraging. The findings presented in this study also have practical implications for conserving natural enemies of insect pests by providing nectar sources in agro-ecosystems.

8.1.1 Food encounter

Parasitic wasps feed because they experience a depletion or shortage in their energy reserves (Zoebelein 1956). The rate of depletion depends on energy demanding activities such as flight and external factors such ambient temperature (Wigglesworth 1972; Chippendale 1978; Friedman 1985). In female *C. rubecula* carbohydrate reserves rather than lipid reserves determine survival and provide fuel for flight (CHAPTER 2). Most or all parasitic wasps readily feed on sugar sources (Jervis *et al.* 1992) and no conversion of carbohydrates into lipids occurs (Ellers 1996; Olson *et al.* 2000; CHAPTER 2). Therefore, the essential function of carbohydrates in survival and flight ability seem to be universal among parasitic wasps. Given the strong relationship between carbohydrates and flight performance, one could speculate that species that fly a lot during their host foraging activities might have either higher carbohydrate reserves at emergence or are "better" sugar foragers than wasps adapted to an environment that requires less flight activity. Currently, the quantity of lipid reserves at emergence is advocated as an indicator for an adaptation to the energy demand of travelling (flight activity) caused by spatial host distribution (large reserves = poor host availability, small reserves = high host availability, Ellers 1996; Ellers and Van Alphen 1997; Ellers *et al.* 1998). As carbohydrates reserves can be maintained by sugar intake, sugar foraging may occur more frequently in wasps adapted to low or unpredictable host availability.

The success of sugar foraging will depend strongly on how well parasitic wasps respond to carbohydrate resources such as flowers and honeydew in the field. Encountering a sugar source could be a completely random process (occurring while searching for hosts) or the result of a directed search, that is mutually exclusive to host searching. It seems to be highly unlikely that parasitic wasps leave a host foraging area for a distant food source (CHAPTER 3). The findings of this study suggest that food sources have to be close to host foraging sites in such way that parasitic wasps such as *C. rubecula* are able to encounter them by chance during host foraging activities. Subsequent nectar experience allows wasps to respond to nearby flowering plants and actively seek them out. But at the same time the presence of hosts will "override" the response to flowers and attract a large proportion of hungry wasps to hosts.

The relative concentration of hosts and nectar cues in the field may shape the foraging strategies of wasps, rather than its "hunger" alone. For example, a high density of hosts produces a high concentration of volatiles attractive to wasps (Kaiser and Carde 1992; Geervliet *et al.* 1998). This could "mask" the odours of flowers present in the same area. Given the same density of floral sugar sources, wasps may search predominantly for hosts in rich host habitats and ignore flowers but would intermittently search for nectar in poorer hosts habitats. It is also possible that a high density of flowers may mask cues from hosts, if low in density, and attract a large number of wasps to flowers. If flowers elicit strong cues but provide little or no reward, wasps may spend a long time searching for food and be "distracted" from host foraging. During this time the risk of predation on flowers (Morse 1986; Maingay *et al.* 1991) or parasitisation of hosts by conspecifics might increase. In this case, the presence of flowers may rather cause harm than benefit to a wasp's survival and reproduction. This has practical implications for the propagation of nectar providing flowers in agro-ecosystems to support natural enemies of insect pests. To plant a high density of flowers increases the chances of sugar encounters, but at times of low host densities foraging for flowers might prevent wasps from finding these hosts. The lack of control at low host numbers might then increase the chances of an outbreak that would not have occurred without the dominant presence of flowers. If parasitoid are not "used to" a high availability of flowers, there might be a danger of confusing the wasp's senses and behaviour.

Even if flowers are highly rewarding and a wasp could leave quickly after a satiating nectar meal, the high concentration of floral cues might trap the forager within the patch of flowers. This is a situation comparable to moths attracted to and trapped by light sources in the dark.

The association of sugar with host plant(s) a parasitic wasp has evolved with, is probably a key element in sugar foraging behaviour. If the location, where hosts are found, provides nectar or honeydew, parasitic wasps might always search for sugar in the vicinity of hosts. For example, female *C. rubecula* are adapted to forage on cruciferous plants (i.e. wild radish, wild mustard) for larval *P. rapae*. Usually these plants flower annually (Tsunoda *et al.* 1980) (although for a limited time during the wasp's foraging season) and might harbour honeydew-producing aphids. Hence, *C. rubecula* wasps may be adapted to environments where plants that bear hosts provide some food. In this situation random food searching behaviour on the host plant makes sense. Generally, parasitoids of herbivorous hosts are likely to have evolved with honeydew, floral or extrafloral nectar on their host plant. The stronger the association of the host plant with a sugar source, the less a parasitoid might search for food away from a host bearing plant. The parasitoid *Evodum puttleri* searching on potato plants for potato beetles did not visit flowering plants nearby because they feed on the honeydew of potato aphids (Idoine and Ferro 1988). The strongest association between hosts and sugar must occur in aphidophagous parasitoids. Their sugar foraging behaviour is likely to be focussed on host occupied areas; host and food foraging in aphidophagous parasitoids might be almost indistinguishable (Grasswitz and Paine 1993). If "host plants" are not providing food itself they might have grown in a plant community that provide certain nectar plants. This plant community could have emerged through common soil requirements and climatic conditions. Parasitic wasps adapted to this kind of environment might search for flowering plants in the vicinity of a host plant.

8.1.2 Food intake and survival gain

Even after having located a potential sugar source, its access might pose the next hurdle in sugar foraging. Plant structure and floral architecture influence how much parasitoids are able to feed (Patt *et al.* 1997; CHAPTER 4). Small-scale feeding experiments are sufficient to test the food value of a particular sugar source, but ultimately the behaviour of a wasp on sugar sources such as flowering plants has to be observed (for example: Idris and Grafius 1997). This is to document how restricted sugar access and intake is and how often a wasp has to revisit a particular sugar source to maintain its energy reserves. Although honeydew in the present study was of lower quality than floral nectar, its accessibility may make it a more available sugar source for parasitoids such as *C. rubecula* than nectar in a concealed flower. If wasps are locating flowers via flight, the structure of the landing area (smaller or larger than wasp's body) may affect the wasp's landing manoeuvre and subsequent sugar access.

Encountering sugar sources in nature may be rather sporadic events and the gain from a single feeding event can be critical. If a single meal lasts a long time, more time is available to forage for hosts and the chances to encounter sugar a second or third time are high. Parasitic wasps such as *C. rubecula* have to locate food once every 1-2 days to achieve maximum expected lifespan given the food source is abundant and of high quality (CHAPTER 5). Sugar sources have to be distributed in such way that parasitic wasps are likely to encounter them during these intervals. The other condition is that food quality and quantity per single meal provide sufficient sugar. In contrast to non-host feeding species, sugar feeding might be less frequent in host feeding species if host hemolymph contributes to some degree to survival. In host-feeding species sugar intake usually increases lifespan greatly as well, but in some, feeding on hosts alone can also extend lifespan (Jervis and Kidd 1986; Heimpel and Collier 1996). The relative benefits of sugar and host feeding vary considerably in host feeding species and are likely to depend on host physiology (hemolymph composition) in conjunction with the type of sugary food consumed. Rivero and Casas (1999a) highlighted the complex nature of nutrients ingested in both sugary foods and host hemolymph: nectar and honeydew are rich in sugars but also contain amino acids, vitamins and other constituents (Baker and Baker 1983), whereas host hemolymph contains proteins, lipids but also carbohydrates among other substances (Mullins 1985). However, sugar concentration of insect hemolymph is extremely low (for example, in larval *Manduca sexta* 1-1.8%, Ziegler (1985)) and rarely exceeds 5% (Wigglesworth 1972). A measurable survival gain in parasitic wasps is usually found in sugar concentrations at or over 10% (Sengonca and Peters 1993; Morales-Ramos *et al.* 1996; Wäckers 1999; CHAPTER 5).

8.1.3 Nectar sources, survival and reproduction - Implications for biocontrol programs

Nectar sources close to plants with hosts resulted in a higher lifetime reproduction of parasitic wasps than without this food source (CHAPTER 6). However, the gain in lifetime and reproduction was only a fraction of potential longevity and reproduction in female *C. rubecula*. The field trials in this study revealed that the effect of flowers on parasitoid reproduction was strongly temperature dependent. The warm to hot conditions in South Australia strongly limited survival and possibly the food foraging behaviour in female *C. rubecula*. It is likely that in cooler climatic regions the availability of nectar producing flowers will increase lifespan and reproduction to a higher degree.

Jervis and Kidd (1999) noticed that the lack of food is currently ranking as a minor cause for pest control failure in biological control literature but point out that this undervalues the real importance of food availability. The failure of achieving higher pest suppression by propagating flowers providing accessible nectar for parasitoids is assumed to be caused rather by inadequate experimental design than a lack of sugar sources increasing parasitoid lifetime reproduction (Jervis *et al.* 1996). The success of propagating flowers in agricultural habitats depends on the careful selection of flower species and their strategic positioning within a crop field (Jervis *et al.* 1996). The findings of this thesis emphasise that even with the correct techniques applied, the provision of sugar sources may still lead to suboptimal results (or even failure) because of climatic conditions of a region and "interference" with the density of hosts when it is high. Several other factors such as inter-specific competition for nectar (M. A. Keller, pers. communication) and the accidental support of pest insects such as Lepidoptera may also limit the effect of sugar sources on pest suppression (Jervis *et al.* 1996; Baggen and Gurr 1998). If food sources cannot be equally distributed within a large cropping area, parasitoids might be drawn away from "untreated" areas (=without food sources), resulting in less pest suppression and higher crop damage in these areas than without food sources provided (Jervis *et al.* 1996). Additionally, if mortality factors other than starvation dominate parasitoid survival than the provision of food sources has little or no effect on pest suppression. The aforementioned factors show that the effect of sugar sources on pest suppression is complex. Each cropping system with its associated fauna in a particular geographical region has to be individually assessed to estimate the expected effect of propagated sugar sources.

8.1.4 Optimal behaviour and sugar foraging

Optimal foraging theory can be helpful in understanding why parasitic wasps should search for food. Optimisation models are frequently used to explore the effects of internal and external factors on foraging strategies (McNamara and Houston 1986; Mangel and Clark 1988). In this thesis, a stochastic-dynamic model was designed to explore the influence of sugar foraging on fitness (number of eggs laid, R_0) (CHAPTER 7). In comparison to the impact of external mortality risks and food availability, the availability of hosts had a marginal effect. This was a rather unexpected outcome (and contradicts intuitions about the impact of host availability). These contradictions may be a fault at the level of the model formulation. Host availability should strongly influence the fitness gain from sugar foraging. For example, if the host encounter rate was low (hosts were scarce) then sugar intake would substantially contribute to reproductive success by extending the energy or time available to find more hosts. If the host encounter rate was high (hosts were abundant), wasps would be able to lay most or all of their eggs quickly and do not need to feed to gain higher survival or searching efficiency.

A crucial argument for the contribution of sugar foraging to reproduction is that initial energy reserves limit the time or energy parasitoids have available to find hosts (CHAPTER 1). However, the need for sugar foraging depends strongly on the extent to which starvation limits parasitoid survival and reproduction in the field. If other mortality factors such as predation, hyperparasitism, disease or adverse weather conditions limit survival in a wasp population more than insufficient sugar intake, then sugar feeding becomes redundant (CHAPTER 6, CHAPTER 7). Only if all mortality factors are considered, and preferably quantified, can the true contribution of sugar intake to reproduction be calculated.

Another important point in regard to the evaluation of sugar foraging is the definition of "time limited" reproduction. The degree of time limitation is usually calculated by the difference between potential egg load and numbers of eggs laid during lifetime (Sevenster *et al.* 1998). However, it is important to consider time limitation relative to host availability as well. For example, limited reproduction in wasps with a potential fecundity of 100 eggs (i.e. in *Cotesia rubecula*) that die with 80 eggs still left to lay, could be caused by premature mortality such as starvation (time limitation) or a low host availability (host limitation). In parasitoids with high egg loads, potential fecundity may lead to an overestimation of time limitation. Most species of parasitic wasps have evolved with egg loads larger than they usually can lay within a lifetime, as hosts are distributed in patches (plants, leaves, fruits) with varying numbers of hosts per patch in an unpredictable manner (Sevenster *et al.* 1998; Ellers *et al.* 2000; but see Heimpel and Rosenheim 1998). In other words, wasps are equipped to handle rare case of high host densities but most of the time, encounter much lower host densities. Therefore, the degree of limited reproduction should be measured not only relative to the maximum potential fecundity but also relative to the most common host density parasitoids are likely to encounter. The "future" fitness gain from sugar foraging depends on how many hosts a parasitoid can encounter within the time gained by sugar intake and less on its maximum egg load.

In regard to sugar foraging, parasitic wasps can be compared with mosquitos that search for hosts (host blood needed to mature eggs) and sugar (maintaining survival) as well. Mosquito research in regard to sugar feeding habits is more advanced than parasitic wasp sugar feeding because of its relevance to human health. Methods in regard to insect sugar research can be adopted from there (as for example lipid and carbohydrate extraction method in CHAPTER 2 originates from mosquito research). A comprehensive and highly recommendable study for parasitic wasp sugar research is a review on "mosquito sugar feeding and reproductive energetics" by Foster (1995). This review shares many parallels in respect to sugar searching, sugar metabolism and its ultimate contribution to fitness. The author concludes, "*the assumption that sugar availability is not limiting to mosquito fitness is undermined by a growing body of circumstantial evidence, but a definitive study has yet to prove otherwise. The wide variation in sugar availability is apparently reflected by the extreme sugar procurement by resourceful generalist species [...]. But whether restricted sugar feeding leads to less successful reproduction is a more tenuous inference supported by few field observations.*" The main difference between mosquito and parasitic wasp sugar foraging is that parasitic wasps of herbivorous hosts may have evolved with an association between nectar/ honeydew sources on or near host plants. Mosquitos have evolved with hosts (mammals) and sugar sources (flowers, honeydew), which are not associated with each other. Therefore, mosquito sugar searching behaviour is likely to be more advanced than in parasitic wasps. Additionally, the mosquito feeding apparatus is specialised to pierce through host skin and suck blood. This is an advantage to reach nectar in a wide range of flowers with a variety of floral architectures that usually limit the majority of parasitic wasps having unspecialised mouthparts. Jervis (1998) demonstrated that an estimated 3% of hymenopteran parasitoids species have evolved with a nectar extraction apparatus, thus indicating that in some cases parasitic wasps have specialised to feed from flowers in their habitat. Interestingly, in dipteran parasitoids approximately 25% of all species have a specialised feeding apparatus, which is attributed mainly to phylogenetic history than ecological "practicality" (Gilbert and Jervis 1998).

In conclusion, the area in a habitat in which parasitic wasps search for sugar, is likely to depend on the degree of association of host plants with nectar or honeydew. Findings of this study support the hypothesis that sugar encounters in the field happen rather opportunistically during host foraging activities. If sugar feeding occurs, the survival gain may be often severely limited by profitability (quality and quantity) of sugary food and extrinsic mortality factors such as adverse weather conditions and predation. The need for food has to be closely studied in relation to a species' adaptation to its environment. The availability of both, hosts and food, as well as food quality and extrinsic mortality factors, influence the benefits and risks of sugar foraging. The costs of finding sufficient sugar to increase expected lifetime may often be larger than its benefits. It is true to say that a high availability of sugar sources in the field, such as by manipulation of flower availability in biological control programs, will increase parasitoid survival and reproduction. However, this increase may be rather marginal and will be sensitive to host and food availability relative to each other and to the aforementioned environmental factors.

8.2 Future research

Although a comprehensive perspective on the role of sugar foraging has been presented, this thesis has opened up new ideas for future research. The following section provides an overview of research possibilities.

- *Resource availability* A major conclusion of this thesis is that variation in the relative availability of host and food may have a strong effect on the incidence of sugar feeding in parasitic wasps and its effect on their reproduction. This hypothesis needs to be experimentally tested further. This research will be useful in regard to biocontrol programs try predicting when flowers contribute most to parasitoid fecundity and pest mortality.
- *Host-sugar association* Each parasitoid-host system is associated with a particular host plant or breeding substrate. Parasitoid sugar foraging is likely to be adapted to the availability of sugar in this system. It may be worthwhile to compare the sugar foraging behaviour of species of parasitoids whose host feeding location is to a varying degree associated with sugar. This may shed light onto the likelihood of the attraction to distant food sources.
- *Host feeding - Sugar feeding* The contribution of sugar intake to survival and reproduction may vary in host feeding and non-host feeding species (Heimpel *et al.* 1997a; Rivero and Casas 1999a). Therefore, between these species, a comparison of

survival gain and egg development (in synovigenic species) after sugar intake, may clarify the need for sugar relative to host feeding activities.

- *Energy metabolism* Further quantification and comparison of parasitoid energy reserves such as lipids and carbohydrates under different environmental conditions (host availability, temperature) is a useful tool to predict why and when a wasp has to feed on sugar.
- *Searching behaviour* To evaluate the effect of certain flowers species on parasitoid survival, more observations are needed on the behaviour on the flower plant in addition to considering only the food quality. In particular, questions have to be answered such as: how does the wasp locate the plant, how does it land on it under natural conditions (i.e. consider wind) and how easily can it move along the leaves, stems and flowers? Additionally, post-feeding observations can reveal how quickly the wasp returns to host foraging activities.
- *Multitrophic context* Sugar sources may not only beneficial for parasitic wasps but also for their pests. The advantage of sugar feeding in the reproduction of parasitic wasps cannot be evaluated alone if adults of its hosts can feed on the same sugar source as well (as in demonstrated in (Baggen and Gurr 1998). In case of *C. rubecula*, which parasitises larvae of the cabbage white butterfly, flowers of or near host plants are also beneficial to the adult "host" butterfly. Along the same line, sugar sources may also benefit predators and hyperparasitoids, thereby limiting the potential fitness gain from sugar intake in parasitic wasps. Future research may be more accurate in predicting the survival gain from sugar intake by considering the ecological community in which a particular parasitoid species must operate.

- *Experiments and parasitoid nutritional state* Although not directly related to this work, the consideration of the parasitoid's nutritional state in host foraging experiments should be given more attention. Almost as a general culturing rule, parasitoids are held with ample sugar conditions (honey or similar). Parasitoids entering experiments are extremely well fed which is an unlikely state under natural conditions. Ample feeding on a rich sugar source not only replenishes depleted energy reserves, it "equips" the wasp with more carbohydrate reserves than at the time of emergence. Wasps with such a high, potential life expectancy are likely to react differently to experimental set ups than unfed wasps (Takasu and Lewis 1995; Stapel *et al.* 1997). Especially, experimenters testing optimal host foraging decisions (i.e. oviposition rates, patch residence time and superparasitism) are advised to control for sugar intake (= life expectancy) in future experiments.

- *Interdisciplinary cooperation* In regard to research on the ultimate contribution of sugar feeding to lifetime reproduction, it is highly advisable to employ optimality modelling to identify factors that play a key role in sugar foraging. Currently, experimentally derived biological information to parameterise models is available from the literature, which may save a great deal of time and labour allocated otherwise to field experiments. Optimality models may help to point out what experimental design to choose. For this purpose the collaboration with applied mathematicians should be sought, to develop a suitable "sugar – foraging model" for a particular parasitoid species.

Appendix

Publication

Hitherto, the following publication has arisen from this thesis:

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

Programming code

Programming code of ‘foraging model’ in Chapter 8 – VBA in MS Excel

```
Sub FoodHost()
Dim L(3) As Single, B(3) As Single              ' resource probability, mortality risk
Dim A(3) As Integer, Y(3) As Integer            ' costs and food reward
Dim R(3) As Single                              ' fitness increment (host parasitised) per period
Dim D(48) As Integer                            ' optimal patch
Dim F0(48) As Single, F1(48) As Single          ' correspond to F(x,t,T) and (F(x,t+1,T)
Dim X As Integer, i As Integer                  ' energy state and patch number
Dim t As Integer                                ' time(life expectancy)
Dim p As Integer                                ' time interval
Dim VI As Single, VM As Single                  ' initial value, Value maximum
Dim Xi As Integer, Xii As Integer               ' state after food found or not found
Dim XC As Integer, C As Integer                 ' minimum and maximum energy state

XC = 6
C = 48

‘ scenario “ hosts are abundant and food is moderately available:

B(1) = 0.01:    B(2) = 0.1:     B(3) = 0.0001
A(1) = 1:       A(2) = 2:       A(3) = 0        ' decision 1 = host foraging
L(1) = 0.77:    L(2) = 0.43:    L(3) = 0        ' decision 2 = food foraging
Y(1) = 0:       Y(2) = 12:      Y(3) = 0        ' decision 3 = resting
R(1) = 1:       R(2) = 0:       R(3) = 0

t = 120

For X = XC + 1 To C                             ' initialise F1
F1(X) = 0
Next X
F1(XC) = 0

' program loop

For t = 120 To 1 Step –1

'Looping:
  For X = XC + 1 To C
    VI = 0
    For i = 1 To 3
      Xi = X - A(i) + Y(i)

      'Chop between C and XC

      If Xi < XC Then Xi = XC
      If Xi > C Then Xi = C
      Xii = X - A(i)
```

```
 'Chop between C and XC

          If Xii > C Then Xii = C
          If Xii < XC Then Xii = XC


 'Calculate the value of patch I

          VM = (1 - B(i)) * (L(i) * (F1(Xi) + R(i))) + (1 - B(i)) * ((1 - L(i)) * F1(Xii))



If VM >= VI Then
          VI = VM
          D(X) = I

          End If


Next i
       F0(X) = VI

   Next X

'Next t
   F0(XC) = 0

'print out results

'For p = t - 1 To 1
   Worksheets("FH1").Range("B2") = "Time"
   Worksheets("FH1").Range("C2") = "State"
   Worksheets("FH1").Range("D2") = "Fitness Parameter "
   Worksheets("FH1").Range("E2") = "Optimal Decision"

   For X = XC + 1 To C

   Worksheets("FH1").Cells(X + (48 - XC) * (t - 1), 2).Value = t
   Worksheets("FH1").Cells(X + (48 - XC) * (t - 1), 3).Value = X
   Worksheets("FH1").Cells(X + (48 - XC) * (t - 1), 4).Value = F0(X)
   Worksheets("FH1").Cells(X + (48 - XC) * (t - 1), 5).Value = D(X)

   Next X

   'update F1(X)

   For X = XC + 1 To C
   F1(X) = F0(X)

   Next X

 Next t


 End Sub
```

References

Altieri, M.A. and Whitcomb, W.A. (1979): The potential use of weeds in the manipulation of beneficial insects. HortScience 14: 12-18.

Andow, D.A., Nicholson, A.G., Wien, H.C., and Willson, H.R. (1986): Insect populations on cabbages grown with living mulches. Environmental Entomology 15: 293-299.

Andow, D.A. and Prokrym, D.R. (1990): Plant structural complexity and host-finding by a parasitoid. Oecologia 82: 162-165.

Asgari, S. and Schmidt, O. (1994): Passive protection of eggs from the parasitoid, *Cotesia rubecula*, in the host, *Pieris rapae*. Journal of Insect Physiology 40: 789-795.

Baggen, L., Gurr, G., and Meats, A. (1999): Flowers in tri-trophic systems: mechanisms allowing selective exploitation by insect natural enemies for conservation biological control. Entomologia Experimentalis et Applicata 91: 155-161.

Baggen, R.L. 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.

Bailey, V.A., Nicholson, A.J., and Williams, E.J. (1962): Interaction between hosts and parasites when some host individuals are more difficult to find than others. Journal of Theoretical Biology 3: 1-18.

Baker, H.G. and Baker, I. (1983): A brief historical review of the chemistry of floral nectar. In: The Biology of Nectaries (eds. B. Bentley and T. Elias), pp. 126-153. Columbia University Press, New York.

Beenakkers, A.M.T. (1969): Carbohydrate and fat as a fuel for insect flight. A comparative study. Journal of Insect Physiology 15: 353-361.

Bernstein, C., Kacelnik, A., and Krebs, J.R. (1988): Individual decisions and the distribution of predators in a patchy environment. Journal of Animal Ecology 57: 1007-1026.

Bigger, D.S. and Chaney, W.E. (1998): Effects of *Iberis umbellata* (Brassicacea) on insect pests of cabbage and on potential biological control agents. Environmental Entomology 27: 161-167.

Blaney, W.M., Schoonhoven, L.M., and Simmonds, M.S.J. (1986): Sensitivity variations in insect chemoreceptors; a review. Experientia 42: 13-19.

Boose, D.L. (1997): Sources of variation in floral nectar production rate in *Epilobium canum* (Onagraceae): Implications for natural selection. Oecologia Berlin 110: 493-500.

Breed, M.D. and Julian, G.E. (1992): Do simple rules apply in honey-bee nestmate discrimination? Nature 357: 685-686.

Briggs, C.J., Nisbet, R.M., Murdoch, W.W., Collier, T.R., and Metz, J.A.J. (1995): Dynamical effects of host-feeding in parasitoids. Journal of Animal Ecology 64: 403-416.

Bursell, E. (1974): Environmental aspects. In: The Physiology of Insecta (ed. M. Rockstein), pp. 284-317. Academic Press, New York.

Cameron, P.J., Walker, G.P., and Keller, M.A. (1995) Introduction of *Cotesia rubecula*, a parasitoid of White Butterfly. In: 48th Annual NZPPS Conference (ed T.N.Z.P.P. Society). The New Zealand Plant Protection Society, Hastings.

Candy, D.J. (1989): Utilization of fuels by the flight muscles. In: Insect Flight (eds. G.J. Goldsworthy and C.H. Wheeler), pp. 300-313. CRC Press, Boca Raton, Florida.

Cappuccino, N., Houle, M.-J., and Stein, J. (1999): The influence of understorey nectar resources on parasitism of the spruce budworm *Choristoneura fumiferana* in the field. Agricultural and Forest Entomology 1: 33-36.

Chippendale, G.M. (1978): The functions of carbohydrates in insect life processes. In: Biochemistry of Insects (ed. M. Rockstein), pp. 2-55. Academic Press, New York.

Clausen, C.P. (1940). Entomophagous insects. McGraw-Hill, New York.

Collier, T.R., Murdoch, W.W., and Nisbet, R.M. (1994): Egg load and the decision to host-feed in the parasitoid, *Aphytis melinus*. Journal of Animal Ecology 63: 299-306.

Collier, T.R. (1995): Adding physiological realism to dynamic state variable models of parasitoid host feeding. Evolutionary Ecology 9: 217-235.

Cook, R.M. and Hubbard, S.F. (1977): Adaptive searching strategies in insect parasites. Journal of Animal Ecology 46: 115-125.

Cowgill, S.E. (1995): Influence of the chickpea cropping system on *Helicoverpa armigera* (Lep.: Noctuidae) populations and their rate of parasitism by *Campoletis chloridae* (Hym.: Ichneumonidae). Entomophaga 40: 307-315.

Crabtree, B. and Newsholme, E.A. (1975): Comparative aspects of fuel utilisation and metabolism by muscle. In: Insect Muscle (ed. P.N.R. Usherwood). Academic Press, London.

DeBach, P. and Rosen, D. (1991). Biological control by natural enemies. Cambridge University Press, Cambridge.

Den Otter, C.J. and Saini, R.K. (1985): Pheromone perception in the tseste fly *Glossina morsitans*. Entomologia Experimentalis and Applicata 39: 155-161.

Den Otter, C.J., Tchicaya, T., and Schutte, A.M. (1991): Effects of age, sex and hunger on the antennal olfactory sensitivity of tsetse flies. Physiological Entomology 16: 173-182.

Dethier, V.G. (1982): Mechanism of host-plant recognition. Entomologia Experimentalis and Applicata 31: 49-56.

Dover, J.W. (1986): The effect of labiate herbs and white clover on *Plutella xylostella* oviposition. Entomologia Experimentalis and Applicata 42: 243-247.

Downer, R.G.H. (1981): Physiological and environmental considerations in insect bioenergetics. In: Energy Metabolism (ed. R.G.H. Downer), pp. 1-17. Plenum Press, New York.

Driessen, G. and Hemerik, L. (1992): The time and egg budget of *Leptopilina clavipes*, a parasitoid of larval *Drosophila*. Ecological Entomology 17: 17-27.

Driessen, G., Bernstein, C., Van Alphen, J.J.M., and Kacelinik, A. (1995): A count-down mechanism for host search in the parasitoid *Venturia canescens*. Journal of Animal Ecology 64: 117-125.

Dyer, L.E. and Landis, D.A. (1996): Effects of habitat, temperature, and sugar availability on longevity of *Eriborus terrebrans* (Hymenoptera: Ichneumonidae). Environmental Entomology 25: 1192-1201

Ellers, J. (1996): Fat and eggs: An alternative method to measure the trade-off between survival and reproduction in insect parasitoids. Netherlands Journal of Zoology 46: 227-235.

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. (1998) Life-history evolution in the parasitoid *Asobara tabida:* on the trade-off between reprodcution and survival. PhD Thesis, Leiden University, Leiden.

Ellers, J., Van Alphen, J.J.M., and Sevenster, J.G. (1998): A field study of size-fitnss relationships in the parasitoid *Asobara tabida*. Journal of Animal Ecology 67: 318-324.

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

Elliott, N.C., Simmons, G.A., and Sapio, F.J. (1987): Honeydew and wildflowers as food for the parasites *Glypta fumiferanae* (Hymenoptera: Ichneumonidae) and *Apanteles fumiferanae* (Hymenoptera: Braconidae). Journal of the Kansas Entomological Society 60: 25-29.

England, S. and Evans, E., W. (1997): Effects of pea aphid (Homoptera: Aphididae) honeydew on longevity and fecundity of the alfalfa weevil (Coleoptera: Curculionidae) parasitoid *Bathyplectes curculionis* (Hymenoptera: Ichneumonidae). Environmental Entomology 26: 1437-1441.

Field, S.A. and Keller, M.A. (1993): Courtship and intersexual signalling in the parasitic wasp *Cotesia rubecula* (Hymenoptera: Braconidae). Journal of Insect Behaviour 6: 737-750.

Finch, S. and Thompson, A.R. (1992): Pests of Cruciferous crops. In: Vegetable Crop Pests (ed. R.G. McKinlay), pp. 87-138. McMillan Press, London.

Fink, U. and Völkl, W. (1995): The effect of abiotic factors on foraging and oviposition success of the aphid parasitoid, *Aphidius rosae*. Oecologia 103: 371-378.

Flanders, S.E. (1950): Regulation of ovulation and egg disposal in the parasitic Hymenoptera. The Canadian Entomologist 82: 134-140.

Fletcher, J.P., Hughes, J.P., and Harvey, I.F. (1994): Life expectancy and egg load affect oviposition decisions of a solitary parasitoid. Proceedings of the Royal Society of London B Biological Sciences 258: 163-167.

Foster, M.A. and Ruesink, W.G. (1984): Influence of flowering weeds associated with reduced tillage in corn on a black cutworm (Lepidoptera: Noctuidae) parasitoid, *Meteorus rubens*. Environmental Entomology 13: 664-668.

Foster, W.A. (1995): Mosquito sugar feeding and reproductive energetics. Annual Review of Entomology 40: 443-474.

Friedman, S. (1985): Intermediary metabolism. In: Fundamentals of Insect Physiology (ed. M.S. Blum), pp. 468-505. John Wiley and Sons, New York.

Geervliet, J., Ariens, S., Dicke, M., and Vet, L. (1998): Long-distance assessment of patch profitability through volatile infochemicals by the parasitoids *Cotesia glomerata* and *Cotesia rubecula* (Hymenoptera, Braconidae). Biological Control: 113-121.

Getz, W.M. and Smith, K.B. (1987): Olfactory sensitivity and discrimination of mixtures in the honeybee *Apis mellifera*. Journal Of Comparative Physiology A Sensory Neural And Behavioral Physiology 160: 239-246.

Gilbert, F. and Jervis, M.A. (1998): Functional, evolutionary and ecological aspects of feeding-related mouthpart specializations in parasitoid flies. Biological Journal of the Linnean Society 63: 495-535.

Godfray, H.C.J. (1994). Parasitoids: behavioural and evolutionary ecology. Princeton University Press, Princeton, New Jersey.

Goodman, L.A. (1968): The analysis of cross-classified data: independence, quasi-independence and interactions in contingency tables with or without missing entries. Journal of American Statistics Association 63: 1091-1131.

Grasswitz, T.R. and Paine, T.D. (1993): Influence of physiological state and experience on the responsiveness of *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Aphidiidae) to aphid honeydew and to host plants. Journal Of Insect Behavior 6: 511-528.

Greathead, J.D. (1986): Parasitoids in classical biological control. In: Insect Parasitoids (eds. J. Waage and D.J. Greathead). 13th Symposium of the Royal Entomological Society of London, pp. 289-318. Academic Press, London.

Gurr, G.M., Wratten S.D., Irvin N.A., Hossein Z., Baggen L.R., Mensah R.K. and Walker, P.W. (1998). In: Pest Management - Future Challenges. Sixth Australasian Applied Entomological Research Conference (ed M.P. Zalucki, R.A.I. Drew and G.G. White).The University of Queensland, Brisbane.

Haccou, P. and Meelis, E. (1995). Statistical Analysis of Behavioural Data. Oxford University Press, New York.

Hagley, E.A.C. and Barber, D.R. (1992): Effect of food sources on the longevity and fecundity of *Pholetesor ornigis* (Weed) (Hymenoptera: Braconidae). Canadian Entomologist 124: 341-346.

Harborne, J.B. (1992). Biological Chemistry. Academic Press, London.

Harcourt, D.G. (1961): Spatial pattern of the imported cabbageworm, *Pieris rapae* (L) (Lepidoptera: Pieridae), on cultivated cruciferae. The Canadian Entomologist 18: 954-952.

Hassel, M.P. (1978). The Dynamics of Arthropod Predator-Prey Systems. Princeton University Press, Princeton.

Heimpel, G.E. and Collier, T.R. (1996): The evolution of host-feeding behaviour in insect parasitoids. Biological Reviews 71: 373-400.

Heimpel, G.E., Rosenheim, J.A., and Mangel, M. (1996): Egg limitation, host quality, and dynamic behavior by a parasitoid in the field. Ecology 77: 2410-2420.

Heimpel, G.E., Rosenheim, J.A., and Kattari, D. (1997a): Adult feeding and lifetime reproductive success in the parasitoid *Aphytis melinus*. Entomologia Experimentalis et Applicata 83: 305-315.

Heimpel, G.E., Rosenheim, J.A., and Mangel, M. (1997b): Predation on adult *Aphytis* parasitoids in the field. Oecologia 110: 346-352.

Heimpel, G., E., Mangel, M., and Rosenheim J. A. (1998): Effects of time limitation and egg limitation on lifetime reproductive success of a parasitoid in the field. The American Naturalist. 152: 273-289.

Heimpel, G., E. and Rosenheim, J., A. (1998): Egg limitation in parasitoids: A review of the evidence and a case study. Biological Control 11: 160-168.

Hendrix, D.L., Wei, Y.A., and Leggett, J.E. (1992): Homopteran honeydew sugar composition is determined by both the insect and plant species. Comparative Biochemistry And Physiology B Comparative Biochemistry 101: 23-27.

Hern, A., Edward-Jones, G., and McKinley, R.G. (1996): A review of the pre-oviposition behaviour of the small cabbage white butterfly, *Pieris rapae* (Lepidoptera: Pieridae). Journal of Applied Biology: 349-371.

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

Hohmann, C.L., Luck, R.F., and Oatman, E.R. (1988): A comparison of longevity and fecundity of adult *Trichogramma platneri* (Hymenoptera: Trichogrammatidae) reared from eggs of the cabbage looper and the angoumois grain moth, with and without access to honey. Journal of Economic Entomology 81: 1307-1312.

Hougardy, E. and Gregoire, J.C. (2000): Spruce stands provide natural food sources to adult hymenopteran parasitoids of bark beetles. Entomologia Experimentalis et Applicata 96: 253-263.

Hubbard, S.F. and Cook, R.M. (1978): Optimal foraging by parasitoid wasps. Journal of Animal Ecology 47: 593-604.

Idoine, K. and Ferro, D.N. (1988): Aphid honeydew as a carbohydrate source for *Edovum puttleri* (Hymenoptera: Eulophidae). Environmental Entomology 17: 941-944.

Idris, A.B. and Grafius, E. (1995): Wildflowers as nectar source for *Diadegma insulare* (Hymenoptera: Ichneumonidae), a parasitoid of diamond back moth (Lepidoptera: Yponomeutidae). Environmental Entomology 24: 1735-1735.

Idris, A.B. and Grafius, E. (1997): Nectar-collecting behavior of *Diadegma insulare* (Hymenoptera: Ichneumonidae), a parasitoid of diamondback moth (Lepidoptera: Plutellidae). Environmental Entomology 26: 114-120.

Idris, A.B. and Grafius, E. (1998): Diurnal flight activity of *Diadegma insulare* (Hymenoptera: Ichneumonidae), a parasitoid of the diamondback moth (Lepidoptera: Plutellidae), in the field. Environmental Entomology. April 27: 406-414.

Ives, A.R. (1995): Spatial heterogeneity and host-parasitoid population dynamics: Do we need to study behavior? Oikos 74: 366-376.

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

Jakobsen, H.B. and Kristjansson, K. (1994): Influence of temperature and floret age on nectar secretion in *Trifolium repens* L. Annals of Botany London 74: 327-334.

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

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

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. and Kidd, N.A.C. (1995): Incorporating physiological realism into models of parasitoid feeding behaviour. Trends in Ecology and Evolution 10: 434-436.

Jervis, M.A., Kidd, N.A.C., and Heimpel, G.E. (1996): Parasitoid adult feeding behaviour and biocontrol - a review. Biocontrol News and Information 17: 11N-26N.

Jervis, M.A. (1998): Functional and evolutionary aspects of mouthpart structure in parasitoid wasps. Biological Journal of the Linnean Society 63: 461-493.

Jervis, M.A. and Kidd, N.A.C. (1999): Parasitoid adult nutritional ecology: implications for biological control. In: Theoretical Approaches to Biological Control (eds. B.A. Hawkins and H.V. Cornell), pp. 131-147. Cambridge University Press, Cambridge.

Jervis, M.A., Heimpel, G.E., Ferns, P.N., Harvey, J.A., and Kidd, N.A.C. (2001): Life-history strategies in parasitoid wasps: a comparative analysis of 'ovigeny'. Journal of Animal Ecology 70: 442-458.

Joerges, J., Kuttner, A., Galizia, G., and Menzel, R. (1996) Visualisation of spatial neuronal codes for odours and their mixtures by calcium imaging. In: Second Meeting of European Neuroscience, Vol. Abstract 51.09. European Neuroscience Association.

Johanowicz, D.L. and Mitchell, E.R. (2000): Effects of sweet alyssum flowers on the longevity of the parasitoid wasps *Cotesia marginiventris* (Hymenoptera: Braconidae) and *Diadegma insulare* (Hymenoptera: Ichneumonidae). Florida Entomologist 83: 41-47.

Jones, R.E. (1977): Movement patterns and egg distribution in cabbage butterflies. Journal of Animal Ecology 46: 195-212.

Jones, R.E. (1981): The cabbage butterfly, *Pieris rapae* (L.): 'a just sense of how not to fly'. In: The Ecology of Pests: Some Australian Case Histories (eds. R.L. Kitching and R.E. Jones). CSIRO, Melbourne.

Josens, R.B., Farina, W.M., and Roces, F. (1998): Nectar feeding by the ant *Camponotus mus*: Intake rate and crop filling as a function of sucrose concentration. Journal of Insect Physiology. 44: 579-585.

Kaiser, L. and Carde, R.T. (1992): In-flight orientation to volatiles from the plant-host complex in *Cotesia rubecula* (Hym.: Braconidae): Increased sensitivity through olfactory experience. Physiological Entomology 17: 62-67.

Keller, M.A. (1990): Responses of the parasitoid *Cotesia rubecula* to its host *Pieris rapae* in a flight tunnel. Entomologia Experimentalis Et Applicata 57: 243-250.

Kevan, P.G. and Baker, H.G. (1999): Insects on flowers. In: Ecological Entomology (eds. C.B. Huffaker and A.P. Gutierrez). John Wiley & Sons, New York.

Kingsolver, J.G. and Daniel, T.L. (1995): Mechanics of food handling by fluid-feeding insects. In: Regulatory Mechanisms in Insect Feeding (eds. R.F. Chapman and G. de Boer), pp. 32-53. Chapman and Hall, New York.

Kloen, H. and Altieri, M.A. (1990): Effect of mustard (*Brassica hirta*) as a non crop plant on competition and insect pests in broccoli (*Brassica olearacea*). Crop Protection 9: 90-96.

Kobayashi, S. (1966): Process generating the distribution pattern of eggs of the common cabbage butterfly *Pieris rapae crucivora*. Researches on Population Ecology 8: 51-61.

Krebs, J.R. and Davies, N.B. (1993). An Introduction to Behavioural Ecology. Blackwell Scientific Publications, Oxford.

Krivan, V. and Sirot, E. (1997): Searching for food or hosts: The influence of parasitoids behavior on host-parasitoid dynamics. Theoretical Population Biology 51: 201-209.

Lang, T.A. and Secic, M. (1997). How To Report Statistics in Medicine. American College of Physicians, Philadelphia.

LaSalle, J. and Gauld, I.D. (1991): Parasitic hymenoptera and the biodiversity crisis. Redia 74: 315-334.

LaSalle, J. and Gauld, I.D. (1993). Hymenoptera and Biodiversity. CAB International Press, Wallingford, U.K.

Leius, K. (1961a): Influence of food on fecundity and longevity of adults of *Itoplectis conquisitor* (Say) (Hymenoptera: Ichneumonidae). The Canadian Entomologist 93: 771-780.

Leius, K. (1961b): Influence of various foods on fecundity and longevity of adults of *Scambus buolianae* (Htg.) (Hymenoptera: Ichneumonidae). The Canadian Entomologist 93: 1079-1084.

Leius, K. (1967): Food sources and preferences of adults of a parasite, *Scambus buolianae* (Hym.: Ichn.), and their consequences. The Canadian Entomologist 99: 865-871.

Lewis, W.J. and Takasu, K. (1990): Use of learned odours by a parasitic wasp in accordance with host and food needs. Nature 348: 635-636.

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

Maingay, H.M., Bugg, R.L., Carlson, R.W., and Davidson, N.A. (1991): Predatory and parasitic wasps (Hymenoptera) feeding at flowers on sweet fennel (*Foeniculum vulgare* Miller var. dulce Battandir & Trabut, Apiaceae) and spearmint (*Mentha spicata* L., Lamiaceae) in Massachusetts. Biological Agriculture and Horticulture 7: 363-383.

Mangel, M. and Clark, C.W. (1988). Dynamic Modeling in Behavioural Ecology. Princeton University Press, Princeton.

Mangel, M. (1989): Evolution of host selection in parasitoids: Does the state of the parasitoid matter? The American Naturalist 133: 688-705.

Maurizio, A. (1975): How bees make honey. In: Honey. A comprehensive survey (ed. E. Crane), pp. 17-26. Heinemann, London.

Mayr, E. (1982). The Growth of Biological Thought. The Belknap Press of Harvard University Press, Cambridge.

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.

McNamara, J. and Houston, A.I. (1986): The common currency for behavioural decisions. The American Naturalist 127: 358-378.

Miller, J.R. and Strickler, K.L. (1984): Finding and accepting host plants. In: Chemical Ecology of Insects (eds. W.J. Bell and R.T. Carde). Chapman and Hall, London.

Mohr, N.A. and Jay, S.C. (1990): Nectar production of selected cultivars of *Brassica campestris* L. and *Brassica napus* L. Journal of Apicultural Research 29: 95-100.

Morales-Ramos, J.A., Rojas, M.G., and King, E.G. (1996): Significance of adult nutrition and oviposition experience on longevity and attainment of full fecundity of *Catolaccus grandis* (Hymenoptera: Pteromalidae). Annals of the Entomological Society of America 89: 555-563.

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

Müller-Schärer, H., Potter, C.A., and Hurni, B. (1992): Untersaaten bei Herbstlauch. Der Gemüsebau 10: 4-7.

Mullins, D.E. (1985): Chemistry and physiology of the haemolymph. In: Comprehensive Insect Physiology, Biochemistry, and Pharmacology (eds. G.A. Kerkut and L.I. Gilbert), pp. 355-400. Pergamon Press, Oxford.

Murdoch, W.W., Briggs, C.J., and Nisbet, R.M. (1997): Dynamical effects of host size- and parasitoid state-dependent attacks by parasitoids. Journal of Animal Ecology 66: 542-556.

Nayar, J.K. and Van Handel, E. (1971): The fuel for sustained mosquito flight. Journal of Insect Physiology 17: 471-481.

Nealis, V. (1985): Diapause and the seasonal ecology of the introduced parasite, *Cotesia rubecula* (Hymenoptera: Braconidae). Canadian Entomologist 117: 333-342.

Nealis, V.G. (1986): Responses to host kairomones and foraging behavior of the insect parasite *Cotesia rubecula* (Hymenoptera: Braconidae). Canadian Journal of Zoology 64: 2393-2398.

Nealis, V.G. (1990): Factors affecting the rate of attack by *Cotesia rubecula* (Hymenoptera: Braconidae). Ecological Entomology 15: 163-168.

Neukirch, A. (1982): Dependence of the life span of the honeybee (*Apis mellifica*) on flight performance and energy consumption. Journal of Comparative Physiology B Biochemical Systemic and Environmental Physiology 146: 35-40.

Nicholls, C.I., Parrella, M.P., and Altieri, M.A. (2000): Reducing the abundance of leafhoppers and thrips in a northern California organic vineyard through maintenance of full season floral diversity with summer cover crops. Agricultural and Forest Entomology 2: 107-113.

Nilssen, A.C. (1997): Factors affecting size, longevity and fecundity in the reindeer oestrid flies *Hypoderma tarandi* (L.) and *Cephenemyia trompe* (Modeer). Ecological Entomology 22: 294-304.

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.

Passera, L. and Keller, L. (1990): Loss of mating flight and shift in the pattern of carbohydrate storage in sexuals of ants (Hymenoptera; Formicidae). Journal of Comparative Physiology B Biochemical Systemic and Environmental Physiology 160: 207-212.

Patt, J., Hamilton, G., and Lashomb, J. (1999): Responses of two parasitoid wasps to nectar odors as a function of experience. Entomologia Experimentalis et Applicata 90: 1-8.

Patt, J.M., Hamilton, G.C., and Lashomb, J.H. (1997): Foraging sucess of parasitoid wasps on flowers: interplay of insect morphology , floral architecture and searching behavior. Entomologia Experimentalis and Applicata 83: 21-30.

Pleasants, J.M. (1989): Optimal foraging by nectarivores: A test of the marginal-value theorem. American Naturalist 134: 51-71.

Powell, W. (1986): Enhancing parasitoid activity in crops. In: Insect Parasitoids (eds. J. Waage and D. Greathead), pp. 319-340. Academic Press, London.

Rabb, R.L., Stinner, R.E., and Van den Bosch, R. (1976): Conservation and augmentation of natural enemies. In: Theory and Practice of Biological Control (eds. C.B. Huffaker and P.S. Messenger), pp. 233-249. Academic Press, New York.

Reznik, D. (1985): Costs of reproduction: an evaluation of the empirical evidence. Oikos 44: 257-267.

Rice, W.R. (1989): Analyzing tables of statistical tests. Evolution 43: 223-225.

Rivero, A. and Casas, J. (1999a): Incorporating physiology into parasitoid behavioral ecology: the allocation of nutritional resources. Researches on Population Ecology 41: 39-45.

Rivero, A. and Casas, J. (1999b): Rate of nutrient allocation to egg production in a parasitic wasp. Proceedings of the Royal Society of London - Series B: Biological Sciences 266: 1169-1174.

Roff, D.A. (1992). The Evolution of Life Histories. Chapman and Hall, New York.

Roitberg, B.D., Sitcom, J., Roitberg, C.A., van Alphen, J.J.M., and Mangel, M. (1993): Life expectancy and reproduction. Nature 364: 108.

Sahragard, A., Jervis, M.A., and Kidd, N.A.C. (1991): Influence of host availability on rates of oviposition and host-feeding, and on longevity in *Dicondylus indianus* Olmi (Hymenoptera, Dryinidae), a parasitoid of the rice brown planthopper, *Nilaparvata lugens* Stal (Hemiptera, Delphacidae). Journal of Applied Entomology 112: 153-162.

Sartori, M., Keller, L., Thomas, A.G.B., and Passera, L. (1992): Flight energetics in relation to sexual differences in the mating behaviour of a mayfly, *Siphlonurus aestivalis*. Oecologia 92: 172-176.

SAS Institute (1995). JMP - Statistics and Graphics Guide, Version 3.1. SAS Institute Inc., Cary, NC.

Sengonca, C. and Peters, G. (1993): Biology and effectiveness of *Apanteles rubecula* Marsh. (Hym., Braconidae), a solitary larval parasitoid of *Pieris rapae* (L.) (Lep., Pieridae). Journal of Applied Entomology 115: 85-89.

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

Shahjahan, M. (1974): *Erigeron* flowers as a food and attractive odour source for *Peristenus pseudopallipes*, a braconid parasitoid of the tarnished bug. Environmental Entomology 3: 69-72.

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

Simmonds, F.J., Franz, J.M., and Sailer, R.I. (1976): History of biological control. In: Theory and Practice of Biological Control (eds. C.B. Huffaker and P.S. Messenger), pp. 17-35. Academic Press, New York.

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.

Sirot, E. and Bernstein, C. (1997): Food searching and superparasitism in solitary parasitoids. Acta Oecologica 18: 63-72.

Sirot, E., Ploye, H., and Bernstein, C. (1997): State dependent superparasitism in a solitary parasitoid: Egg load and survival. Behavioral Ecology 8: 226-232.

Smith, B.H. and Cobey, S. (1994): The olfactory memory of the honeybee, *Apis melifera*. II. Blocking between odorants in binary mixtures. Journal of Experimental Biology 195: 91-108.

Sokal, R.R. and Rohlf, F.J. (1981). Biometry: the principle and practice of statistics in biological research. Freeman, W.H., San Francisco.

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.

Stearns, S.C. (1992). The Evolution of Life Histories. Oxford University Press, Oxford.

Steele, J.E. (1981): The role of carbohydrate metabolism in physiological function. In: Energy Metabolism (ed. R.G.H. Downer), pp. 101-133. Plenum Press, New York.

Stephens, D.W. and Krebs, J.R. (1986). Foraging Theory. Princeton University Press, Princeton.

Stoffolano Jr., J.G. (1995): Regulation of a carbohydrate meal in the adult Diptera, Lepidoptera, and Hymenoptera. In: Regulatory Mechanics in Insect Feeding (eds. R.F. Chapman and G. de Boer). Chapman and Hall, New York.

Syme, P.D. (1975): The effects of flowers on the longevity and fecundity of two native parasites of the European pine shoot moth in Ontario. Environmental Entomology 4: 337-346.

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.

Tatar, M. and Carey, J.R. (1995): Nutrition mediates reproductive trade-offs with age-specific mortality in the beetle *Callosobruchus maculatus*. Ecology 76: 2066-2073.

Tenhumberg, B., Keller, M.A., Tyre, A.J., and Possingham, H.P. (2001): The effect of resource aggregation at different scales: optimal foraging behaviour of *Cotesia rubecula*. The American Naturalist 158:505-518.

Teraoka, T. and Numata, H. (2000): Effects of feeding on reproduction and overwintering in female adults of *Ooencyrtus nezarae* Ishii (Hymenoptera: Encyrtidae). Applied Entomology and Zoology 35: 361-367.

Thompson, J.N. (1987): Variance in number of eggs per patch: Oviposition behaviour and population dispersion in a seed parasitic moth. Ecological Entomology 12: 311-320.

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

Topham, M. and Beardsley, J.W. (1975): Influence of nectar source plants on the New Guinea sugar cane weevil parasite, *Lixophaga sphenophori* (Villeneuve). Proceedings of the Hawaiian Entomological Society 22: 145-154.

Tsunoda, S., Hinata, K., and Gomez-Campo, C. (1980) Brassica crops and wild allies: biology and breeding. Japan Scientific Society Press, Tokyo.

Van Alphen, J.J.M. and Galis, F. (1983): Patch time allocation and parasitization efficiency of *Asobara tabida*, a larval parasitoid of Drosophila. Journal of Animal Ecology 52: 937-952.

Van Alphen, J.J.M. and Vet, L.E.M. (1986): An evolutionary approach to host finding and selection. In: Insect Parasitoids (eds. J. Wagge and D.J. Greathead). Academic Press, London.

Van Driesche, R.G. and Bellows Jr, T.S. (1996). Biological Control. Chapman and Hall, New York.

Van Emden, H.F. (1963): Observations on the effect of flowers on the activity of parasitic Hymenoptera. Entomologist's Monthly Magazine 98: 265-270.

Van Emden, H.F. (1990): Plant diversity and natural enemy efficiency. In: Critical Issues in Biological Control (eds. M. Mackauer, L.E. Ehler and J. Roland), pp. 63-80. Intercept, Andover.

Van Handel, E. (1985a): Rapid determination of glycogen and sugars in mosquitoes. Journal of the American Mosquito Control Association 1: 299-301.

Van Handel, E. (1985b): Rapid determination of total lipids in mosquitoes. Journal of the American Mosquito Control Association 1: 302-304.

Van Handel, E. and Day, J.F. (1988): Assay of lipids, glycogen, and sugars in individual mosquitos: correlation with wing length in field collected *Aedes vexans*. Journal of the American Mosquito Control association 4: 549-550.

Van Lenteren, J.C., Van Vianen, A., Gast, H.F., and Kortenhoff, A. (1987): The parasite-host relationship between *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* Westwood (Homoptera: Aleuyrodidae). XVI. Food effects on oogenesis, lifespan and fecundity of *Encarsia formosa* and other hymenopterous parasites. Journal of Applied Entomology 103: 69-84.

Van Noordwijk, A.J. and de Jong, G. (1986): Acquisition and allocation of resources: their influence on variation in life history tactics. The American Naturalist 128: 137-142.

Vandermeer, J. (1989). The Ecology of Intercropping. Cambridge University Press, Cambridge.

Vet, L.E.M., De Jong, R., Van Giessen, W.A., and Visser, J.H. (1990): A learning-related variation in electroantennogram responses of a parasitic wasp. Physiological Entomology 15: 243-247.

Vet, L.E.M. (1995): Parasitoid Foraging and Learning. In: Chemical Ecology of Insects (eds. R.T. Carde and W.J. Bell).

Visser, M.E., Van Alphen, J.J.M., and Nell, H.W. (1992): Adaptive superparasitism and patch time allocation in solitary parasitoids: The influence of pre-patch experience. Behavioral Ecology and Sociobiology 31: 163-171.

Völkl, W., Liepert, C., Birnbach, R., Huebner, G., and Dettner, K. (1996): Chemical and tactile communication between the root aphid parasitoid *Paralipsis enervis* and trophobiotic ants: Consequences for parasitoid survival. Experientia 52: 731-738.

Völkl, W. and Kroupa, A.S. (1997): Effects of adult mortality risks on parasitoid foraging tactics. Animal Behaviour 54: 349-359.

Vos, M. (1999) Foraging under incomplete information: parasitoid behaviour and community dynamics, PhD Thesis, Wageningen University, Wageningen.

Waage, J.K. (1979): Foraging for patchily-distributed hosts by the parasitoid, *Nemeritis canescens*. Journal of Animal Ecology 48: 353-371.

Wäckers, F.L. and Swaans, C.P.M. (1993): Finding floral nectar and honeydew in *Cotesia rubecula*: random or directed. Proceedings of Experimental and Applied Entomology, N.E.V. Amsterdam 4: 67-72.

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. 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: 105-112.

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.

Warburg, M.S. and Yuval, B. (1996): Effects of diet and activity on lipid levels of adult Mediterranean fruit flies. Physiological Entomology 21: 151-158.

Weisser, W., W., Völkl, W., and Hassell, M., P. (1997): The importance of adverse weather conditions for behaviour and population ecology of an aphid parasitoid. Journal of Animal Ecology 66: 386-400.

Wheeler, C.H. (1989): Mobilization and transport of fuels to the flight muscles. In: Insect Flight (eds. G.J. Goldsworthy and C.H. Wheeler), pp. 274-301. CRC Press, Boca Raton, Florida.

White, P.R. (1989): Factors affecting the antennal and behavioral responses of the saw-toothed grain beetle *Oryzaephilus surinamensis* to food odor and aggregation pheromone. Physiological Entomology 14: 349-359.

Wigglesworth, V.B. (1972). The Principles of Insect Physiology. Chapman and Hall, London.

Wilson, F. and Huffaker, C.B. (1976): The philosophy, scope and importance of biological control. In: Theory and Practice of Biological Control (eds. C.B. Huffaker and P.S. Messenger), pp. 3-15. Academic Press, New York.

Wolcott, G.N. (1942): The requirements of parasites for more than hosts. Science 96: 317-318.

Woodring, J.P. (1985): Circulatory systems. In: Fundamentals in Insect Physiology (ed. M.S. Blum), pp. 5-56. Wiley-Interscience, New York.

Wratten, S.D. and van Emden, H.F. (1995): Habitat management for enhanced activity of natural enemies of insect pests. In: Ecology and Integrated Farming Systems (eds. G.M. Glen, M.P. Greaves and H.M. Anderson), pp. 117-145. John Wiley, Chichester, UK.

Yuval, B. and Schlein, Y. (1986): Leischmanias in the Jordan Valley III. Nocturnal activity of *Phlebotomus paptasi* (Diptera: Psychodidae) in relation to nutrition and ovarian development. Journal of Medical Entomology 23: 411-415.

Ziegler, R. (1985): Metabolic energy expenditure and its hormonal regulation. In: Environmental Physiology and Biochemistry of Insects (ed. K.H. Hoffmann), pp. 95-118. Springer-Verlag, Berlin.

Zoebelein, G. (1956): Der Honigtau als Nahrung der Insekten. Zeitschrift für angewandte Entomologie 38: 369-416.