Patch Exploitation by the Parasitoids of *Plutella xylostella* (L.): From Individual Behaviour to Population Dynamics

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To my family

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Summary

The diamondback moth, *Plutella xylostella* (L.), is the most destructive pest of brassica crops worldwide. Since this pest has developed resistance to all major classes of insecticides in many parts of the world, including China and Australia, integrated management of *P. xylostella*, particularly by enhancing the impact of its two major larval parasitoids *Cotesia plutellae* and *Diadegma semiclausum*, has been emphasized in many parts of the world. The work presented in this thesis aims to investigate the behaviour and ecology of these two parasitoids.

This thesis has two complementary lines of investigation. From a theoretical perspective, I used *D. semiclausum* as a model species to focus my investigation on a central issue of foraging theory in parasitoids: patch time allocation. That is how an individual parasitoid allocates its foraging time over patches of differing profitability, including patch selection decisions (which patches to visit), oviposition decisions (whether or not to oviposit upon encountering a host), and patch-leaving decisions (how long to stay in a given patch). Within this framework, I tested a number of the proposed foraging models in parasitoids. From a practical perspective, the behavioral approach I used has identified some of the effective behavioral attributes that both parasitoid species use to exploit hosts.

D. semiclausum is a specialist parasitoid of its host, while *C. plutellae* is an oligophagous species. Both parasitoids were shown to employ antennal search and ovipositor search when seeking hosts, but *D. semiclausum* also seemed to use visual perception in the immediate vicinity of hosts. Larvae of *P. xylostella* avoid detection by parasitoids by moving away from damaged plant parts after short feeding bouts. When they encounter parasitoids, the larvae wriggle vigorously as they retreat and often hang from silk strands after dropping from a plant. These two parasitoids differed in their responses to the host defenses. *D. semiclausum* displayed a 'wide-area' search around feeding damage, and waited near the silk strand for a suspended host to climb up and then attacked it again. *C. plutellae* displayed an 'area-restricted' search, and usually pursued the host down the silk strand onto the ground. Analysis of behavioral data showed that *D. semiclausum* has a fixed behavioral pattern leading to oviposition, but *C. plutellae* exhibits a more plastic behavioral pattern. The time spent by the two parasitoids on different plants increased with increasing host density, but the time spent either on all plants or a single plant by *D. semiclausum* was typically longer than that of *C. plutellae*. *D. semiclausum* visited individual plants more frequently than *C. plutellae* before it left the patch, and stung hosts at more than twice the rate of *C. plutellae*. The results indicated that the host location strategies employed by *D. semiclausum* were better adapted to the host's defensive behaviour, and thus it was more effective at detecting and parasitising the host than *C. plutellae*.

The oviposition decisions of *D. semiclausum* were investigated with single wasps that searched a patch containing initially unparasitised host and hosts parasitised previously either by a conspecific female or the female herself. The parasitoids were either naive or experienced with oviposition in unparasitised hosts prior to the experiments, and were allowed to freely leave the patch by providing an alternative host resource patch. *D. semiclausum* tended to oviposit into a newly encountered host (threshold tactic), and evaluated a host by ovipositor probing (internal discrimination). The influences of pre-patch experience, host quality, patch time, rate of oviposition and egg depletion on the probability of host acceptance were analyzed using logistic regression. The parasitoid's decisions to accept or reject a host were dependent on host quality, and these decisions were dynamically affected by the patch residence time and egg depletion. No other experience had a statistically significant effect on oviposition decisions. The parasitoid was able to distinguish between hosts: unparasitised, parasitised by a conspecific female, parasitised by herself one day previously, and by herself in the present foraging bout. However, contrary to the theoretical predictions of a static optimal model and an evolutionarily stable strategy (ESS) model of oviposition decisions in solitary parasitoids, both self- or conspecific- superparasitism were very common in *D. semiclausum*. The common occurrence of superparasitism in *D.*

semiclausum may confer an adaptive advantage in terms of relatively high cost involved in locating hosts and overcoming host defense.

A number of proposed patch-leaving models in parasitoids such as the Marginal Value Theorem, simple 'rules of thumb', incremental mechanisms and count-down mechanisms were tested using *D. semiclausum*. The foraging behaviour of *D. semiclausum* was directly observed in a wind tunnel, and the patch-leaving tendency was analyzed by means of Cox's proportional hazards model. The patch-leaving tendency decreased with increased host density or presence of host damage, clumped host distribution, unsuccessful encounters with hosts, and increasing inter-patch distances. Successful oviposition, self-superparasitism, rejection of parasitised hosts, and unsuccessful search time since last oviposition increased the patch-leaving tendency. None of the simple rules of thumb such as fixed searching time, fixed oviposition number, or fixed giving-up time was likely to have been employed by *D. semiclausum*. The results agreed with the general predictions of the Marginal Value Theorem that both patch residence time and number of ovipositions of *D. semiclausum* increased with increasing inter-patch distance and host density. The incremental influence of oviposition on the patch-leaving tendency regardless of host density and distribution, indicated that a count-down mechanism gave the best description of the patch-leaving decisions of *D. semiclausum*. A conceptual model of the patch-leaving decisions by *D. semiclausum* was developed.

However, at different spatial levels in a multi-plant environment, the parasitoid appeared to use different patch-leaving rules. At the largest of the spatial levels (groups of plants), the parasitoid appeared to gather less information or to use fewer cues to decide when to leave a given area. This suggested that the parasitoid may perceive a multi-plant environment as a hierarchical patch system, and its patch-leaving decisions might be spatially hierarchical.

I also tested the optimal foraging predictions and the population consequences of the differential patch exploitation by groups of female *D. semiclausum*, foraging for patchily distributed host resources under laboratory and field conditions. In the laboratory test, the parasitoids displayed an unchanging pattern of aggregation in patches of varying host densities during patch exploitation. In contrast to the optimal foraging predictions, patches were neither exploited in order of profitability nor exploited differentially until the same oviposition rate was achieved in each patch. The resulting pattern of parasitism was density-independent, which mainly depended on the interplay between the tendency to aggregate and the conflicting mechanisms that determine the relative foraging efficiency such as density dependent host defense and handling time. In addition, adaptive superparasitism in this parasitoid also outweighed the density-dependence of parasitism. In the field, the parasitoid population also showed a positive aggregation response to plants with a high host density over time at two spatial scales: single plants and groups of plants. However, the resultant patterns of parasitism depended on the relative degree of aggregation to host density at a given spatial scale.

The results suggest that the direct link between the optimal foraging predictions and aggregation responses appears to be weak in *D. semiclausum*, and the spatial density-dependence in parasitism could depend on the relative degree of aggregation in the field.

Finally, I assessed the impact of *D. semiclausum* in suppressing *P. xylostella* using a cage exclusion method in a winter broccoli field in Queensland, Australia. Of the larvae that were recovered from the field, 71.7 to 93.6% were found to be parasitised by *D. semiclausum* in the uncaged treatments, indicating that the parasitoid substantially reduced the *P. xylostella* population during the winter.

This study has given new insight into the decision making of individual parasitoids, especially *D. semiclausum*. From both practical and theoretical perspectives, the study contributes the understanding of foraging efficiency of the parasitoids of *P. xylostella*. Incorporating more biologically realistic behavioral attributes such as those identified in this thesis into host-parasitoid interaction models could provide a link between individual behaviour and population dynamics in the future.

Declaration

The work presented in this thesis contains no material which has been accepted for the award of any degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this thesis, when deposited in the University Library, being available for loan or photocopying, provided due acknowledgments are given in the instance of any reference to this work.

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Preface

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Publications

At the time of printing, the following manuscript based on work present in Chapter 2 of this thesis had been accepted for publication:

Wang X.G & Keller, M.K. A comparison of the host-searching efficiency of two larval parasitoids of *Plutella xylostella. Ecological Entomology* (in press).

Chapter 1 General introduction

1.1 Introduction

Parasitoids have long been a model system for behavioral ecologists and evolutionary biologists (Godfray, 1994; Godfray & Shimada, 1999). Practical work on the use of parasitoids for biological control of agricultural pests has focused on parasitoid behaviour as a major determinant of their effectiveness. An effective parasitoid should concentrate search in areas of high host density to maximize the rate of host attack (Waage, 1983). Thus, a knowledge of parasitoid foraging behaviour could help in the evaluation of parasitoids for biological control (Luck, 1990). Theoretical work on biological control concerns the persistent suppression of the host populations at low equilibrium levels by parasitoids and the stability of the host-parasitoid population dynamics (Hassell & May, 1973, 1974; Beddington *et al.*, 1978; May, 1978; Chesson & Murdoch, 1986; Pacala *et al.*, 1991; Ives, 1995; Murdoch & Briggs, 1996; Godfray & Müller, 1997; Hawkins & Cornell, 1999).

Because of the direct link between successful searching and the production of offspring, parasitoid foraging behaviour is likely to be strongly influenced by natural selection. Thus, the foraging behavior of parasitoids is also an ideal subject for testing optimality hypotheses in the context of behavioral ecology and evolutionary biology (van Alphen & Vet, 1986; Godfray, 1994). In foraging theory for parasitoids, one key issue is to understand how individual parasitoids allocate their foraging time over patches of different profitability to maximize their reproductive rate (Charnov, 1976a, 1976b; Cook & Hubbard, 1977; Comins & Hassell, 1979; Waage, 1979; McNair, 1982; Sutherland, 1983; Lessells, 1985; van Alphen & Vet, 1986; Godfray, 1994). Optimal patch exploitation involves three basic decisions: patch selection (which patch to visit) (Charnov, 1976b; Waage, 1979), oviposition decisions (whether or not to accept a host upon encountering it) (van Alphen & Visser, 1990; van Alphen & Jervis, 1996); and patch leaving (how long to stay in a patch after arriving there)

(Charnov, 1976b; Waage, 1979; Stephens & Krebs, 1986; Crawley & Krebs, 1992; Godfray, 1994; Driessen *et al.*, 1995; van Alphen & Jervis, 1996; Driessen & Bernstein, 1999).

Understanding of these adaptive patch behaviors in parasitoids should give insight into the factors that govern parasitoid foraging behaviour, and provide a link between the individual behavior of parasitoids and host-parasitoid population dynamics. Certain behaviour patterns are adaptive and help to maximize the numbers of offspring of individual foragers or enhance population stability (Royama, 1971; Hassell & May, 1973, 1974; Murdoch & Oaten, 1975; Charnov, 1976b; Cook & Hubbard, 1977; Comins & Hassell, 1979; Waage, 1979; Hassell, 1982; Murdoch & Briggs, 1996; Vet, 1996; Taylor, 1996).

In this chapter, I briefly review the host-parasitoid system chosen for investigation of foraging behaviour, particularly patch exploitation by the parasitoids. Then I review the literature on the major issues of foraging theory related to patch exploitation in parasitoids, and the population consequences of parasitoid foraging behaviour in the context of host-parasitoid interactions. This chapter concludes by introducing the aims of this thesis.

1.2 The host-parasitoid system

1.2.1 *The pest status of Plutella xylostella*

The host-parasitoid system I chose is suitable for studies on the foraging behaviours of parasitoids, from both a practical and theoretical perspective. The diamondback moth (DBM), *Plutella xylostella* (Lepidoptera: Plutellidae), is the most destructive insect pest of cruciferous crops worldwide, particularly in temperate regions, the tropics and subtropics (Waterhouse & Norris, 1987). The annual cost for managing DBM on cruciferous crops was estimated to be U.S. $ 1 billion in 1992 (Talekar & Shelton, 1993). The pest has developed resistance to almost all major classes of insecticides, including *Bacillus thuringiensis* Berliner, over the last three decades in many tropical and subtropical areas of the world (Talekar *et al.*; 1986; Cheng, 1988; Tabashnik *et al.*, 1990; Talekar & Shelton, 1993; Liu & Sun, 1998), making it one of the most difficult pests to manage. Such a crisis has led to increasing interest in the development of biologically based integrated pest management system for DBM (Talekar & Shelton, 1993; Liu & Sun, 1998). The three international conference proceedings of DBM workshops have given a comprehensive review of current research and development on management of this pest (Talker & Griggs, 1986; Talekar, 1992; Sivapragasam *et al.*, 1997).

Both in China and Australia, DBM has been the most serious threat to cruciferous crops since the 1980's, due in part to the development of resistance to a range of insecticides used in its control (Liu *et al.*, 1995; Baker & Kovaliski, 1999). National and international cooperative programs on integrated pest management of DBM have been undertaken since the early 1990's to try and better manage this pest in both countries (Liu *et al.*, 1995; Heisswolf *et al.*, unpublished report). In these IPM programs, maximizing the role of natural control measures has been emphasized as one of the ways to reduce reliance on insecticides.

More than 90 hymenopterous parasitoids are associated with DBM worldwide (Talekar & Shelton, 1993). Among larval parasitoids, *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae) and *Diadegma semiclausum* (Hellén) (Hymenoptera: Ichneumonidae) are the two most important species which have been successfully introduced into many subtropical and tropical countries to regulate DBM populations (Waterhouse & Norris, 1987; Talekar & Shelton, 1993). In Australia, *D. semiclausum* is a widely established and abundant parasitoid (Goodwin, 1979; Waterhouse & Norris, 1987) while in China as well as in many other Asian counties, *C. plutellae* is a common indigenous species (Waterhouse & Norris, 1987; Talekar & Shelton, 1993; Wang *et al.*, 1998; Liu *et al.*, 2000). Both species have been introduced extensively among the Asian-Pacific countries (Waterhouse & Norris, 1987). In order to determine whether biological control would be enhanced by introducing one of the two species into an area where the other has established, it is important to evaluate the conditions under which one species is superior over the other and the nature of their interactions.

1.2.2 *General biology of P. xylostella*

P. xylostella occurs on a large number of plant species mostly in the Family Brassicaceae (= Cruciferae). The hosts include cabbage, broccoli, cauliflower, collards, rapeseed, mustard, and Chinese cabbage (Lin *et al.*, 1983; Talekar & Griggs, 1986; Talekar & Shelton, 1993; Verkerk & Wright, 1997) and a variety of weeds of this family (Muhamad *et al.*, 1994; Hatami, 1996). The presence of mustard oils and their glucosides, compounds characteristic of the Brassicaceae, influence the susceptibility of host plants to DBM. These chemicals are also utilized by larval DBM as phagostimulants (Gupta & Thorsteinson, 1960 a, b) and by adults as oviposition stimulants (Reed *et al.*, 1989). The phylloplane waxes on the leaf surfaces of host plants influence resistance to and oviposition preference by DBM (Eigenbrode & Espelie, 1995; Spencer, 1996; Justus *et al.*, 2000). For example, oviposition by DBM was increased on surfactant-treated broccoli which changed the structure of leaf waxes (Riggin-Bucci & Gould, 1996; Riggin-Bucci *et al.*, 1998).

The adults emerge during the first 8 hr of photophase and become active during the night, and mating occurs at dusk of the same day (Tabashnik, 1985; Pivnick *et al*, 1990; Talekar & Shelton, 1993). Female moths begin laying eggs on the day of emergence (Harcourt, 1957) or soon after copulation (Moller, 1988). The eggs of DBM are generally laid singly or in groups of two to four often along the mid-ribs or principal veins on the undersides of leaves or indented surfaces near smaller veins (Bhalla & Dubey, 1986; Chelliah & Srinivasan, 1986), or in groups of up to eight, mainly on the upper surface of leaves (Waterhouse & Norris, 1987).

DBM has four instars (Harcourt, 1957). Larval DBM wriggle rapidly and often drop from the plant on a silk thread when disturbed. First instars are leaf-miners while older larvae feed by scraping the epidermis of leaves, preferentially the younger leaves in the middle and inner part of the host plant (Harcourt, 1957). Each instar can be distinguished by the width of the head capsule (Herminanto, 1995). Larvae construct a loosely spun cocoon and spend a two-day period in a quiescent prepupal stage. The development from egg to adult was reported to require 283 day-degrees with a threshold of 7.3 • C (Harcourt, 1957). Although 17 to 25 • C is considered the optimum temperature range of DBM (Atwal, 1955), it has an ability to survive in a wide range of temperatures (Ooi, 1986; Shirai, 2000).

1.2.3 *C. plutellae* and *D. semiclausum*

C. plutellae (junior synonym: *Apanteles plutellae*) is a solitary endoparasitoid of larval DBM. The parasitoid is an oligophagous species (Nixon, 1974; Cameron *et al.*, 1998). Although *C. plutellae* was believed to be host specific (e.g. Potting *et al.*, 1999), it has been recorded from or reared on a number of other species of Lepidoptera besides *P. xylostella*: *Agdistis benneti*, *Aglais urticae*, *Anthocharis cardamines*, *Corcyra cephalonica*, *Crocidolomia binotalis*, *Ephestia cautella*, *Hellula hydralis*, *Hyphantria cunea*; *Maniola jurtina*, *Malacosoma castrensis*, *Ocnogyna baeticum*, *Spilosoma urtica*, *Thaumetopea herculeana*, *Trichoplusia ni* in the laboratory (Joshi & Sharma, 1974; Nixon, 1974; Lim, 1986; Waterhouse & Norris, 1987; Fitton & Walker, 1992; Cameron *et al.*, 1998), but less commonly recovered from alternative hosts that have been collected from the field (see Cameron *et al.*, 1998). This species was believed to be native to Europe, and has been introduced into a number of Pacific countries to control DBM (Waterhouse & Norris, 1987; Fitton & Walker, 1992). In fact, *C. plutellae* commonly occurs in most Asian countries (e.g. Noda *et al.*, 1996; Wang *et al.*, 1998), and South Africa (Kfir, 1998) where no introduction was ever recorded. Some hyperparasitoids are known to attack *C. plutellae* and their activities could adversely affect the efficiency of the parasitoid (Ooi, 1979; Liu *et al.*, 2000). A total of 12 species of hymenopterous hyperparasites were recorded from *C. plutellae* (Ooi, 1979; Sviapragasam & Rashid, 1994) in Malaysia, one in the Philippines (Velasco 1982), four in South Africa (Kfir, 1994), and five in China (Wang *et al.*, 1998).

D. semiclausum (junior synonyms: *D. cerophagus, D. eucerophaga*) is also a solitary endoparasitoid of larval DBM. The parasitoid is specific to DBM (Gauld, 1984), and is native to Europe (Waterhouse & Norris, 1987). *D. semiclausum* has been successfully introduced from Europe into several Asian-Pacific countries for enhancing biological control of *P. xylostella* (Waterhouse & Norris, 1987; Talekar & Shelton, 1993; Liu & Sun, 1998).

The two species show much overlap in their fundamental niches based on their geographical range, temperature requirements and host stage suitability (Talekar & Yang, 1991; Talekar & Shelton, 1993; Verkerk & Wright, 1996). An important difference is their suitable temperature ranges. *D. semiclausum* is believed to be more effective in parasitization at relatively lower temperatures (15-25 • C) than *C. plutellae* (20-30 • C) (Talekar & Yang, 1991; Yang *et al.*, 1993; Herminanto, 1995; Shi & Liu, 1999). This appears to explain the successful establishment of *D. semiclausum* and the substantial suppression of DBM in highlands of Indonesia, Malaysia, and Taiwan. Under suitable temperature conditions, functional response experiments showed that *D. semiclausum* was superior to *C. plutellae* (Chua & Ooi, 1986), although the results present in the study were preliminary. Field studies showed that host plant species differentially influenced parasitism of *P. xylostella* by the two parasitoids (Yang *et al.*, 1993; Verkerk & Wright, 1997). In addition, populations of *D. semiclausum* are often strongly male-biased (ca. 25% female) which is considered a factor in preventing them from regulating populations of DBM (Chau & Ooi, 1986; Fox *et al.*, 1990; Yang *et al.*, 1993). Recently, Noda (2000) reported the detection of diploid males in *D. semiclausum*, which could partly account for the male-based sex ratio.

Traditionally, evaluation of the two parasitoids for biological control has been focused on comparing the differences in aspects of their biology, characteristics of parasitism, and field ecology (Chua & Ooi, 1986; Talekar & Yang, 1991; Talekar & Shelton, 1993; Verkerk & Wright, 1996; Shi & Liu, 1999). We have a thorough understanding about the basic biology of both the host and parasitoids (reviewed in Waterhouse & Norris, 1987; Talekar & Shelton, 1993). However, there is limited understanding of the behavior of these two parasitoids. Both utilize volatile semiochemicals to locate host-infested plants (Davis, 1987; Bogahawatte & van Emden, 1996; Potting *et al.*, 1999; Shiojiri *et al.*, 2000). In order to predict levels of parasitism by these wasps, a thorough understanding of the behavioral ecology of the tritrophic interactions involved is needed (Verkerk & Wright, 1996).

1.3 Foraging theory

1.3.1 *Patch time allocation*

Foraging theory began with a recognition of the importance of the patchiness of the environment (MacArthur & Pianka, 1966), and the development of theoretical models that addressed the optimal exploitation of patchy resources by foragers (Fretwell & Lucas, 1970; Charnov, 1976a, 1976b). A number of patch time allocation models have been proposed (see Godfray, 1994). These studies have been taken three major approaches: (1) constructing theoretical models such as the Marginal Value Theorem (Charnov, 1976b) and dynamic evolutionary models of patch exploitation (Mangel & Clark, 1988); (2) proposing models based on behavioral observation such as simple 'rules of thumb' (see Stephens & Krebs 1986) and behavioral mechanism models (Waage, 1979; Driessen *et al.*, 1995); and (3) deducing behavioral rules using statistical modeling (Haccou *et al.*, 1991). These studies have sought to determine how a forager estimates information about the distribution and abundance of both resources and competitors (Krebs & Kacelnik, 1991).

The best-known theoretical model is the Marginal Value Theorem (Charnov, 1976b), which deals with the optimal patch residence time of individual foragers in a given patch. The model assumes that a forager has universal environmental information and can correctly access patch quality instantaneously while foraging in the patch. If the forager searches for prey randomly, it experiences decreased resource availability with every successful harvest. As a result, the cumulative gain function (e.g. net energy intake) is presumed to be a negatively accelerated increase over time leading to patch depletion. Therefore, the model predicts that an optimal forager should leave each patch when the instantaneous resource harvest rate falls to the average maximum rate expected for the habitat. This produces two main predictions: (1) a forager should spend more time in better quality patches when travel time between patches is constant or negligible, and should stay longer when travel times between patches become longer in the presence of patches of equal quality but with varying travel times between patches (Figure 1.1). The general predictions of the model, that the forager should spend more time in better quality patches, often shows qualitative agreement with empirical observations (e.g. Alonso *et al.*, 1995; Bonser *et al.*, 1998; Lei & Camard, 1999).

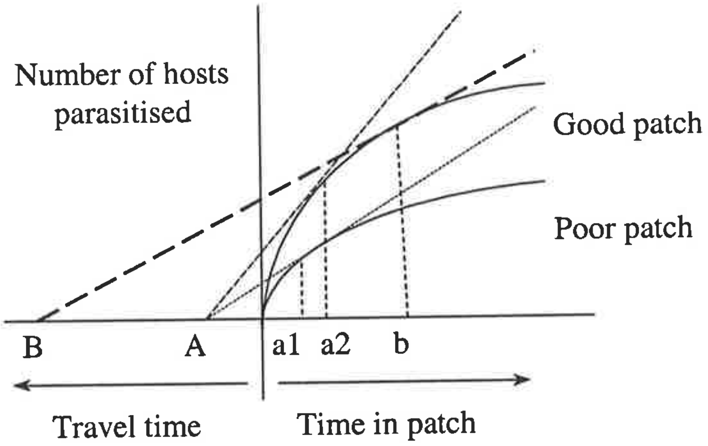


Figure 1.1 The marginal value theorem (Charnov, 1976b). The curves represent gain function of the cumulative resource harvest (here the number of hosts parasitised) over time while foraging within a patch. When patches are identical, patch residence time (right side from the origin) increases with increasing travel time between patches (left side from the origin). The optimal residence time can be found by constructing a line tangent to the gain function that begins at the point on the travel axis. Longer travel time (B) leads to longer residence time (b). When patches vary in quality, the parasitoid should leave a patch when its marginal rate of gain (the slope of the cumulative gain curve) falls to the long-term average maximum gain rate expected in that environment. Parasitoids should remain longer in good patches (a2) than in poor patches (a1).

In situations where foragers have either no or incomplete information about the distribution and abundance of resources, a variety of simple behavioral 'rules of thumb' have been proposed (see Stephens & Krebs, 1986). For instance, leaving after encountering a certain number of prey or hosts (fixed number), or leaving after a certain time has elapsed since entering that patch (fixed patch time) or encountering a host (fixed giving-up time) (Figure 1.2). A number of theoretical analyses have focused on the utility of such simple patch-leaving rules, in which foragers could achieve nearly optimal patch-leaving decisions (Iwasa *et al.*, 1981; McNair, 1982; Green, 1984). For example, the fixed number rule may be an adaptive solution when the variation in host density among patches is low (Iwasa *et al.*, 1981). However, empirical evidence of the general use of those rules is rare in real species. When patches vary in density or other factors, the first two rules of thumb are not appropriate (van Alphen & Vet, 1986; Godfray, 1994).

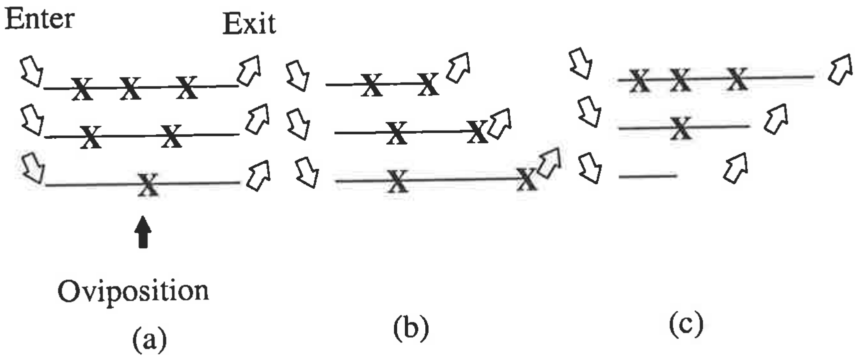
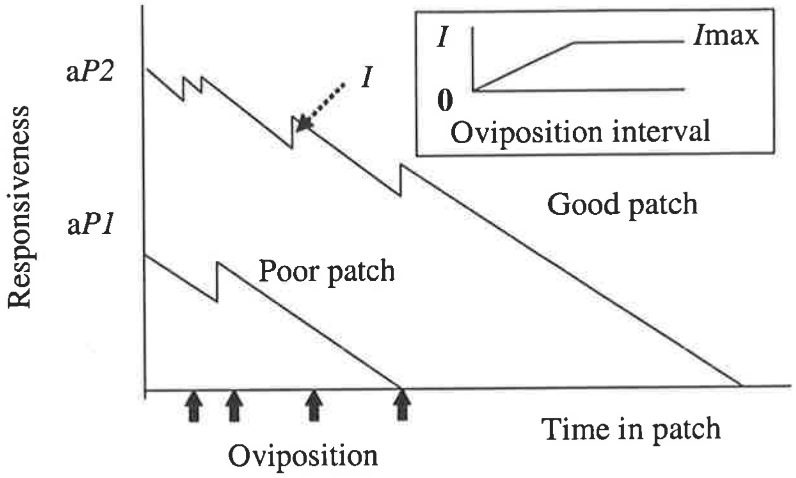


Figure 1.2 Patch-leaving rules of 'thumb'. (a) Fixed time, (b) fixed number of hosts or prey (c) fixed giving-up time. Arrows indicate the beginning and the ending of patch visits, and X indicates the time and order of oviposition.

Given an insect parasitoid has limited prior information about patch quality, foraging experience could help to decide when to give up a patch. Two simple behavioral models considering the effect of the rewarding oviposition experience have been proposed, based on an empirical study of the parasitoid *Venturia canescens* (Waage, 1979; Driessen *et al.*, 1995). Waage's model (1979) assumes that the parasitoid has a basic tendency to remain in a given patch as a response to the kairomone concentration, which decays with time. Oviposition increases this responsiveness by a set amount that is assumed to be a linearly increasing function of unsuccessful search time since the last oviposition with an asymptotic cut-off. Thus, patch time in this model is determined primarily by the patch kairomone concentration and the numbers and timing of ovipositions (Figure 1.3). In contrast, Driessen *et al.* (1995) proposed an opposite mechanism, in which an oviposition decreases the responsiveness level, and the effect of each subsequent oviposition decreases with increasing ovipositions (Figure 1.3). Many empirical studies have supported either of above models (see Driessen & Bernstein, 1999). From a functional point of view, which of these mechanisms should be used by a parasitoid depends on the parasitoid's information processing ability. If the parasitoid is able to correctly assess the patch quality, a count-down mechanism should be favored (Shaltiel & Ayal, 1998; Vos *et al.*, 1998; Driessen & Bernstein, 1999).

The Marginal Value Theorem model has shaped modern foraging theory, while the simple behavioral models do incorporate some realistic aspects of parasitoid biology and behavior, and have explained many observations. No doubt, these behavioral models are very successful in initial attempts to explain the responses of parasitoids to particular patch systems. However, these simple models are inherently based on assumptions about the information sources available to parasitoids and their ability to utilize such information. When testing these rules in the real world, there are a number of potential problems. It can be expected that the rules used by a forager could depend largely on features of its natural environment, and that patch-leaving behaviour should be variable or adjustable (Vos *et al.*, 1998), making generalization difficult.



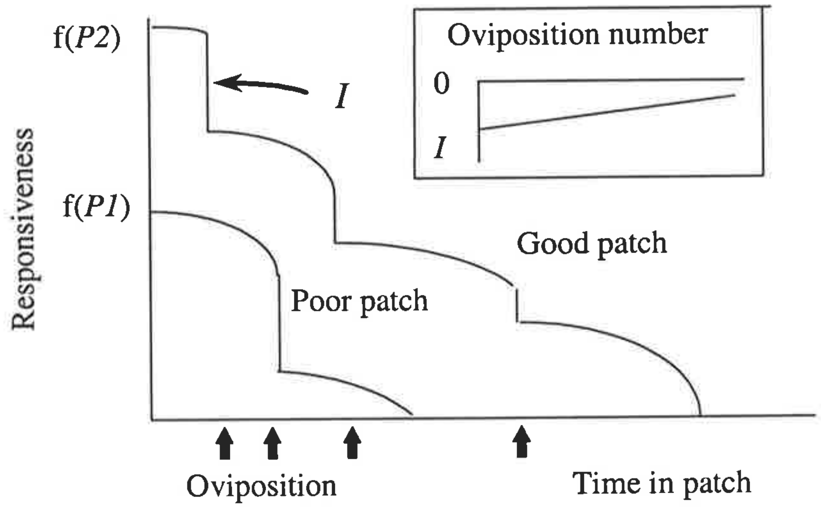


Figure 1.3 Graphical representation of Waage's incremental mechanism model (Top) and Driessen's count-down mechanism model (Bottom). Both models assume that parasitoids have a basic tendency to stay in a given patch as a response to the kairomone concentration, which is a function of host density. The responsiveness decreases over time, until the parasitoids leave the patch when responsiveness level drops to a critical threshold. Waage's model predicts that oviposition increases the responsiveness and the increment value *I* depends on the time elapsed since the last oviposition, up to a maximum I_{max} (see insert). In contrast, Driessen's model predicts that oviposition results in a decrease in the responsiveness and the value *I* decreases with oviposition number (see insert).

There are two important problems that should be addressed when studying the foraging decisions of parasitoids. Firstly, parasitoids' foraging behavior might be very complicated. Many factors could influence a parasitoid's behavior, including previous foraging experience (van Lenteren, 1976; Waage, 1979; van Lenteren, 1981; Roitberg & Prokopy, 1984; Dicke *et al.*, 1985; Bakker *et al.*, 1990; Haccou *et al.*, 1991; van Lenteren, 1991; van Roermund *et al.*, 1994; Nelson & Roitberg, 1995; van Alphen & Jervis, 1996; van Steenis *et al.*, 1996; Vos *et al.*, 1998), adaptive superparasitism or imperfect host discrimination (Bakker *et al.*, 1990; van Alphen & Visser, 1990; Visser *et al.*, 1990; Rosenheim & Mangel, 1994), physiological state of the parasitoid (e.g. Mangel & Clark, 1988; Fletcher *et al.*, 1994), and the nature of the environment (e.g. Waage, 1978; van Lenteren & Bakker, 1978; Waage, 1979; Iwasa *et al.*,1981; Dicke *et al.*, 1985; van Lenteren, 1991; Visser *et al.*, 1992a; Driessen *et al.*, 1995; Bernstein & Driessen, 1996; Longley & Jepson, 1996; van Steenis *et al.*, 1996). It seems that all information about the patch and habitat quality available to the parasitoid will influence the parasitoid's foraging behaviour, and all these factors could influence patch use decisions. For example, Waage's and Driessen's models have presumed that a parasitoid estimates host density by kairomone concentration. Although many parasitoids respond to host-associated cues such as kairomones (Vet & Dick, 1992), in nature the kairomone concentration could only serve as a rough estimation of the initial host density rather than host quality. Furthermore, the estimation largely depends on the information detectability and processing of the parasitoid within a particular environment. Therefore, in the light of the complexity of behavioral cues and parasitoid's ability to respond, these simple and *a priori* behavioral models are limited in their applications in complicated environments such as multiple patches. In the real world, there may be confounding factors which have influenced the outcomes predicted by theoretical models, as the effects of some factors can not be tested separately (van Alphen & Jervis, 1996).

Secondly, more importantly and rarely explored in previous studies, the boundaries of patches that a parasitoid perceives while foraging in a given area must be identified? A general view of patch is a surface area differing from its surroundings in nature or appearance (Kotliar & Wiens, 1990). In terms of this definition, patch implies a discrete and internally homogeneous area. However, in nature this kind of patch is rarely observed. Instead, hierarchical mosaics of patches within patches occur over a broad range of scales such as the heterogeneity among individual leaves, plants, and plant clumps of various sizes. Thus studies restricted to different spatial levels may yield different results (Kotliar & Wiens, 1990; Keller & Tenhumberg, 2000). For instance, predictions of patch giving-up times derived from the Marginal Value Theorem will largely depend on the scales over which a parasitoid samples its environment. However, most tests of optimal foraging theory have been made in situations involving either very simple arenas where the parasitoid's perception of the 'patch' is unambiguous or simple assumptions on the parasitoid's perception of host patch (Waage, 1979; Ayal, 1987; van Roermund *et al.*, 1994; Rosenheim *et al.*, 1989; Vos *et al.*, 1998).

Haccou and Hemerik (1985) first introduced Cox's proportional hazards model (Cox, 1972) for the analysis of the foraging behaviour of the red wood ant *Formica polyctena,* and then analyzed the effects of various factors on the patch-leaving tendency of the parasitoid *Leptopilina heterotoma* (Haccou *et al.*, 1991). The statistical analysis of patch-leaving tendency by means of the proportional hazards model enables quantitative estimation of the influence of various factors on the patch-leaving tendency directly from the data, with few *a priori* assumptions (Haccou *et al.*, 1991). Several other studies have used this model to analyze the patch leaving tendency of parasitoids (Hemerik *et al.*, 1993; van Roermund *et al.*, 1994; van Steenis *et al.*, 1996; Vos *et al.*, 1998; Driessen & Bernstein, 1999; Wajnberg *et al.*, 1999, Keller & Tenhumberg, 2000).

Statistical modeling helps illustrate the complexity of the behavioral rules used by parasitoids. In practical, the statistical method is an extension to the usual *a priori* modeling. On the one hand, *a priori* considerations are used to determine which covariates are potentially relevant. On the other hand, the results of such analysis may provide important directions for further tests or the development of theoretical models (Hermerik *et al.*, 1993).

Comparing the predictions of evolutionary searching models with the performance of statistically derived behavioral rules may accelerate the development of more realistic models of foraging behaviour (Godfray, 1994).

1.3.2 *Oviposition decisions*

Two major issues of the oviposition decisions of parasitoids, which have received growing theoretical attentions during the last decades, are the interdependent phenomena of superparasitism and host discrimination, and their effects on optimal patch utilization strategies (van Alphen & Visser, 1990; Speirs *et al.*, 1991; Godfray, 1994). In solitary parasitoids, only one larva can successfully complete development in a host. Oviposition into an already parasitised host (superparasitism) generally yields a lower fitness return than an unparasitised host. The adaptive value of recognizing a host thus parasitised (host discrimination), is largely self-evident (reviewed in van Alphen & Visser, 1990). Superparasitism is now widely accepted as an adaptive oviposition strategy under many closely defined conditions, and has been investigated in several recent theoretical studies by either static and dynamic optimality models within the framework of evolutionarily stable strategies (ESS) (Hughes, 1979; Iwasa *et al.*, 1984; Charnov & Skinner, 1985; Hubbard *et al.*, 1987; Mangel & Roitberg, 1989; van Alphen & Visser, 1990; Mangel, 1992; Visser *et al*, 1992a; Godfray, 1994).

Both static optimality models and ESS models of superparasitism in solitary parasitoids assume that the parasitoids are time-limited rather than egg-limited, and they should maximize their lifetime reproduction. Static models predict the dependent relationship between host quality, host recognition time and host acceptance (Hughes, 1979; Iwasa *et al.*, 1984; Harvey *et al.*, 1987; Janssen, 1989; van Alphen & Visser, 1990). Because a resource habitat often is exploited simultaneously by more than one parasitoid, adaptive strategies of superparasitism are dependent on the decisions of competitors, and therefore superparasitism decisions have been modeled as components of ESS (Hubbard *et al.*, 1987; van Alphen & Visser, 1990; Mangel, 1992; Visser *et al.*, 1992a). The ESS approach considers directly the interdependence of superparasitism and patch time allocation in the decision-making process, and adds to the static model the predictions that when there is no competition, self-superparasitism should never occur, and the optimal strategy is to reject parasitised hosts initially, but later to superparasitise when the rate of gain of offspring from the patch drops to a certain threshold (Hubbard *et al.*, 1987; Visser *et al.*, 1992a, Visser, 1995).

Such rate maximization models may not be suitable for parasitoids that are not only time-limited, but also may be egg-limited or both (Godfray, 1994; Rosenheim, 1996). By incorporating state-dependent variables relating to the physiological conditions of the foraging parasitoids such as egg-load, dynamic models of superparasitism in solitary parasitoids predict a parasitoid's decision to superparasitise is influenced by the number of mature eggs available for oviposition, as evidenced by both theoretical and empirical studies (Iwasa *et al.,* 1984; Mangel & Roitberg, 1989; Mangel, 1992; Minkenberg *et al.*, 1992; Fletcher *et al.*, 1994; Hughes *et al*, 1994; Sirot *et al.*, 1997).

Empirical studies have documented many other factors that affect oviposition decisions such as the risk of mortality when attacking a defensive host (e.g. Potting *et al.*, 1997), the mating status of females (Minkenberg *et al.*, 1992; Michaud, 1994), life expectancy (Roitberg *et al.*, 1993; Fletcher *et al.*, 1994).

A major conclusion has been that the oviposition decisions of a parasitoid should be fundamentally dynamic and thus vary in response to (1) its physiological state (Iwasa *et al.*, 1984; Rosenheim & Rosen, 1991; Minkenberg *et al.*, 1992; Roitberg *et al.*, 1993; Fletcher *et al.*, 1994; Henneman e*t al.*, 1995; Sirot *et al.*, 1997), and (2) its informational state about the environment, including all kinds of experiences accumulated or learned during the course of foraging, such as host availability, risk of mortality, and competition (Iwasa *et al.,*1984; Hubbard *et al.*, 1987; Mangel & Roitberg, 1989; Rosenheim & Rosen, 1991; Mangel, 1992; Visser *et al.*, 1992a; Henneman *et al.,*1995; Visser, 1995; Hubbard *et al.*, 1999). However, many theoretical models have a limited scope due to the limited range of assumptions on which they are based. Thus theoretical models can not be used to assess the relative impacts or interactions among all of the possible variables that may affect oviposition decisions in the real world (Henneman *et al.*, 1995; Rosenheim, 1996; Seventer *et al.*, 1998; Rosenheim, 1999). Statistical modeling offers an alternative means of determining the effects of egg depletion, experience and other time-related variables on oviposition decisions (Rosenheim & Rosen, 1991; Visser, 1995; Horng *et al*, 1999).

1.4 Link between individual behaviors and population dynamics

Optimal foraging models assume that natural selection has shaped parasitoid foraging behaviour in such a manner that it maximizes oviposition rate (Charnov, 1976b; Hubbard & Cook, 1978). If this assumption is true, then the population consequences of parasitoid foraging behaviour can be predicted. An optimally foraging parasitoid should be expected to concentrate searching on highly profitable patches, which should lead to an aggregation response on high host density patches (e.g. Hassell & May, 1974), or and Ideal Free Distribution of foragers over patches (Fretwell & Lucas, 1970; Sutherland, 1983; van Baalen & Sabelis, 1993; Krivan, 1997; Bernstein *et al.*, 1988,1991, 1999).

Theoretical models that seek to explain the population consequences of host-parasitoid interactions suffer from the use of overly simplistic assumptions about foraging behaviour (Ives, 1995; Sutherland, 1996). Any parasitoid behaviour that cause spatially heterogeneous parasitism contributes to the persistence of the interacting species (Beddington *et al.*, 1978, May, 1978; Chesson & Murdoch, 1986; Pacala *et al.*, 1990; Ives, 1995).

Since the active aggregative response of insect parasitoids has been demonstrated in many laboratory studies (e.g. Waage, 1979; van Alphen & Galis, 1983), and field observations (Stamp, 1982; Wagge, 1983; Smith & Maelzer, 1986; Thompson, 1986; Jones & Hassell, 1988; Casas, 1989; Ives *et al.*, 1999), a huge amount of theoretical work has been devoted to explaining the population consequences of parasitoid aggregation (Beddington *et al.*, 1978;

May, 1978; Hassell, 1980; Murdoch *et al.*, 1985; Reeve & Murdoch, 1985; Chesson & Murdoch, 1986; Strong, 1988; Murdoch & Stewart-Oaten, 1989; Pacala *et al.*, 1990; Godfray & Pacala, 1992; Ives, 1995; Murdoch & Briggs, 1996). The positive aggregation response by parasitoids should generate a direct density-dependent parasitism, if the parasitoids are not strongly egg-limited (Comins & Hassell, 1979; Hassell, 1980, 1982; Waage, 1983; Lessells, 1985), or if interference between individuals is not very strong (Sutherland, 1983). However, empirical studies have often failed to detect density-dependent parasitism, although the parasitoids were observed more frequently on high-density patches (e.g. Morrison & Strong, 1980; Waage, 1983; Smith & Maelzer, 1986). Parasitism by insect parasitoids displays highly variable patterns from direct, inverse density-dependent to density-independent (reviewed in Lessells, 1985; Stiling, 1987; Walde & Murdoch, 1988).

Models have explained the lack of density-dependence in parasitism as a consequence of behavioural or physiological limitations of the foraging parasitoids, such as the low availability of eggs and handling time when foraging in high host density patches (e.g. Hassell, 1982; Waage, 1983; Lessells, 1985); stochastic variations in patch time allocation (Morrison, 1986); and other forms of aggregation response (e.g. Walde & Murdoch, 1989; Reeve *et al.*, 1989). However, it seems to be impossible to produce a universal explanation.

Some studies have attempted to infer statistically the aggregation response of parasitoids indirectly based on the observed levels of parasitism (e.g. Heads & Lawton, 1983). But it could be very difficult to distinguish behaviorally mediated aggregation from demographically mediated aggregation (Rosenheim *et al.*, 1989). Furthermore, the aggregation response of a parasitoid or predator is sensitive to the spatial scale and thus the level of parasitism (Morrison & Strong, 1980; Walde & Murdoch, 1988; Rosenheim *et al.*, 1989; Sheehan & Shelton, 1989; Ives *et al.*, 1993). It is necessary to understand the parasitoid's perception of ‘patchiness’ or spatial scale at which the parasitoids recognize and respond to differences in host density and distribution (Walde & Murdoch, 1988; Rosenheim *et al.*, 1989).

Unfortunately, as is often acknowledged (e.g. Hassell, 1980; Waage, 1983; Walde & Murdoch, 1988; Rosenheim *et al.*, 1989; Ives *et al.*, 1999), empirical studies that quantify the dynamic pattern of patch exploitation based on direct observations of the foraging behaviour of parasitoids are not common (Hubbard & Cook, 1978; Hassell, 1980). While optimal foraging models have made the specific assumptions about a parasitoids' ability to assess information, a thorough understanding of the parasitoid's patch use rules is necessary to elucidate their role in the host's population dynamics (Godfray, 1994). Thus, laboratory research is needed to obtain a thorough understanding of a parasitoid's patch use rules and to assess critically a parasitoid's limitations to determine the relationship between foraging behaviour, aggregation response and parasitism. In addition, experimentally oriented field work should be particularly valuable for determination of the aggregation response of the parasitoids to local variations in host density over time and space. Most population ecology studies are concerned with the pattern of parasitism, not the foraging behaviour *per se*. However, conclusions concerning relationships between parasitoid searching behaviour and the spatial pattern of parasitism should be drawn from a direct examination of parasitoid foraging rather than indirectly through an examination of spatial patterns of parasitism. This would lead to incorporation of more biologically realistic behavioral attributes into theory-driven host-parasitoid interaction models. Thus, such an approach could bridge the gap between individual behavioral studies and ecological studies of host-parasitoid population dynamics.

1.5 Aim of this project

This project aimed to investigate the foraging behaviour of two larval parasitoids of *P. xylostella*: *C. plutellae* and *D. semiclausum*. There were two primary goals: a practical focus on the efficacy of the two parasitoids as biological control agents, and empirical tests of foraging theory models using *D. semiclausum* as a model species. Although some existing foraging models have been tested with a number of parasitoids species, an integrated approach that provides a through understanding of the decision-making involved in a parasitoid's patch exploitation within the broader context of both behaviour and population dynamics has been lacking.

This thesis includes eight chapters that present experimental research. Chapter 2 describes and compares the foraging behaviour of *C. plutellae* and *D. semiclausum*. The purpose of this work was to establish a foundation upon which the behavioral mechanisms observed in the subsequent experiments could be interpreted. From a practical perspective, the behavioral approach was used to identify the effective behavioral attributes that both parasitoid species employ to exploit hosts and overcome host defense. This contributes to the understanding of the relative suitability of the parasitoids for biological control.

Chapter 3 is a dynamic analysis of the oviposition decisions by *D. semiclausum* using statistical modeling. This chapter also forms the basis for interpretation of the patch-leaving decisions in later chapters.

From a theoretical perspective, the following three chapters (4-6) investigate the patch time allocation, particularly the patch leaving decisions in *D. semiclausum*. The effects of various factors on the patch-leaving tendency of the parasitoid were analyzed by direct statistical comparisons and by means of Cox's proportional hazard models. A number of foraging models such as Charnov's Marginal Value Theorem model (Charnov, 1976b), simple 'rules of thumb' (Stephens & Krebs, 1986) and two behavioral models (Waage, 1979; Driessen *et al.*, 1995) were evaluated to determine how well they predict the observed patterns of behavior. These studies provide a more thorough understanding of the decision making of the individual parasitoids, from which an adjustable patch leaving model in *D. semiclausum* was proposed.

Another important, yet relatively unstudied issue in parasitoid foraging ecology is the spatial variation of foraging behaviour, *i.e.*, how the selected spatial scales might influence the results on the analysis of patch-leaving decisions. Thus, chapter 7 addresses the importance of spatial scale for elucidation of patch-leaving decisions. The data collected on the foraging behaviour of *D. semiclausum* in a multi-patch environment were analyzed on different spatial scales from a single leaf, to a single plant and a group of plants.

Chapter 8 evaluates the optimal foraging predictions of a group of parasitoids foraging in a multi-patch environment, both under laboratory and field conditions (Charnov, 1976b; Cook & Hubbard, 1977; Sutherland, 1983). This work provides a link between the individual behaviour and host-parasitoid population dynamics. A conceptual model was constructed to analyze the relationship between patch exploitation and density-dependence in resultant patterns of parasitism.

The impact of natural enemies, particularly *D. semiclausum,* on *P. xylostella* in a winter broccoli field in Queensland, Australia is evaluated using cage exclusion methods in chapter 9.

Finally, the major results of this study are reviewed and integrated in a general discussion in chapter 10.

Chapter 2 Foraging efficiency of *C. plutellae* and *D. semiclausum*

2.1 Introduction

Insect parasitism is the result of a sequence of directed searching behaviors at progressively finer environmental levels from host habitat location to host location and host suitability (Vinson, 1976). Host-searching behaviour is directly related to a parasitoid's capacity to locate and parasitise hosts under various conditions. A knowledge of host searching behaviour may help to define the effective attributes used by parasitoids to exploit host resources efficiently, and thus to evaluate the suitability of parasitoids for biological control of insect pests (Luck, 1990).

Because of the direct link between successful searching and the production of offspring, a parasitoid's host-searching behaviour is likely to be under strong natural selection (Vet & van Alphen, 1985; van Alphen & Vet, 1986; Godfray, 1994). Comparative studies of host-searching behaviour in related species sharing similar ecological niches can reveal the extent to which the interspecific differences at all levels of host-searching have influenced their relative foraging efficiency (Wiskerke & Vet, 1994), their extrinsic competitive interactions and potential co-existence (van Dijken & van Alphen, 1998; De Moraes *et al.*, 1999). An analysis of these differences in behaviour combined with other aspects of parasitoid biology might also give insight into the evolutionary dynamics of host selection and location strategies in parasitoids (Vet & van Alphen, 1985; van Alphen & Vet, 1986; Vet & Dicke, 1992; Godfray, 1994; Vinson, 1998).

The host range of parasitoids varies between extreme specialists that attack only one species and generalists that attack a wide range of species sharing taxonomic affinity or similar ecological niches. Specialization is thought to confer superior host location efficiency involving both long-range and short-range cues and greater ability to overcome host defenses (Vet & Dicke, 1992; Vet *et al.*, 1993; Godfray, 1994; Geervliet *et al.*, 1996; Cortesero *et al.*, 1997). Thus a host specialist should locate and parasitise more hosts than a generalist in a given arena. This chapter tested these predictions by comparing the host searching behaviour of *C. plutellae* and *D. semiclausum.*

C. plutellae is an oligophagous parasitoid (Nixon, 1974; Cameron *et al.*, 1998). Although *C. plutellae* was assumed to be host specific, it has been recorded from or reared on several other species of Lepidoptera (Fitton & Walker, 1992; Cameron *et al.*, 1998). *D. semiclausum* is specific to *P. xylostella* (Gauld, 1984) and has been successfully introduced from Europe into several Asian countries for enhancing biological control of *P. xylostella* (Talekar & Shelton, 1993). Together these two species are the most important larval parasitoids of *P. xylostella* in most areas of the world (Talekar & Shelton, 1993).

Both parasitoids use volatile semiochemicals to locate host-infested plants (Davis, 1987; Potting *et al.*, 1999; Shiojiri *et al.*, 2000). Laboratory studies have shown that host plant cultivars influence the host location behaviour of *C. plutellae* (Bogahawatte & van Emden, 1996), while field-based studies have shown that host plant species differentially influence parasitism of *P. xylostella* by the two parasitoids (Talekar & Yang, 1991; Verkerk & Wright, 1997). In order to predict levels of parasitism by these wasps, a thorough understanding of the behavioral ecology of the tritrophic interactions involved is needed (Verkerk & Wright, 1996).

This chapter aims to describe quantitatively how the host-searching behaviour is integrated and expressed by *C. plutellae* and *D. semiclausum* in patches of host plants infested by different densities of larval *P. xylostella.* The pattern of movement of each searching wasp and the resultant parasitism over patches as a response to local variation in host density among plants was analyzed. It showed how the different host searching strategies at all levels of host searching could ultimately result in differences in the encounter rate with hosts and levels of parasitism achieved by the two species. One particular interest was the different species-specific adaptations of host-location strategies against host defensive behaviour, which could be associated with the different degrees of host specificity.

2.2 Materials and methods

2.2.1 *Insects and host plant*

A laboratory population of the diamondback moth (DBM) *P. xylostella* was established from a field collection in Adelaide, South Australia, and maintained on potted cabbage plants in an insectary (24 °C, 14L: 10D, 50-70% RH). *C. plutellae* was imported from Taiwan in 1998 and had been reared for 1-2 generations prior to the experiments. *D. semiclausum* was collected as parasitised larval DBM in crucifer vegetable fields in Adelaide in October 1997. Field-collected *D. semiclausum* were added to the culture periodically to maintain genetic diversity. Both parasitoids were reared on larval DBM under the same conditions as DBM. For detailed procedures of the culture of both the host and two parasitoids see Appendix.

Common cabbage (*Brassicae oleracea* var. *capitata*, cv. Green Coronet) was grown in pots in a greenhouse. Second and third instar DBM larvae and young potted cabbage plants with 5-6 fully extended leaves were used in the experiments.

2.2.2 *Open wind tunnel design*

All experiments were conducted in an open wind tunnel designed to provide a free-flight environment for the parasitoids (Figure 2.1). The tunnel was located in a windowless room (1.8 x 2.2 x 2.8 m) with controlled temperature of 24-25 °C. The walls were coated with cream matt paint. The test arena had a wooden floor, which was covered with sand. Plants that were grown in 8 cm diameter plastic cups were fitted into holes in the floor so they were flat on the ground. A mirror on the far side of the test section facilitated viewing of wasps that were otherwise out of sight. Two vertical wooden frames (54 x 80 cm) covered with Terylene voil were fitted to the two ends of the board. At the upwind end, a cardboard honeycomb (used as a spacer inside hollow doors) fitted inside the frame reduced air turbulence. Wind was produced by an electric fan fixed behind the downwind Terylene screen. Air was

hylene tunnel (50 cm diameter) which went under the test arena and
wooden frames. In this experiment, the wind speed was set at 30-
al arena was lit by 4 cool white 40 W fluorescent tubes hanging

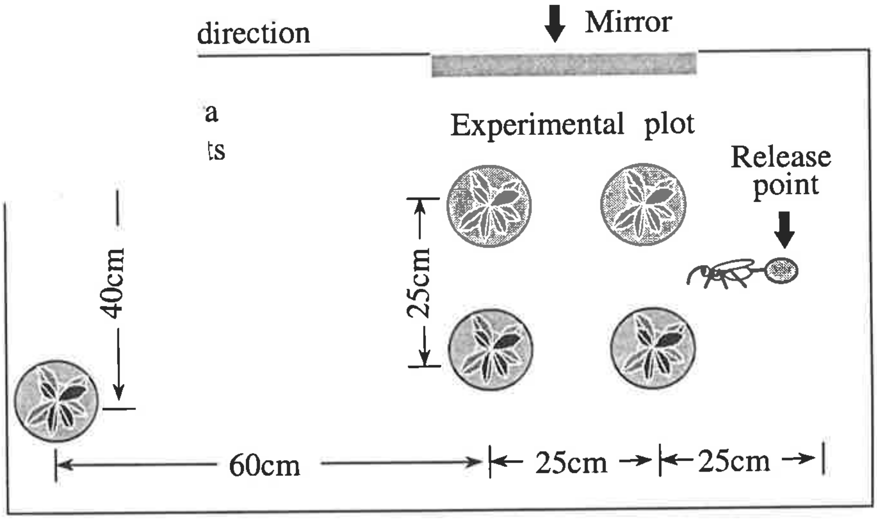


Figure 2.1 Layout of the testing section of the open wind tunnel (140 x 64 cm).

2.2.3 *Parasitoid handling*

The parasitoids used in this experiment were 2- to 3- day-old, mated female *C. plutellae* and *D. semiclausum*. Parasitoid cocoons were collected, and maintained individually in glass vials (7x 2 cm). Upon emergence, individual female wasps were caged with 5 males until each mated. The mated females were then isolated in vials again with a drop of honey prior to the experiments. Immediately before release, individual parasitoids were primed for 5 min. by placing them in an 80 mm glass Petri dish containing a piece of cabbage leaf with fresh feeding damage produced by one 3rd instar DBM. This exposure gave experience with host-related cues to the experimental parasitoid and increased the parasitoid's in-flight orientation towards host-related cues (Davis, 1987; Kaiser & Cardé, 1992; Potting *et al.*, 1999). Individual wasps were then transferred to a clean glass vial for release.

2.2.4 *Experimental procedures*

Each leaf of the experimental plants was numbered sequentially from bottom to top to permit rapid identification. Four plants were infested with either zero, one, two, or four randomly distributed larval DBM one day before the experiments, and were placed randomly into a 2 x 2 grid (Figure 2.1). Just prior to the experiments, each leaf was examined and the number of the larvae, their position, and the presence of host damage and frass were recorded. The position of each larva was distinguished as either on damage, off damage, or moving into the heart leaves (larvae rarely moved between leaves during the course of an observation). Two extra plants infested by 4 larval DBM were placed upwind of the experimental plants to provide an alternative landing site for the wasps when they left the experimental plants. Wasps might spend excessive amounts of time on the experimental plants if there were no alternative sources of semiochemicals. Individual wasps were released from a 7 x 2 cm glass vial on a stand at about the same height as the feeding host larvae. All experiments were conducted between 9:00 and 16:00. On each date, one or two *C. plutellae* and *D. semiclausum* females were observed in a random order. Occasionally the wasp first landed away from the experimental plants and hence the observation was failed. In total, 24 of 39 releases in *C. plutellae* and 28 of 31 releases in *D. semiclausum* were successfully completed at the first time.

2.2.5 *Behavioural observation and records*

A behavioural catalogue was developed through preliminary observations of searching behaviour of the two parasitoids on a cabbage plant infested with hosts in a wind tunnel. The behaviour expressed by the two parasitoids was similar; thus the same catalogue of 8 distinctive elements of behaviour was used for both species (Table 2.1). For simplicity, in recording of the behaviours, probe was pooled with antennate, and attack was pooled with sting. Therefore, six types of behaviours were recorded: antennate, fly, groom, still, sting, walk.

Once the wasp flew from the release vial, its behaviour was observed continuously and recorded by a portable event-recording computer The Observer for Windows' (Noldus, 1991), until the wasp left the experimental patches and landed on the extra upwind plants or outside the test arena. Three types of data were recorded: type of behaviour, location among plants, and leaf number when on a plant. The positions of all larvae that were stung were recorded and these were dissected to determine parasitism on the same day.

2.2.6 *Data analysis*

A • ^{2} test was used to analyse the frequencies of first landing in relation to plant positions and host densities in the open wind tunnel. The mean duration, frequency, and proportion of time (arcsin square root transformed) devoted to each type of behaviour were calculated for each individual. For each type of behaviour, these statistics were compared between species using the Kruskal-Wallis test.

The temporal patterns of behaviour were examined by constructing behavioural flow diagrams (Field & Keller, 1993). The goal of this analysis was to gain insight into how behaviour was organised rather than to develop a robust model of behaviour. First-order behavioural transition matrices were constructed for each individual, with rows corresponding to preceding behaviours and columns to following behaviours. Data from all individuals were pooled in the analysis. By pooling, it was assumed that there were no significant differences among individuals. The principal diagonal elements of these matrices were logical

Table 2.1 A catalogue of behavioural acts of *C. plutellae* and *D. semiclausum*

Event	Descriptions
Antennate	The wasp palpates the substrate with both antennal tips while either moving or stationary, and usually moves rather slowly and in varying directions. This behaviour is associated with intensive searching activity as arrested by host feeding damage.
Attack*	Following antennal contact with a host, both parasitoids spread their antennae laterally, and the anterior portion of the body is raised while approaching the host in this posture. Additionally, *D. semiclausum* detects host by visual contact in the immediately vicinity of the host, and slowly approaches the host while maintaining the above posture.
Sting	The wasp curls its abdomen under the body, holds the host, raises its wings, and inserts the ovipositor into the host. Oviposition usually occurs during wrestling between wasp and host.
Probe	The abdomen is curled forward under the body and the ovipositor is pushed forward or down. The wasp pierces the substrate often near feeding damage, and host frass while antennating.
Fly	Any airborne activity.
Groom	The wasp repeatedly brushes the antennae with the forelegs, thorax and midlegs, and the abdomen, ovipositor, and wings with the hindlegs, rubs legs together, strokes the face and antennae with legs, i.e. any actions involving cleaning the body.
Still*	The wasp is motionless with the antennae stretching out in front of the head. *D. semiclausum* often remains still when waiting for a host near silk, but the antennae are pointed backwards at 45 °C over their heads.
Walk	The wasp moves along the leaf surface at a relatively constant and fast speed with the antennae extended and waving alternately up and down in front of the head.

* Indicates differences between the two species.

zeros since behaviours could not follow themselves in our records. The expected values of the matrix cells were found using the iterative proportional fitting method of Goodman (1968).

The statistical significance of the overall table was evaluated using a log-likelihood ratio (G) test. Yates' correction for continuity was applied throughout. When deviations in the overall table were found to be statistically significant, significant transitions were found by collapsing the table into a 2 x 2 matrix around each transition and a G test performed. The significance of these individual tests was adjusted to a table-wide level of 5% using the sequential Bonferoni method (Rice, 1989). The results of analysis of behavioural transition matrices were presented graphically in kinetograms (van Hoof, 1982; Field & Keller, 1993).

Patterns of movement were examined in relation to host densities on whole plants. The time allocation to clean vs. infested leaves, over plants infested by different densities of host larvae (arcsin square root transformed), and the frequency of visits, attacks, and stings per unit time were calculated for each individual, and were compared between species using the Kruskal-Wallis test.

2.3 Results

2.3.1 *First landing*

After taking off from the release site, *D. semiclausum* flew significantly more often to a high host-density plant than to a clean or low density plant, and landed on the plants nearest to the release point at a significantly higher frequency than on the more distant plants (Table 2.2). In contrast, *C. plutellae* landed more often on the nearest plants irrespective of host density.

2.3.2 *Host defensive behaviour*

Larval DBM avoided parasitoids in four ways: (1) Larvae often responded to the vibrations in the leaf caused by parasitoids searching nearby, and dropped off the leaf along a silk strand before being attacked by a wasp. (2) Larvae often left their damage behind after a short feeding bout (Table 2.3) and made many small and widely dispersed feeding injuries. This could have reduced the efficiency of wasps that searched unoccupied feeding sites. (3) Larvae usually fed on the underside of the leaves where they were less accessible to wasps. (4) Encounters with wasps caused larvae to wiggle vigorously away from the feeding area or dropped from the plant onto a silk thread.

Table 2.2 Frequency of the first landing of *C. plutellae* and *D. semiclausum* on plants bearing different host densities and in different positions in the open wind tunnel

Parasitoid	No of larvae /plant	No of first landings	Position 1#	Position 2
C. plutellae	0	12	12*	0
	1 (or 2)	12	11*	1
	4	15	13*	2
D. semiclausum	0	3	2	1
	1 (or 2)	8	7	1
	4	20*	17*	3

* Significantly higher frequencies (• ^{2} tests, $p < 0.05$).

Position 1 refers to the row nearest the release site; position 2 was further away.

Table 2.3 Feeding patterns of larval *P. xylostella* on cabbage leaves with different host densities

Density (No. of larvae /leaf)	No. observations	% On damage	% Off damage	% Moving into heart leaves
1	271	0.52	0.41	0.07
2	149	0.60	0.29	0.11
3	58	0.60	0.19	0.21

*A larva resting away from the damage site was counted as off damage.

2.3.3 *Foraging behaviour*

The foraging behaviour of *D. semiclausum* differed from that of *C. plutellae* in several ways that were reflected in the efficiency of the two wasps. When taking off from the release site, *D. semiclausum* flew upwind towards an infested plant (28 of 31 releases; Table 2.2), and usually landed on a leaf having feeding damage or frass (18 of 28 times). A wasp often palpated the surface with the tips of its antennae on the site of feeding damage, and at times probed the leaf with its ovipositor while continuing to antennate. In the immediate vicinity of the host, the wasp turned, slowly approached the host, then attacked, suggesting that the wasp used visual cues. When the host dropped down and hung on a silk strand, the wasp reacted by waiting for the host near the silk strand, and attacked the host when it climbed up to the leaf. This waiting last up to 970 s (244.6 ± 38.9 s, n = 34) until the wasp either successfully attacked the host or left the leaf. The termination of waiting was, in part, dependent on the behaviour of the host. The observed waiting time (W) was approximately an exponential distribution, i.e. a linear relationship between logarithm of waiting time (-Log Survival ($W > t$) and t (long-rank test on survival analysis; Kaplan-Meier procedure) (Haccou & Meelis, 1994).

If *D. semiclausum* did not encounter a host around the feeding site, it searched the wider area on the undamaged leaf. Searching females exhibited a stereotyped sequence of behaviour (antennate-groom-still-fly) while moving over the plant before encountering hosts, and there was a direct behavioural path between flight and oviposition in *D. semiclausum* (Figure 2.2a). After oviposition, females often immediately flew to another part of the same leaf, another leaf, or another plant.

In contrast, *C. plutellae* more often landed first on the plants nearest the release site, regardless of host density (36 of 39 releases; Table 2.2). If the wasp landed on a clean leaf surface, it then displayed the sequence walk-still-fly before finding a site with damage or frass (Figure 2.2b). At such sites, the wasp showed a very strong arrestment response. A searching female intensively palpated the substrate containing host frass or feeding damage, slowly moved and sharply turned around the site, while at times the ovipositor probed the substrate. The wasp only searched a very narrow area around the damage hole, within about 1cm of the perimeter of the feeding damage. Once damage was located, antennating and grooming usually alternated until the wasp encountered a host (Figure 2.2b). It only attacked and oviposited if it first contacted the host with its antennae. When a host dropped off the plant, the wasp walked down the silk strand and dropped onto the ground if the silk was broken. On the ground, the wasp quickly walked and palpated the wider area near where the host landed. *C. plutellae* exhibited a relatively plastic behavioural pattern leading to oviposition (Figure 2.2b), with no strong statistical linkage between flight and oviposition.

The wider-area search of *D. semiclausum* enabled it to detect more host larvae resting away from the feeding damage (Table 2.3). The narrow-area search (a strong local arrestment response to feeding damage) of *C. plutellae* was relatively inefficient, particularly at low host densities, as larval DBM tend to leave their damage after feeding, which may increase with decreasing host density (Table 2.4). 79 of 101 encounters (79%) between *D. semiclausum* and larval DBM resulted in oviposition whereas only 18 of 31 encounters (58%) were successful for *C. plutellae*. 51 of 79 ovipositions (65%) by *D. semiclausum* were made at the first attack, while 29 ovipositions occurred following waiting.

Table 2.4 The relationship between the host larvae positions in relation to feeding damage and the percentage of detection by *C. plutellae* and *D. semiclausum*

Parasitoid	Host density (No. of larvae /leaf)	No. observations	Percentage detected On damage	Percentage detected Off damage
C. plutellae	1	36	0.11	0.03
	2	46	0.18	0.06
	3	40	0.27	0.00
D. semiclausum	1	27	0.36	0.15
	2	29	0.29	0.42
	3	18	0.62	0.20

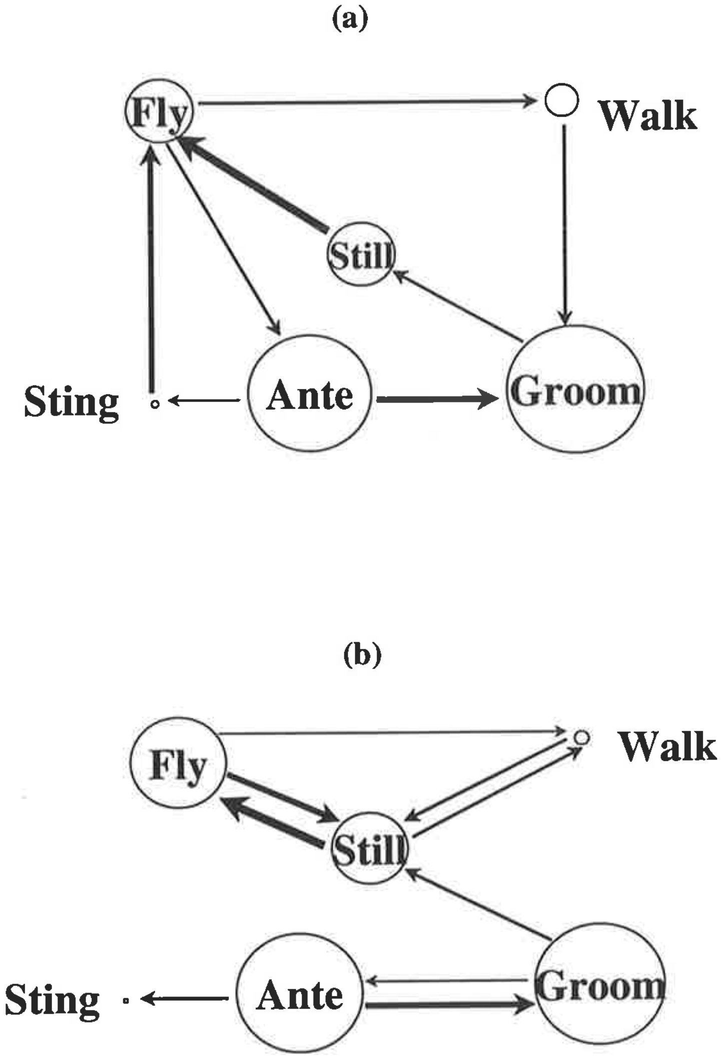


Figure 2.2 Kinetograms of (a) *D. semiclausum* and (b) *C. plutellae* searching in patches of cabbage plants infested by *P. xylostella* larvae. Ante.= antennate. The areas of circles are proportional to the overall frequency of each behavioural event. The arrows represent the significant behavioural flows ($P < 0.05$), the widths of arrows are proportional to the standardised residual of positive deviations from expected values:

Standardised residuals = (observed-expected) / $\sqrt{Expected}$

The frequency, mean duration, and proportion of time devoted to each type of behaviour differed between the two parasitoids (Table 2.5). *D. semiclausum* spent the greatest proportion of its time still, while *C. plutellae* spent a greater proportion of its time grooming and flying. These differences were related to their different host-searching behaviour. The greater proportion of time that *D. semiclausum* remained still was a consequence of the tendency of this species to wait for hosts. Although *C. plutellae* was still more frequently than *D. semiclausum*, the mean duration of still was much shorter than that of *D. semiclausum*. The greater percentage of time spent grooming and flying by *C. plutellae* was due to the longer mean duration of grooming and more frequent flight respectively. *D. semiclausum* also walked more frequently, while *C. plutellae* walked and antennated longer. However, these differences did not affect the overall proportions of time devoted to these behaviours.

2.3.4 *Patch time allocation*

The total residence time in the patch of four plants by *D. semiclausum* was 3195 ± 303.5 s (n = 28), more than twice that of *C. plutellae* (1472 ± 201.7s, n = 24). Both parasitoid species spent most of their time on infested leaves (Figure 2.3). The percentage time both parasitoids spent on different plants increased with increasing host density, and did not differ significantly between species (Figure 2.4). In comparison, *C. plutellae* spent significantly a greater proportion of time on the ground (17.7 ± 3.6 s/observation) than *D. semiclausum* (4.4 ± 1.1 s /observation), searching for hosts. The mean number of visits, attacks, and stings by both parasitoids on different plants all increased with host density (Figure 2.5). But the number of visits per unit time per plant did not differ significantly between the two species, except on the plant infested by one larva, *D. semiclausum* paid more visits per unit time than *C. plutellae*. However, *D. semiclausum* attacked and stung at a significantly higher rate than *C. plutellae*, either per plant searched or per unit time. This indicated further that *D. semiclausum* was more efficient than *C. plutellae*.

Table 2.5 Mean ± SE frequency and duration of each type of behaviour of *C. plutellae* and *D. semiclausum*

Behaviour	*D. semiclausum*		*C. plutellae*	
	N	Mean ± SE	N	Mean ± SE
Frequency (no. /hour)				
Antennate	28	67.5 ± 5.96	24	69.8 ± 4.55
Fly*	28	37.4 ± 3.45	24	52.9 ± 3.09
Groom	28	74.9 ± 6.20	24	70.5 ± 3.59
Still*	28	37.2 ± 2.20	24	41.9 ± 3.17
Walk*	28	18.9 ± 3.20	24	6.4 ± 1.42
Sting*	28	3.9 ± 0.41	24	1.5 ± 0.52
Duration (s)				
Antennate*	28	9.5 ± 0.49	24	11.0 ± 0.53
Fly	28	14.2 ± 1.85	24	14.8 ± 0.97
Groom*	28	13.8 ± 1.46	24	17.7 ± 1.46
Still*	28	47.9 ± 7.90	24	21.8 ± 2.65
Walk*	21	4.5 ± 0.33	24	5.9 ± 1.14
Sting	28	4.6 ± 0.52	13	4.7 ± 0.47

* Significant differences between means (Kruskal-Wallis tests, $P < 0.05$).

Although both parasitoids concentrated their searching on the plants bearing the highest host densities, the rate of parasitism was density-independent (Figure 2.6)

2.4 Discussion

Host specialist parasitoids often display specialised adaptations for host location, while parasitoids with a wider range of potential hosts often display a relatively plastic foraging behaviour (e.g. Vet *et al.*, 1993; Wiskerke & Vet, 1994; Geervliet *et al.*, 1996; Cortesero *et al.*, 1997). This study showed that *C. plutellae* and *D. semiclausum* differed substantially in their host searching behaviour and response to host defence. The host specialist parasitoid *D.*

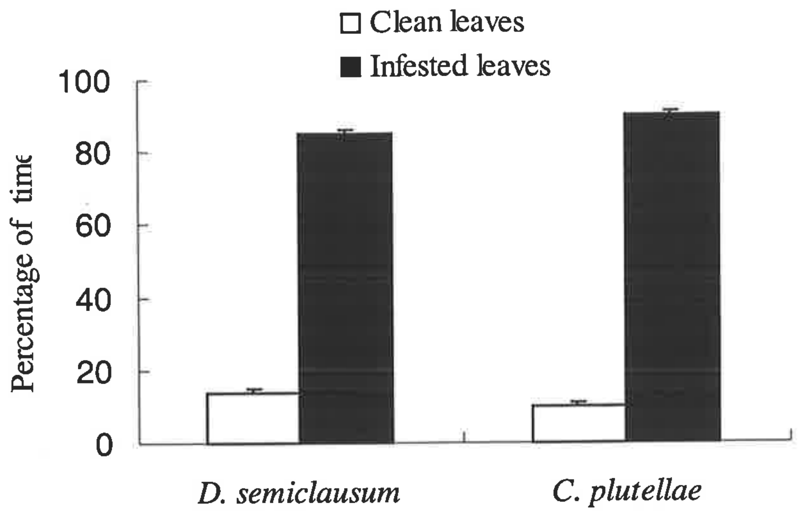


Figure 2.3 Time allocation to clean leaves vs. infested leaves by *C. plutellae* (n = 24 wasps) and *D. semiclausum* (n = 28 wasps) in patches of cabbage plants infested by *P. xylostella* larvae. Mean and SE are expressed as percentage of time spent on each type of leaf.

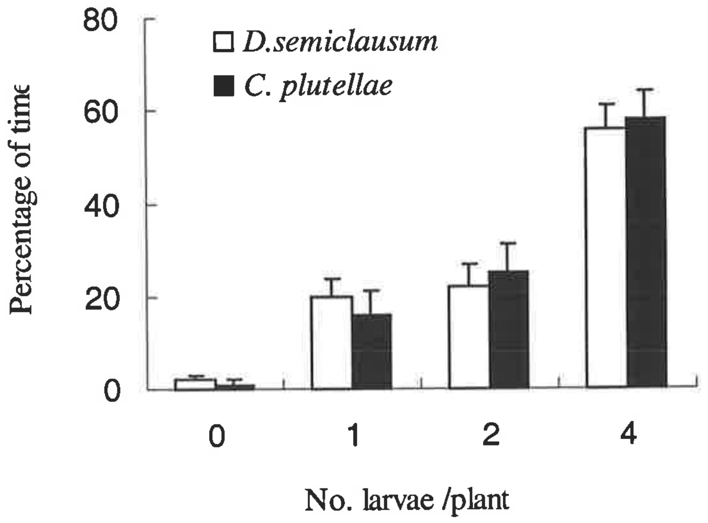


Figure 2.4 Time allocation by *C. plutellae* (n = 24 wasps) and *D. semiclausum* (n = 28 wasps) over plants infested by different density larvae of *P. xylostella*. Mean and SE are expressed as percentage of time spent on each plant.

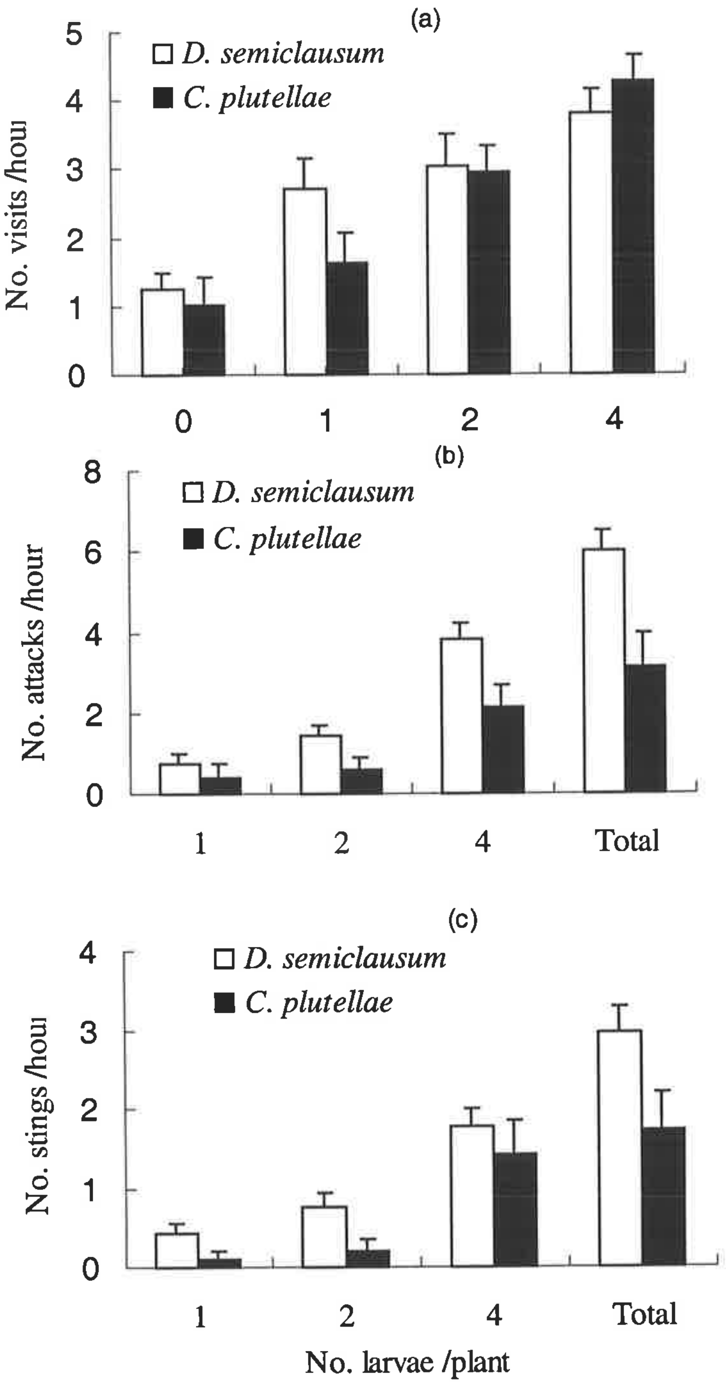


Figure 2.5 The mean number of (a) visits, (b) attacks, (c) stings by *C. plutellae* (n = 24 wasps) and *D. semiclausum* (n = 28 wasps) within the patches of cabbage plants infested by *P. xylostella* larvae.

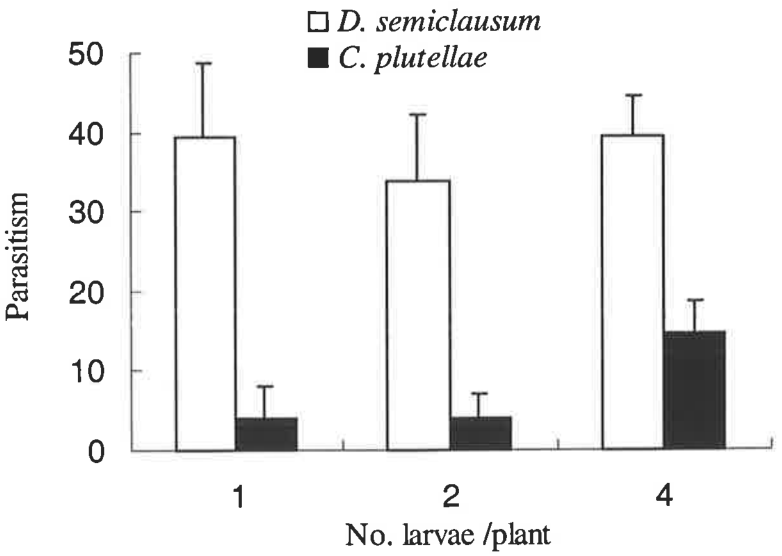


Figure 2.6 The percent parasitism by *C. plutellae* (n = 24 wasps) and *D. semiclausum* (n = 28 wasps) over the patches of cabbage plants infested by different density larvae of *P. xylostella* larvae.

semiclausum was much more effective than the oligophagous species *C. plutellae*, both in locating DBM and overcoming host defence.

Larval *P. xylostella* avoid parasitoids in a number of ways: leaf-mining in the first instar, feeding preferentially in relatively more sheltered heart leaves, leaving damage behind, and dropping off the plant or retreating vigorously when encountering parasitoids. These defensive behaviours are common in caterpillars and could influence the foraging success of their parasitoids and the resulting levels of parasitism (Waage, 1983; Davis, 1987; Lederhouse, 1990; Mauricio & Bowers, 1990; Gross, 1993; Weseloh, 1993).

During the in-flight orientation to the host habitat, *D. semiclausum* was able to more accurately locate the most profitable source at the first landing than *C. plutellae* (Table 2.2). Upon arriving at a potential host habitat, both parasitoids responded to feeding damage and faeces by palpating the microhabitat with their antennae while regularly probing the leaf surface with their ovipositors. This response was similar to that of a number of other parasitoids of caterpillars such as *Venturia canescens* (Grav) (Waage, 1978), *Cardiochiles nigriceps* Vier. (Strand & Vinson, 1982), and *Cotesia rubecula* (Nealis, 1986). In addition, *D.*

semiclausum also seemed to detect hosts visually in the immediate vicinity of the host. Ovipositor search enabled both parasitoids to detect leaf-mining first instar DBM, and larvae feeding in the heart leaves. Antennal search seems to be a response to contact kairomones which thus reduces the potential search area and increases the probability of detecting hosts that are feeding on the surface. However, antennal search is not very efficient in locating larval DBM, as the larvae often escaped before the parasitoids attack them. Visual detection by *D. semiclausum* is an efficient approach to its evasive host.

In the analysis of behavioural transitions, there was a direct link between fly and oviposition in *D. semiclausum* but not in *C. plutellae* (Figure 2.2). This suggests that *D. semiclausum* is able to more effectively use cues such as volatile semiochemicals and plant damage to locate hosts. In contrast, the behavioural pattern of *C. plutellae* was more random which seems to have reduced its searching efficiency.

C. plutellae was strongly arrested by the presence of host-associated cues such as feeding damage, as evidenced by a significantly longer duration of antennate behaviour (Table 2.5), and displayed an area-restricted search. *D. semiclausum* usually first searches the feeding damage and then the wider area around plant damage, as shown by the higher walk frequencies (Table 2.5). The wide-area search employed by *D. semiclausum* enabled it to detect more hosts resting away from the feeding damage than *C. plutellae*, particularly at low host densities (Table 2.3). The strong local arrestment response of *C. plutellae* to feeding damage could be more effective at relatively higher host densities where hosts would not have much space to retreat.

D. semiclausum usually waited for a host that was discovered and hanging on a silk strand. The time taken to oviposit was 3-5 s (Table 2.5) while the total handing time including the time spent waiting for a host could be up to 970 s. This sit-and-wait strategy employed by *D. semiclausum* seems to be a specialised adaptation to the host's defensive behaviour.

After a successful oviposition, the wasp usually immediately flew away from the attack site. In 53 of 72 successful ovipositions, they flew to either another leaf or another plant after ovipositing, and only in 19 of them did the wasps return to the same leaves, where in most such cases more healthy hosts were still available. Such behaviour was similar to that observed in *C. nigriceps* (Strand & Vinson, 1982) and *C. rubecula* (Nealis, 1986) for a response to oviposition, which could be interpreted as a mechanism to increase the parasitoid's chance of finding unexploited hosts and to avoid superparasitism particularly in this low host density environment.

The relationship found here between host defence of larval DBM and its parasitoids' counter play could be used to predict or account for different consequences of the behavioural interactions between the two species and their hosts. Larger instars could be considerably more susceptible to parasitoid attack than early-instar larvae (Lloyd, 1940) and more likely to move a greater distance away from the point of the initial landing when dropping on the soil, thus reducing the chance of being re-encountered by both parasitoids. The smaller instar larvae, however, will be located easily by both species, particularly by *C. plutellae*. However, this benefit may be balanced by the reduced probability of the host being detected in the smaller larvae as a result of less damage and fewer chemical cues, compared with larger larvae. Studies on the pattern of host stage acceptance by both parasitoids assumed that both parasitoids preferred specific larval instars (Talekar & Yang, 1991), however, this preference could have reflected the comprehensive outcome of behavioural interactions between the host and parasitoids (Harvey & Thompson, 1995). Thus the differences in the behaviour of host larvae can result in different risks of being parasitised, but the difference in parasitoid ability to overcome the host defence could reduce extrinsic competition for host use and improve coexistence of the two species. The defensive behaviour elicited by the presence of parasitoids significantly increased larval movement and thus contact with infective units of the entomopathogenic fungus, *Zoöphthora radicans* Brefeld (Furlong & Pell, 1996). Clearly, there could be a greater increase in the probability of infection when *D. semiclausum* is present than *C. plutellae* as a consequence of the wide-area searching behaviour of *D. semiclausum*. If this is so, the synergism between fungal pathogens and *D. semiclausum* would make this species a better agent of biological control.

Optimal foraging theory predicts that parasitoids of patchily distributed hosts should concentrate searching in the more profitable patches (see Godfray, 1994). The patch time allocation of both parasitoids agreed with this general prediction, i.e. the aggregation of searching time on patches of high host density (Figure 2.3 & 2.4). However, the resulting patterns of parasitism were locally density-independent in both species (Figure 2.6). In many situations where the parasitoid aggregation did not lead to density-dependent parasitism (e.g. Waage, 1983), the level of parasitism in these areas was highly variable (e.g. Morrison *et al.*, 1980). Waage (1983) argued that long handing time resulting from the occasional tendency of *Diadegma* spp. wasps to wait for returning larvae could be a factor contributing to the density-independent pattern of parasitism. In this study, it was found that one potential source of variation could result in hosts not being encountered at a rate proportional to their density. The rate of encounter was more influenced by host defensive behaviour than host density. Whether or not a host is resting away from feeding damage will directly influence detection by a parasitoid. Although the actual host density could not change, the effective host density depends on the feeding behaviour of the host. Particularly for *C. plutellae*, the parasitoid would be expected to spend proportionally more time detecting hosts at lower density as the percentage of larvae resting away feeding damage increased with decreasing host density (Table 2.3). Also *C. plutellae* spent more time examining feeding damage without increasing its rate of encountering a host because of its area-limited searching around feeding damage. Therefore, any factors which change the density of detectable hosts to feeding damage, such as age-specific host defence behaviour, will obscure the relationship between host density and the rate of parasitism in *C. plutellae*. In addition, the rate of oviposition is largely influenced by the random occurrence of waiting for an encountered host in *D. semiclausum.* All these factors could influence the variability in the number of hosts attacked and the time spent by individual parasitoids on plants. Factors that are beyond the control of parasitoids, such as age-specific host defence and density-dependent host defence obscure the relationships between the rate of parasitism and foraging time or host density (Nealis, 1986; 1990; Weisser, 1995). A large body of theoretical work on parasitoid-host population dynamics concerns the behavioural processes and consequences of parasitoid behaviour (e.g. Hassell & May, 1974; Murdoch, 1996). Whether there is random or non-random variability in foraging behaviour could be important in order to link the individual behaviour to population interactions (Ives, 1995; Ives *et al.*, 1999).

In summary, *D. semiclausum* was much more effective than *C. plutellae* in parasitising DBM in such a low host density environment, and its behaviours were well adapted to the host behaviour. *C. plutellae* is obviously not efficient at finding hosts at low host densities, but its behaviour would be relatively effective in searching in a high host density environment with relatively early instar composition. Differences in the behaviour of host larvae can result in different risks of being parasitised. Older larvae would be more susceptible to aggressive attack and thus are less likely to be parasitised by *C. plutellae* than by *D. semiclausum.* In most Asian counties, where *C. plutellae* and another species *Oomyzus sokolowskii* (Kudj.) usually occur (Wang *et al.,* 1999) the desired level of DBM control is not achieved alone by native parasitoids. Thus, the introduction of *D. semiclausum* into such areas may result in the exploitation of different densities of the host population and different parts of the population. Thus, differences in search behaviour between the two species could reduce overlap in host use and improve coexistence of the two species, and may potentially contribute to the overall biological control of DBM.

Chapter 3 Oviposition decisions by *D. semiclausum*

3.1 Introduction

The major issues of the oviposition decisions in insect parasitoids, which have received growing theoretical attention during recent decades, are the interdependent phenomena of superparasitism and host discrimination, and their effects on optimal patch utilization strategies (van Alphen & Visser, 1990; Spiers *et al*., 1991; Godfray, 1994). In solitary parasitoids, oviposition into an already parasitised host (superparasitism) generally yields a lower fitness return than an unparasitised host, the adaptive value of recognizing a host thus parasitised (host discrimination) is largely self-evident (reviewed in van Alphen & Visser, 1990). However, superparasitism occurs frequently under laboratory and natural conditions, although in many species parasitoids discriminate parasitised from unparasitised hosts (Salt, 1961; van Lenteren, 1981; Janssen, 1989). Superparasitism is now widely accepted as an adaptive strategy of oviposition behaviour under certain conditions, and has been investigated in recent theoretical studies by static or dynamic optimality models and evolutionarily stable strategy (ESS) models (Hughes, 1979; Iwasa *et al*., 1984; Charnov & Skinner, 1985; Hubbard *et al*., 1987; Mangel & Roitberg, 1989; van Alphen & Visser, 1990; Mangel, 1992; Visser *et al*., 1992; Godfray, 1994).

Both static optimality and ESS models of superparasitism in solitary parasitoids assume that the parasitoids are time-limited rather than egg-limited, and they should maximize their lifetime reproduction. A static model integrates the dependent relationship between host quality, host recognition time and host acceptance (Hughes, 1979; Iwasa *et al*. 1984; Harvey *et al*., 1987; Janssen, 1989; van Alphen & Visser, 1990). The model predicts that (1) superparasitism should not occur, unless the encounter rate with unparasitised hosts is low or unparasitised hosts are not available; and (2) parasitised hosts are either always or never accepted by a parasitoid capable of host discrimination. Because a resource habitat is often being exploited simultaneously by more than one parasitoid, adaptive strategies of superparasitism are dependent on the decisions of competitors, and therefore superparasitism decisions were also modeled as components of ESS (Hubbard *et al.*, 1987; van Alphen & Visser, 1990; Mangel, 1992; Visser *et al.*, 1992). The ESS model considers directly the interdependence of superparasitism and patch time allocation in the decision-making process, and adds to the static model the following predictions that: (1) when there is no competition, self-superparasitsm should never occur; and (2) the optimal strategy is to reject parasitised hosts initially, but later superparasitise when the rate of gain of offspring from the patch drops to a certain threshold (Hubbard *et al*., 1987; Visser *et al*., 1992, Visser, 1995).

The rate maximization models may not be suitable to parasitoids that are not only time-limited, but also egg-limited or both (Godfray, 1994; Rosenheim, 1996). By incorporating state-dependent variables of the physiological conditions of the foraging parasitoids such as egg-load, dynamic model of superparasitism in solitary parasitoids predicts a parasitoid's decision to superparasitise is influenced by the number of mature eggs available for oviposition in both theoretical and empirical studies (Iwasa *et al.,* 1984; Mangel & Roitberg, 1989; Mangel, 1992; Minkenberg *et al*., 1992; Fletcher *et al*., 1994; Hughes *et al*., 1994; Sirot *et al*., 1997).

Empirical studies have documented many other factors that may influence the frequency of superparasitism, such as risk of mortality when attacking a defensive host (e.g. Potting *et al*., 1997), mating status of females (Minkenberg *et al*., 1992; Michaud, 1994), life expectancy (Roitberg *et al*., 1993; Fletcher *et al*.,1994).

Under many circumstances superparasitism should be favored by parasitoids capable of host discrimination (Van Alphen & Visser, 1990; Godfray, 1994), as there is a finite chance of the superparasiting progeny outcompeting the earlier parasitoid (Sirot, 1996; Scott *et al.*, 1997; Yamada & Miyamoto, 1998). Thus a major conclusion has been that the oviposition decisions of a parasitoid should be fundamentally dynamic (Papaj, 2000), and thus vary in response to (1) its physiological state (Iwasa *et al.*, 1984; Rosenheim & Rosen, 1991; Minkenberg *et al.*, 1992; Roitberg *et al.*, 1993; Fletcher *et al.*, 1994; Henneman e*t al.*, 1995; Sirot *et al.*, 1997); (2) its informational state about the environment, including all kinds of experiences accumulated or learnt during the course of foraging, such as host availability, risk of mortality, and competition (Iwasa *et al.*,1984; Hubbard *et al.*, 1987; Mangel & Roitberg, 1989; Rosenheim & Rosen, 1991; Mangel, 1992; Visser *et al.*, 1992; Henneman *et al.*,1995; Visser, 1995; Hubbard *et al.*, 1999). Therefore, a real model of oviposition decisions, to be valid, might need to incorporate all aspects of both physiological and information state variables. The observed propensity to superparasitsm in a parasitoid could partly reflect innate tendencies, change in physiological state, accumulation of experience or all of these, which depends upon the ecological and social conditions surrounding the parasitoids (Rosenheim & Rosen, 1991; Henneman *et al*, 1995; Visser, 1995).

However, neither traditional analysis methods for superparasitism and host discrimination, which are usually based on egg distribution or acceptance /encounter ratios (e.g. van Leteren, 1981), nor the above mentioned models, can assess the potential impacts of all variables and predict their relative or interacting effects on oviposition decisions in an realistic world. Two notorious problems which are still debated are (1) state-dependent vs. experience-dependent decisions (Rosenheim & Rosen, 1991; Henneman *et al*., 1995), and (2) time-limited vs. egg-limited life histories (Rosenheim, 1996; Seventer *et al*., 1998). Oviposition generally involves costs in both time and eggs in relation to future reproduction, particularly in pro-ovigenic species (Rosenheim, 1996, 1999). In the field, a parasitoid could be at risk of becoming time or egg limited (Casas *et al*., 2000). The relative effects of egg depletion, experience, and time-related variables on oviposition decision are incompletely understood.

In this chapter, the oviposition decisions of *D. semiclausum* were investigated using a logistical regression model. This statistical model is able to assess the relative effects of various variables on an insect's oviposition decisions (Visser, 1995; Horng *et al*., 1999). The host-searching behaviour of this parasitoid has been described in Chapter 2. Here, the oviposition behaviour of the parasitoid was directly observed in a wind tunnel. Single wasps were released onto a host plant containing both unparasitised and parasitised hosts previously attacked by either a conspecific female or the female herself. The wasp was allowed to freely leave the experimental plant for an alternative host plant placed upwind of the experimental plant. First, the basic data was analyzed to determine whether the parasitoid (1) tends to oviposit into a newly encountered host, i.e. threshold tactic in host selection; (2) employs internal discrimination; and (3) encounters hosts randomly. Then the main purpose of this chapter was to investigate the effects of potential variables on the oviposition decisions of *D. semiclausum*, in this case the probability of host acceptance, using a logistical regression model. With statistical modeling of a fitted logistical model, it was able to further investigate if the parasitoid could distinguish between different types of hosts. The adaptive significance and dynamic view of the oviposition decisions by *D. semiclausum* were discussed.

3.2 Materials and methods

3.2.1 *Insects and plants*

For detailed rearing procedures of both the host and parasitoid, see Appendix. All experiments used 2nd and 3rd instar *P. xylostella*, the most suitable host stages for parasitization by *D. semiclausum* (Yang & Taleker, 1991), and 2-3 day-old, mated female *D. semiclausum*. Parasitoid cocoons were collected and maintained individually in vials until emergence. One to two days prior to the experiments, individual female wasps were held with 5 males in a cage (20 x 20 x 20 cm) until the female mated. Mated females were always kept individually with honey in vials (7 x 2 cm) before the experiments. The cabbage plants with 5-6 fully extended leaves (*Brassicae oleracea* var. *capiata*, cv. Green Coronet) grown in 8 cm pots in a greenhouse were used in the experiments.

3.2.2 *Experimental design and procedures*

D. semiclausum is a pro-ovigenic species (Yang *et al*., 1994). A preliminary experiment was conducted to investigate the egg maturation of the parasitoid at different ages after emergence. Wasps were held individually in vials supplied with honey for between one and six days without oviposition. Individual wasps were killed and immediately dissected in a drop of water on a cavity side. The number of mature eggs in the ovaries was counted. Dissection revealed that females emerged with a substantial complement of eggs, and slowly matured more over time. Their average egg-load after 1-2 days, 3-4 days and 5-6 days were 27.7 ± 3.1 (n = 23), 32.2 ± 3.9(n = 19) and 35.4 ± 4.0 (n = 20), respectively.

All experiments were conducted in a wind tunnel (Figure 3.1, for wind tunnel design, see Keller, 1990), which was located in a room with controlled conditions of 24-25°C and 50-70% RH. A single female was released onto the experimental plant containing 12 hosts, half were unparasitised, and the other half were parasitised one day prior to the experiments by either the female herself or a conspecifc female. All hosts were placed on the experimental plants one day prior to the experiments. In order to increase the overall encounter rate, a relatively high host density was set-up by confining the 12 hosts on two middle leaves of the experimental plant, with six hosts each (three parasitised and three unparasitised). The two leaves having hosts were numbered to permit rapid identification during the experiment.

The experiments consisted of three treatments. (1) A naive wasp was provided with six unparasitised hosts and six hosts parasitised one day prior to the experiments by a conspecific female. (2) An experienced wasp was provided with six unparasitised hosts and six hosts parasitised one day prior to the experiments by a conspecific female. (3) An experienced wasp was provided with six unparasitised hosts and six hosts parasitised one day prior to the experiments by the female herself.

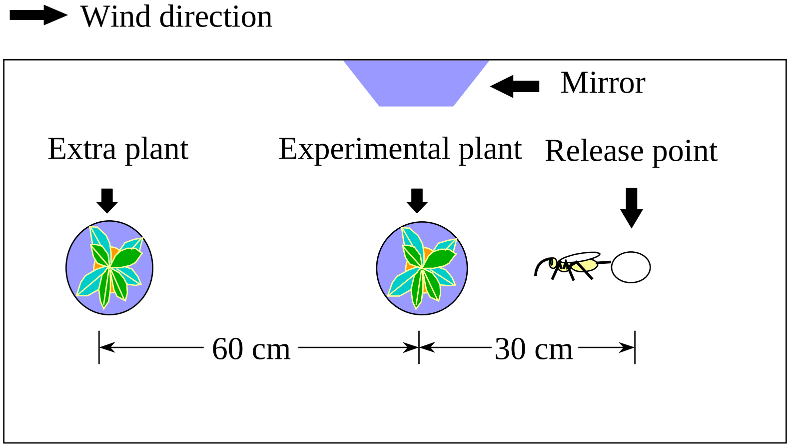


Figure 3.1 Layout of the experiment in the test section of a wind tunnel. A wasp was released 30 cm downwind of the experimental plant. A mirror was place behind the experimental plant, which allowed observation of the searching wasp on the leaves opposite to the observer. An extra plant was placed 60 cm upwind of the experimental plant to provide an alternative landing resource for the searching wasp.

One day before the experiments, four standard female wasps were pre-experimentally treated and catalogued as (1) A 'naive' wasp without any oviposition experience. (2) A 'self ' wasp allowed sequentially to oviposit 6 times into unparasitised hosts in a Petri dish (8 x 2 cm) containing a cabbage leaf and one larval DBM. The six parasitised hosts thus were provided for herself. (3) A 'conspecific' wasp allowed sequentially to oviposit 6 times into unparasitised hosts, but the six parasitised hosts were provided for the 'naive' wasp as conspecific-parasitised hosts. (4) Another female wasp was used to produce six parasitised hosts as above and the parasitised hosts were provided for the ‘conspecific’ wasp. Following above procedure, the three experimental wasps were individually maintained in a vial with a drop of honey for the experiments. For convenience, hereafter the three wasp treatments were referred as 'Naive', 'Conspecific' and 'Self', respectively.

Unparasitised hosts were marked with red ink on the end of the host abdomen using a fine brush pen under a microscope, so that they were easy to be followed during the experiments. The marking did not influence host selection of the parasitoid. Six host larvae (3 unparasitised and 3 parasitised as above) were placed on each of the two selected leaves of the experimental plants. In order to limit the movements of host larvae among the leaves, a paper barrier was wrapped around the stems of the two infested leaves. In the morning immediately prior to the experiments, each leaf was examined to check the number of hosts present. Few larvae moved away from the selected leaves, and these plants were discarded. One extra plant infested with 12 unparasitised larval DBM was placed 60 cm away upwind of the experimental plant (see Figure 3.1), to provide an alternative source of semiochemicals for the free-searching wasps. Otherwise, the wasp might spend an excessive amount of time on the experimental plant, which could potentially result in the level of superparasitism being higher than might occur naturally. Thus, the observed levels of superparasitism should reflect the realistic conditions in which the wasp was allowed to disperse when it encountered extensively parasitised hosts. A mirror was placed 25 cm behind the experimental plants, which allowed observations of the searching behaviors of the wasp on the leaves opposite to the observer. Wasps were released from a 7 x 2 cm vial 30 cm downwind of the experimental plants on a stand about the same height above floor as the infested leaves (Figure 3.1). Immediately before release, the parasitoid was held in an 80 mm Petri dish containing a piece of cabbage leaf with fresh feeding damage produced by one larval DBM for 5 minutes. This exposure was designed to give experience with host-related cues to the experimental parasitoid, and increased the parasitoid orientation towards host-related cues (Chapter 2). The wind speed was set at 32.5 cm/s.

Once the wasp flew away from the release point, it was followed continuously, until it left the experimental plant and landed on the extra plant, when the observation was terminated. The time and sequence of each encounter (attack) with and sting in a host were recorded. Every time a larva was observed to be stung, it was followed until the end of the experiment. Then these larvae were collected and dissected immediately after the experiment to determine the presence of parasitoid eggs. The size of eggs differed between those deposited one-day prior to the experiments and those during the experiments, so it was easy to distinguish the past eggs from recently laid eggs. The total patch time was also recorded. All experiments were conducted between 9:00 and 16:00. On each date, 1 or 2 wasps for each of the three treatments were released in a random order. Each treatment was replicated 20 times. In a few cases, final dissection revealed that a few 'parasitised hosts' prepared one day prior to the experiments were actually not parasitised, thus these replications were not included in the analysis. There were 17 complete replications in total for each of the three treatments.

3.2.3 *Basic analysis of the data*

Upon arrival onto the experimental plant, the wasp may first encounter a host either unparasitised (U), parasitised previously by a conspecifc female (PC, treatment 1 or 2) or by the female herself (PS, treatment 3) if she randomly searches for host. With the exploitation of the searching female on the patch, the unparasitised hosts become parasitised, while initially parasitised host may become superparasitised. Thus, until the wasp left the experimental plants, she could have sequentially encountered and examined various types of hosts (Table 3.1).

Table 3.1 Various types of hosts initially presented to and sequentially encountered by the parasitoids during the course of patch visit in the three treatments

Initial host types	Current host types
Unparasitised (U)	Unparasitised (U)
	Self-parasitised once (U-S1)
	Self-superparasitised more than once (U-S2)
Parasitised by a conspecific female (PC)	Conspecific-parasitised (PC)
	Conspecific-superparasitised once (PC-S1)
	Conspecific- and self-superparasitised (PC-S2)
Parasitised by the female herself (PS)	Self-parasitised in the past (PS)
	Self-superparasitised once currently (PS-S1)
	Self-superparasitised more than once (PS-S2)

When a host was observed to be stung more than once during the experiment, but the final dissection only found one newly laid egg in the host, it was unknown at which sting the egg was laid. *D. semiclausum* oviposits very quickly, and oviposition usually occurs during wrestling between wasp and host (Chapter 2). It is impossible to determine whether or not an egg is laid based on the duration of the sting. There were two possible selection tactics: comparison and threshold (Real, 1990; Horng *et al*., 1999). With comparison tactic, an individual wasp may not lay an egg into a host at the first sting, but returns to lay an egg at the second sting when she finds the quality of other hosts is relatively low. In this case, a host may be accepted at any point during the sequence of stinging. With threshold tactic, an individual wasp's decision to accept a host is based on her expected ‘threshold’ of acceptance, which could be constant or adjusted by previous experience. She always oviposits into a newly encountered host, and thus the only egg should be laid at the first sting into the host. Similarly, If a host was stung for three times, but the final dissection only found two newly laid eggs, the two eggs should be laid at the first two stings if the parasitoid employed a threshold tactic. Thus, it was necessary to provide evidence that the parasitoid uses a threshold tactic, in order to estimate the frequency of host acceptance to different types of hosts when they were stung more than once.

The basic data on frequency of host encounter, sting and oviposition were first analyzed. An encounter was defined as direct contact with a host or waiting for a detected host nearby a silk thread on which the host was suspending. A sting was defined as an ovipositor insertion, and an oviposition following a sting defined as acceptance. The basic data were analyzed to address the three questions: (1) threshold tactic; (2) internal discrimination; and (3) random encounter.

3.2.3.1 *Threshold tactic*

The ratio of stings to acceptances of the hosts that only received one sting by the parasitoid was analyzed, by pooling all data within a treatment. These were the three types of hosts: unparasitised (U), parasitised previously by a conspecific female (PC, treatment 1 or 2), or by the female herself (PS, treatment 3). If a comparison tactic was used by *D. semiclausum*, it should be less likely to lay an egg into a parasitised host at the first sting on the host. In contrast, if a threshold tactic was used, she should readily accept an unparasitised host for oviposition at the first sting, and could lay an egg into a parasitised host at the first sting.

3.2.3.2 *Internal discrimination*

If the parasitoid could use external cues to recognize a parasitised host, she should avoid stinging it after external examination, unless the wasp decides to superparasitise the host. In contrast, internal discrimination requires ovipositor probing, and a sting does not necessarily result in oviposition, except that the parasitoid is not capable of host discrimination. Thus, two groups of data were analyzed: (1) the frequency of first encounter with unparasitised (U) vs. parasitised host (PC or PS), to determine whether the first encounter with three different types of hosts were random; and (2) the overall ratio of encounter to sting, sting to acceptance of the different types of hosts, by pooling all the data within a treatment.

If the parasitoid could not discriminate the status of the host by external cues, both the unparasitised and parasitised hosts should have equal probability of being encountered, and there should be no difference in the encounter / sting ratio between different types of hosts, given that the proportion of hosts which escaped being located was independent of host type. The ratio of stinging to oviposition in different types of hosts should give an overall indication of the parasitoid's host discrimination ability, but a more sensitive analysis is needed to determine how discrimination varies according to changing internal and external factors.

3.2.3.3 *Random encounter*

Many models of random search commonly used in foraging theory are based on the exponential distribution (e.g. Murdoch & Oaten, 1975; Marschall *et al.,* 1989). If n hosts are left in a patch, then the time T_n before the next host encounter has the exponential distribution.

$$P\ (T_n > t) = e^{-\alpha nt}$$

Where α is some positive constant (Murdoch & Oaten, 1975). This assumes that (1) each host is encountered independently, such that the time to encounter any particular host is not affected by the type of host present; and (2) the probability of finding a host during the next interval of time depends only on the length of that interval. These assumptions lead to the exponential distribution for the time to encounter a particular individual host and the independence of encounter interval for different hosts. According to this model, T_n should have an exponential distribution with constant parameter α.

Each encounter interval for each individual wasp in the three treatments was calculated. First, the variance of encounter intervals among individual wasps was analyzed, i.e. testing the homogeneity of variance by using Bartlett's statistic, which is designed for testing differences among variances for normally distributed data. Because there was no significant difference in the variance of encounter intervals among individual wasps and treatments (d.f. = 50, $p > 0.05$, JMP, SAS), all the data of the encounter intervals were pooled, and then the logarithm of the encounter interval (-Log Survival ($T_n > t$) was plotted against t. It should be a straight line if the distribution was exponential.

Also the average patch residence time and number of ovipositions were compared among treatments.

3.2.4 *Statistical modeling*

The data were further analyzed using logistic regression (SAS / STAT, SAS 1994) to determine the influence of certain variables on oviposition decisions (rejection or acceptance of a host for oviposition).

Each individual decision to accept or reject a host of a given quality is dichotomous. However, the observed overall pattern of host acceptance showed a 'partial preference' that deviated from a dichotomous rule, in contrast to the predictions of some models of superparasitism decisions in solitary parasitoids, which predict that a parasitoid should always accept or reject a given quality of host (see introduction). Such partial preference can be explained by statistically incomplete or erroneous information, or several kinds of heterogeneity, but now could be explained as dynamic pattern of adaptive oviposition (e.g. Visser, 1995). Basic data analyses already showed that such partial preference occurred at an individual level (Table 3.4). It was assumed that in *D. semiclausum* there is a probability value of p from 0 to less than or equal to 1 in the decision to accept a host following a sting. p is dependent of a number of potential explanatory variables. Using logistic regression, the relationship between the dependent variables and p is as follows:

$$p = \frac{\exp(\alpha + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_i x_i)}{1 + \exp(\alpha + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_i x_i)}$$

Where α = constant, β_i = the regression coefficients that give the relative contribution of i covariates. Four types of variables were considered: pre-patch experience, egg depletion, host availability, and time-related variables (Table 3.6). Each of the specific questions for each of the variables selected is addressed below.

3.2.4.1 *Pre-patch experience*

Both the experiences of the wasps prior to the experiment and the first sting experience once upon the experimental plant may influence their propensity to superparasitise, and were considered as two potential variables. Naive wasps without oviposition experience may be more willing to oviposit into a parasitised host (e. g. van Lenteren & Bakker, 1975; Hughes *et al.*, 1994; Henneman *et al.*, 1995; Potting *et al.*, 1997). Naive wasps may have not learned the relative quality of a host, and thus will more readily oviposit into the first encountered host irrespective of the host types, whereas experienced wasps that have oviposited into a healthy host will subsequently avoid laying eggs into parasitised hosts (van Lenteren & Bakker, 1975). A functional approach explains this as an adaptive superparasitism rather than a matter of learning (van Alphen *et al.* 1987), as the pre-patch experience could influence the parasitoid's expectation. The first sting experience could be an important indicator of the future patch quality, particularly for a naive wasp. If they first encounter a parasitised host, they may perceive healthy hosts to be rare in the environment and hence make the decision to superparasitise.

3.2.4.2 *Egg depletion*

Preliminary experiments showed that the egg-load of 1-2 day old wasps was relatively high but eggs matured slowly. In this experiment, egg depletion was rapid, on average a parasitoid laid about 7 eggs per patch visit within the short period of time (see Table 3.5), relative to the rate of egg maturation. Under normal temperatures, *D. semiclausum* can continue to lay eggs up to 28 days when food is provided, and can only live three days without food (Yang *et al.* 1994). There was large variation in the total number of eggs laid among the wasps tested (range from 2 to 12 eggs). It was possible that the decisions to accept a parasitised host could be influenced by egg depletion. Thus, the number of eggs laid was considered as a potential variable.

3.2.4.3 *Host availability*

Host availability includes parameters concerned with host quality. Theory predicts that a parasitoid would pay if it invests time in sampling poor quality habitats and in laying eggs in less-profitable hosts (Rosenheim, 1999). But how do parasitoids obtain, process and employ information about habitat quality, as usually their foraging environment is unpredictable? If a parasitoid could track the changes in the environment as patches are depleted, then her decision to accept or reject a newly encountered host may be modified by previous experience. Thus as a wasp forages for hosts, it may update a 'memory window' (McNamara, 1987). Such a “memory window” would allow a parasitoid to average or weight its recent experience of encountering hosts of different quality (Mangel & Roitberg, 1989; Visser, 1995). Wasps that have experienced different sequences in encountering different types of hosts should estimate the habitat to have a different quality. Superparasitism should decrease at lower rates of encounter with unparasitised hosts (Mangel & Roitberg, 1989). It was assumed a simple form of the parasitoid's memory of previous experience would involve 'averaging' previous host quality, defined as the average number of parasitoid eggs present in the previously encountered hosts. It was unknown how far the parasitoid could 'remember' previous host quality. Here the previous three sting experiences were considered. The first three stings in an experiment did not; however, have three previous sting experiences. In these cases the average for the third and second sting was taken over the previous two and the previous sting, respectively. The very first sting of an experiment obviously has no previous sting experience on the patch. Therefore, first encounter was excluded from the analysis.

The decision to superparasitise may be instantaneous, and thus dependent on the quality of currently encountered hosts as predicted by a static diet model (Hughes, 1979). It was hypothesized that the parasitoid could estimate the current host quality based on the number of eggs present, and distinguish unparasitised from parasitised hosts. At the first test, the parasitoid was assumed to be able to distinguish between unparasitised hosts and each of five types of parasitised hosts (Table 3.6).

3.2.4.4 *Time-related variables*

Rate maximization models of superparasitism assume that host acceptance is mainly influenced by host recognition time, travel time between patches, which influence the rate of host encounter and thus the life-time reproduction (Hughes, 1979; Visser *et al.*, 1992; Glaizot & Arditi, 1999; Horng *et al.*, 1999). Here three variables were included: elapsed time since entering the patch, length of present oviposition bout (time since last sting), and number of unsuccessful encounters (without stings) with hosts during an oviposition bout.

SAS Proc Logistic procedure can perform sequential analyses that provide the likelihood ratio statistics between the null model (without variables) and full model (all variables included), and the difference in -2 log likelihood between the two models is assessed as chi-squared distribution with degree of freedom = k-1, where k is the number of variables. Model chi-square assesses the overall logistic model but does not indicate which variable is more important or significant than others are. This can be done, by comparing the difference in -2 Log Likelihood between the full model and a nested model, which drops one of variables. Non significant variables whose removal does not cause a significant increase in deviance can then be omitted from the models. The final model included only the significant variables, and the residual plot was checked.

3.3 Results

3.3.1 *Threshold tactics*

At their first sting wasps readily accepted the unparasitised hosts in each of three treatments (Table 3.2). Although the frequency of accepting hosts previously parasitised by a conspecific female was significantly higher than that of accepting the hosts parasitised previously by the female herself at the first sting, a substantial proportion of both types of host were accepted. Thus the comparison tactic was unlikely to be used by *D. semiclausum*.

3.3.2 *Internal discrimination*

In total, there were 655 encounters, 508 stings and 368 ovipositions. The frequency of first encounter with either an unparasitised or a parasitised host was not different in all the three treatments (Table 3.3), indicating that the parasitoid randomly encountered hosts. There was no significant difference between the overall proportion of an encounter resulting in a sting in hosts of different types, but were significant difference in the overall ratios of sting to acceptance of different types of host, when compared either within or among treatments (Table 3.4). The wasps stung an encountered host irrespective of the host types, but they did not always lay an egg into a parasitised host following a sting, indicating that *D. semiclausum* can discriminate parasitised hosts, but requires ovipositor insertion.

Table 3.2 Frequency of accepting each type of hosts (U, PC, PS, for host types see table 3.1) that were only stung once by *D. semiclausum* during the experiments.

Treatment	Host types	No. stings	No. accepted	No. rejected	Acceptance (%)*
Naive	U	44	44	0	100 a
	PC	35	26	9	74.3 b
Conspecific	U	42	42	0	100 a
	PC	29	20	9	68.9 b
Self	U	46	44	2	95.7 a
	PS	44	24	20	54.5 c

* Value followed by different letters differed significantly (Multiple chi-squared tests adjusted with the sequential Bonferoni method (Rice, 1989), $p < 0.05$)

About 20-30% of the first encounters did not result in a sting. This happened because of the strong physical defense of the host larvae by wiggling or dropping off the plant. On occasions, the larvae directly dropped onto the ground and escaped being attacked. In some cases the parasitoid gave up waiting. 182 out of 654 encounters resulted in the hosts directly dropping onto the ground, of them 162 followed a sting while in 20 cases there was a direct encounter with the parasitoid but escaped being sting. Unparasitised hosts dropped off plants more often (37.8 %) than previously parasitised hosts (21.9 %) after being stung. Thus, superparasitised hosts may have a greater chance of being re-encountered compared with once parasitised hosts.

Table 3.3 Frequency of first encounter with unparasitised (U) vs. parasitised (PC or PS) hosts upon arriving on the experimental patch, by *D. semiclausum*. These frequencies of encounter did not differ from those expected if encounters were random ($\chi^2 = 0.4901$, $p > 0.05$).

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Treatment	Unparasitised	Parasitised
Naive	9	8
Conspecific	10	7
Self	9	8

Table 3.4 Frequency distribution of encounter with, sting and oviposition into hosts of different types by *D. semiclausum* (for host type, see Table 3.1).

Treatment	Host types	No. encounters	No. stings	% Sting	No. ovipositions	% Acceptance *
Naive	U	86	65	0.76	63	0.97a
	PC	62	46	0.74	35	0.76b
	PC-S1, PC-S2	24	20	0.83	12	0.60c
	U-S1, U-S2	31	26	0.84	10	0.38d
Conspecific	U	77	56	0.73	56	1.00a
	PC	76	54	0.71	42	0.78b
	PC-S1, PC-S2	39	29	0.74	14	0.48c
	U-S1, U-S2	31	24	0.77	13	0.54c
Self	U	79	67	0.85	65	0.97a
	PS	90	73	0.81	41	0.56c
	PS-S1, PS-S2	32	27	0.84	12	0.44c
	U-S1, U-S2	27	21	0.78	5	0.24d

* Percentages of sting were not significantly different within and among treatments, but percentages of acceptance were significantly different within and among treatments (Multiple chi-squared tests with sequential Bonferoni method (Rice,1989), $p < 0.05$).

3.3.3 *Random encounter*

There was linear relationship between logarithm of encounter interval T_n and time t (Figure 3.2), indicating the encounter interval was randomly distributed. Thus, in this experiment, the wasps randomly encountered hosts.

Table 3.5 Mean (± SE) patch residence time and number of ovipositions by *D. semiclausum* per patch visit. Patch time was compared using log-rank test on the survival functions, Kaplan-Meier procedure, JMP, SAS ($p > 0.05$). Mean number of ovipositions was compared using Kruskal-Wallis test ($p > 0.05$).

Treatment	Patch time (s)	Number of ovipositions
Naive	1829 ± 203	7.0 ± 0.62
Conspecific	1960 ± 232	7.2 ± 1.12
Self	1946 ± 209	7.2 ± 0.99

3.3.4 *Statistical modeling*

Host acceptance was significantly affected by present host types, egg depletion and time elapsed since arriving onto the patch (Table 3.7). The wasps can distinguish between unparasitised from parasitised hosts. This agreed with above basic analysis of the data, and generally met the predictions of simple diet model of host acceptance. None of the pre-patch experience had a significant effect on the probability of accepting a host. The wasp seemed unable to track the depletion of the patch, suggesting that the female might not be able to adjust its oviposition decisions using previous experience. However, the dynamic information: elapsed time and egg depletion was used by the parasitoid to adjust its host acceptance probability.

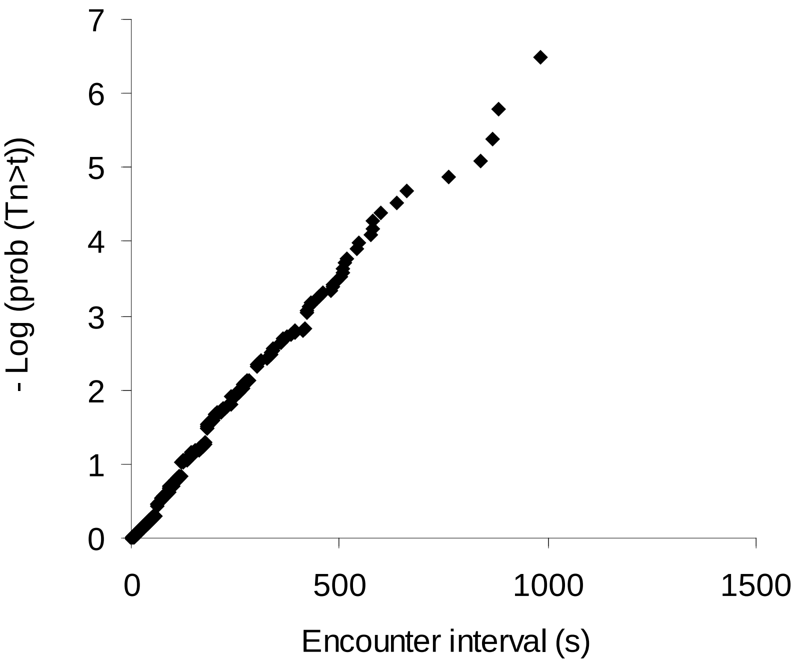

Figure 3.2 Probability distribution of encounter intervals (T_n) across all treatments by *D. semiclausum*.

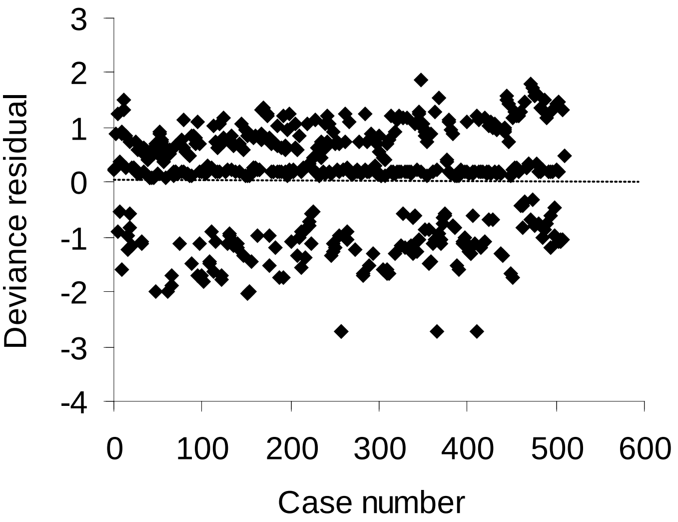

Figure 3.3 Deviance residual of the final fitted model of host acceptance by *D. semiclausum* (For the model parameters, see Table 3.10).

Table 3.6 Explanatory variables selected for testing the effect on host acceptance by *D. semiclausum* using logistic regression model with binomial error (SAS Proc Logistic Program).

Variables	Description (code of binary variables)
Pre-patch experience	
Wasp treatment (WASP)	Naive wasps without oviposition experience (0), or experienced wasps with 6 ovipositions in unparasitised hosts before the experiments (1)
First sting (FCON)	First sting in a host of unparasitised (0), or parasitised previously by a conspecific female(1)
First sting (FSELF)	First sting in a host of unparasitised (0), or parasitised by the female herself (1)
Egg depletion	
Number of eggs laid (EGG)	Number of eggs laid since entered the patch
Host quality	
Previous host quality (PHQ1, PHQ3, PHQ3)	Average number of eggs in previous one to three hosts stung, respectively
Present host type (SElFP)	U = 0, PS = 1 (For host types see Table 3.1)
Present host type (CONP)	U = 0, PC = 1
Present host type CSELF)	U = 0, U-S1 = 1, U-S2 = 1
Present host type (SELF)	U = 0, PC-S1 = 1, PC-S2 = 1
Present host type (SSELF)	U = 0, PS-S1 = 1, PS-S2 = 1
Present host quality (HEGG)	Number of eggs on currently encountered host
Timing	
Unsuccessful encounter with host (ENT)	Number of unsuccessful encounters with hosts since last oviposition
Bout length (BOUT)	Length of present oviposition bout
Patch time (TIME)	Elapsed time since entered the patch

Table 3.7 Statistic model of oviposition decisions by *D. semiclausum*

Variables	Deviance	d.f.	Deviance change*	Significance
Null model	604.5			
Full model	408.0	13	196.5	yes
Present host type (SELFP)	443.9	1	35.4	yes
Present host type (CONP)	422.4	1	14.6	yes
Present host type (CSELF)	448.5	1	40.4	yes
Present host type (SELF)	422.1	1	14.1	yes
Present host type (SSELF)	441.3	1	33.3	yes
First sting (FCON, FSELE)	410.0	2	2.1	no
Wasp treatment (WASP)	408.7	1	0.6	no
Present host quality (HEGG)	411.5	1	3.0	no
Previous host quality (PHQ1-PHQ3)	408.3	3	0.3	no
Encounter (ENT)	408.5	1	0.50	no
Bout length (BOUT)	408.2	1	0.2	no
Patch time (TIME)	433.1	1	20.6	yes
Number of eggs laid (EGG)	431.6	1	23.4	yes

*Deviance changes refer to the difference in -2 Log likelihood between the full model and each nested model which drops the corresponding variable, and the significance is assessed by Log-Likelihood tests ($p < 0.05$).

When the seven significant variables were fitted into a final model, the relative degree of host discrimination among CSELF, SELF and SSELF was not significant based on the fitted coefficients and their standard error (Table, 3.8). In this first test, it was assumed that the parasitoid could distinguish unparasitised from parasitised host of each of the five different types. It was unknown how the parasitoid could distinguish between the different type parasitised hosts. There could be several forms of host discriminations. For example, the wasps could prefer to superparasitise host parasitised by conspecifics (overall) or avoid hosts parasitised by itself in the preceding day (past); in the present foraging bout (present) or overall. To account for this, the hosts were split into three types, and two hypotheses of the parasitoid's ability of host discrimination were tested by re-coding the variables associated host types, and examining the deviance change in the final model (Table 3.9).

Table 3.8 The estimated coefficients of a final model when assuming *D. semiclausum* females could distinguish unparasitised from each of parasitised hosts of five different types as showed as in Table 3.6.

Parameter	d.f.	Estimate	SE	χ^2	*P*
Intercept	1	3.6877	0.5364	47.3	0.0001
Present host type (SELFP)	1	-5.6600	0.7850	51.9	0.0001
Present host type (CONP)	1	-2.7360	0.5635	23.6	0.0001
Present host type (CSELF)	1	-4.2217	0.5789	53.2	0.0001
Present host type (SELF)	1	-4.2533	0.6094	48.7	0.0001
Present host type (SSEF)	1	-3.6234	0.5031	41.4	0.0001
Patch time (TIME)	1	-0.00092	0.0002	20.5	0.0001
Number of eggs laid (EGG)	1	0.2485	0.0498	24.9	0.0001

In comparison, the best prediction about the parasitoid's ability was that the wasp could discriminate among the four types of host: unparasitised (U), parasitised previously by a conspecific female (PC), parasitised by the female herself in the past and parasitised by the female herself in the present (overall) (Table 3.9). A final model of the five parameters was estimated (Table 3.10). The model fitted the data well (Figure 3.3). The order of the probability of accepting a host parasitised was conspecifc-parasitised, self-parasitised in the present (overall) and self-parasitised in the past. The host acceptance probability increased with egg depletion, and decreased with elapsed search time on the patch (Figure 3.4, Figure 3.5).

Table 3. 9 Statistic modeling of the host discrimination ability by *D. semiclausum*: a test of two hypotheses

Hypotheses	Host types (see Table 3.1)	d.f	Deviance Changes*
Final model when (1):		5	187.2
Present host type (SELFP)	U = 0, PS = 1		
Present host type (CONP)	U = 0, PC = 1		
Present host type (CSELF)	U = 0, Others = 1		
Patch time (TIME)			
Number of eggs laid (EGG)			
Final model when (2):		5	165.2
Present host type (SELFP)	U = 0, PS = 1, PS-S1 = 1, PS-S2 = 1		
Present host type (CONP)	U = 0, PC = 1 PC-S1 = 1, PC-S2 = 1		
Present host type (CSELF)	U = 0, U-S1 = 1, U-S2 = 1		
Patch time (TIME)			
Number of eggs laid (EGG)			

* Testing against the null model.

Table 3.10 The final model of host acceptance by *D. semiclausum* and parameter estimation

Parameter	d.f.	Estimate	SE	$\chi 2$	P
Intercept	1	3.7868	0.5350	50.11	0.0001
Present host type (SELFP)	1	-5.5788	0.7807	51.06	0.0001
Present host type (CONP)	1	-2.7358	0.5635	23.57	0.0001
Present host type (CSELF)	1	-3.9479	0.5347	54.51	0.0001
Patch time (TIME)	1	-0.00093	0.0002	21.33	0.0001
Number of eggs laid (EGG)	1	0.2239	0.0470	22.44	0.0001

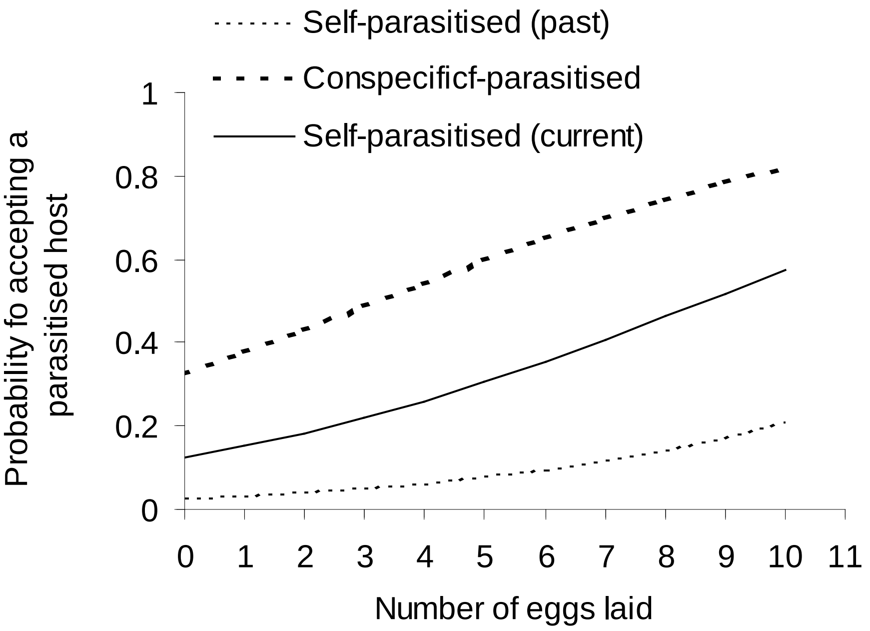

Figure 3.4 Probability of accepting a host parasitised by a conspecific female, the female herself in the past and the female herself in the present (overall), in relation to the number of eggs laid by female *D. semiclausum* during the experiment. The curve was estimated by the final five parameter model, given TIME =1912s (average patch time)

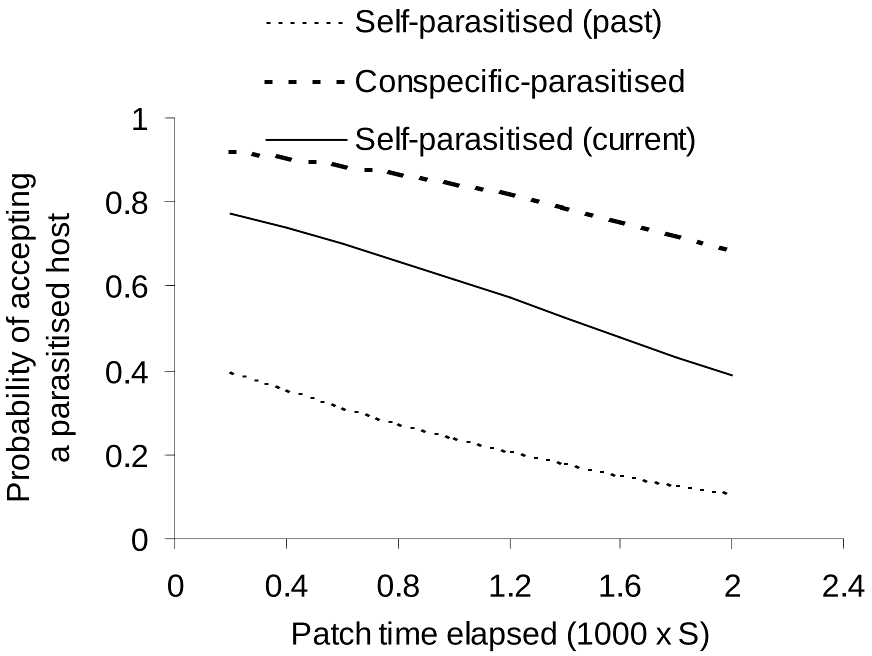

Figure 3.5 Probability of accepting a host parasitised by a conspecific female, the female herself in the past and the female herself in the present (overall), as a function of elapsed searching time by female *D. semiclausum* during the experiment. The curve was estimated by the final five parameter model, given EGG = 7 (average number of ovipositions)

3.4 Discussion

The results show that *D. semiclausum* (1) tends to oviposit into a newly encountered host; (2) discriminates a parasitised host by internal examination; and (3) encounters with hosts randomly in this experiment.

Random encounter could be a consequence of host defense. As observed in Chapter 2, the parasitoid displayed wide-area searching around the feeding sites, which increased the likelihood of detecting hosts resting away from the feeding site, and re-encountering hosts climbing up to the plants. The random encounter intervals could also, in part, result in the random termination of observed waiting periods (Chapter 2).

Parasitoids often leave some individual-specific marking substance on or in a host during oviposition, or even on the patch surface where they searched, to discourage superparasitism by a consepecifc female (Hubbard *et al.*, 1987; van Alphen & Visser, 1990; Bai & Mackauer, 1990; van Dijken *et al*., 1992; Danyk & Mackauer, 1993; Visser, 1993; Potting *et al*., 1997). Internal discrimination by *D. semiclausum* indicated the possible presence of an internal oviposition marker. Internal markers are reliable indicators of previous parasitism, but recognizing a host thus marked involves direct handling of the host by ovipositor probing. It might be impossible for *D. semiclausum* to detect an external marker reliably because of the strong physical defense of its host. Marking the patch is also unlikely employed by this parasitoid because host movement could decrease its reliability, because larval *P. xylostella* is very defensive, avoiding detection by parasitoids by moving away from damaged plant parts after short feeding bouts and by vigorously wiggling and dropping off the plant when being detected (Chapter 2). Obviously, host handling is time-consuming in this parasitoid. The time-saving benefit would be greater if *D. semiclausum* could use a threshold tactics in host selection.

No relationship between the probability of host acceptance and the pre-patch experience was found. Host discrimination in *D. semiclausum* seems to be an innate ability. For several parasitoid species, it has been demonstrated that naive females have the ability to discriminate between parasitised and unparasitised hosts **(**Bai & Mackauer, 1990; Völkl & Mackauer, 1990). In this experiment, the difference between naive wasps and experienced wasps were that the latter had six oviposition experiences into unparasitised hosts one day before the experiments. If host discrimination needs to be learned (van Lenteren & Bakker, 1975), it is possible that the pre-patch experience could have affected the first host acceptance, as the naive wasps acquired quickly experience, the overall effect of pre-patch experience as well as the effect of first sting experience might be not detected in the statistical analysis. In some parasitoids, for example *L. heterotoma*, a single oviposition experience is sufficient to suppress self-superparasitism (van Alphen *et al.*, 1987), whereas in *Venturia canescens* the rate of avoidance of superparasitism gradually rises as successive healthy hosts are encountered (Hubbard *et al*., 1999). It is also possible that the so-called 'experienced' wasps, deprived of hosts for 24 h in vial, could tend to superparasitize more (e.g. Hughes *et al*.,1994). However, if such pre-patch experience does affect its subsequent expectation of future patch exploitation, the parasitoid should have the ability to track environment depletion. Thus to some extent, the effects of pre-patch experience should be consistent with the previous experience. However, none of this previous sting experience had a significant effect on the probability of host acceptance as if the parasitoid *D. semiclausum* did not use previous experience in oviposition decisions.

There was some evidence that *D. semiclausum* showed discrimination by preferring unparasitised hosts (Legaspi, 1986). This study further demonstrated that the parasitoid could discriminate between four different types of hosts: unparasitised, parasitised by a conspcific female, self-parasitised in the past and self-parasitised in the present (overall). The parasitoid preferred unparasitised over parasitised hosts, conspecific-parasitised over self-parasitised hosts, and previously self-parasitised over presently self-parasitised hosts. Host discrimination between unparasitised and parasitised hosts is a common phenomenon among the parasitic Hymenoptera (van Lenteren, 1981; van Alphen & Visser, 1990). A number of parasitoids also have the ability to distinguish self- from conspecifc- parasitised hosts (Hubbard *et al*., 1987; van Dijken *et al*., 1992; Visser, 1993; Ueno, 1994). Self- or conspecifc- discrimination is principally achieved through the perception of individual-specific marks by the wasp (van Dijken *et al*., 1992). Most chemicals of host-discrimination factors are often produced by the female's genital apparatus, particularly the Dufour's gland, and are transferred to the host during oviposition (Hubbard *et al.,* 1987; Gauthier & May, 1998). However, the parasitoid seemed to superparasitize readily the presently self-parasitised hosts. A possible explanation for low rates of avoidance immediately after oviposition is that the chemical marker takes time to diffuse through the host before it is detectable (Hubbard *et al*., 1987).

Contrary to the predictions of static or ESS models (Visser *et al*., 1992; Visser, 1995), the results showed that, although *D. semiclausum* could discriminate several different types of hosts, both self- and conspecific- superparasitism were common (Table 3.4). For a solitary parasitoid, conspecific-superparasitism could prove to be an adaptive strategy if the probability of the offspring from the second egg wining and surviving to adulthood is greater than zero (van Alphen & Visser, 1990; Godfray, 1994). Self-superparasitism, in most circumstances, should not be adaptive since the competition within the host would be between full sibs, of which only one would survive. However, several empirical studies observed that self-superparasitism frequently occurs when (1) encounters with parasitised hosts are rare and the selection pressure for identification of self-parasitised hosts is small (Gates, 1993); (2) there is a low overall host encounter rate due to the presence of a host refuge (Edwards & Hopper, 1999); and (3) there is a high risk associated with foraging (Potting *et al*., 1997).

In *D. semiclausum* there were several possible explanations for the frequency of superparasitism. Firstly, host defense behaviour could place selection pressure on her oviposition decisions. Host handling is obviously time-consuming for the parasitoid. A recent model predicts that the accuracy of host recognition can be increased by increasing the inspection time, and this could generate some degree of non-recognition at the individual level when the time cost of inspection reaches a certain threshold; hence the optimal strategy could be partial acceptance (Glaizot & Arditi, 1998). Thus, assessment of host quality may lead to some superparasitism due to the time cost. Secondly, as the frequency of superparasitism increased in *D. semiclausum*, the proportion of female progeny also increased (Yang *et al*., 1994), thus the total reproductive success of offspring is likely to be higher if she chooses self-superparasitism. Superparasitism may confer an adaptive advantage on reducing the overall time spent in handling hosts. Thirdly, explanation of self-superparasitism is that females expecting to compete with other wasps for a limited host supply may ensure possession of the host by self superparasitism (Danyk & Mackauer, 1993). Because host examination requires considerably more time than oviposition in *D. semiclausum*, it may be adaptive for a female to lay an egg in an examined host regardless of variations in host quality.

Both egg depletion and elapsed searching time influenced the host acceptance probability of *D. semiclausum*. The effects of these interacted. Host acceptance probability increased with egg depletion, and decreased with elapsed search time on the patch. Static or ESS models of oviposition decisions have assumed the parasitoids are time-limited (Hughes, 1979; van Alphen & Visser, 1990; Visser *et al.*, 1992; Godfray, 1994). This has been argued mainly in the context of pro-ovigenic parasitoids species. Species like *D. semiclausum* produce mature egg slowly over time and their rate of egg depletion within a short term depends on the host encounter rate. In this experiment, the egg depletion was relatively quick. As this wasp always accepted an unparasitised host when it was encountered, oviposition in unparasitised hosts appeared to not be limited by egg complement (Table 3.4). Some individuals may become quickly egg-limited. When a group of female *D. semiclausum* was released into a multiple host plant environment, containing abundant unparasitised hosts, surprisingly most wasps quitted out the habitat after a about 10-15 ovipositions (see Chapter 8). When kept without food, the parasitoid could only live three days (Yang *et al*., 1993). Thus in the field, the parasitoids could be generally time-limited, i.e. die with plenty of eggs left in her body.

The probability of accepting a host by *D. semiclausum* decreased with elapsed time but increased with decreasing egg-load (Figure 3.4; Figure 3.5). The decreasing probability of host acceptance over time could reduce the risk of egg limitation. However, the higher search costs because of (1) the wasp could not track the environment change in host depletion; (2) random encounter; and (3) internal discrimination, could favored acceptance of a newly located host. Thus the effects of these two factors on oviposition decision could be balanced and continuously adjusted by the timing system and rate of egg depletion, the later depends on host density and rate of encounter. The extreme situation is that if the egg-load is high or the cost of egg-laying is small, the decision to oviposit may switch to being absolutely time-limited (Danyk & Mackauer, 1993). Because the handling time are similar for both parasitised and unparasitised hosts in *D. semiclausum*, the decision whether or not to superparasitism should be strongly influenced by the number of mature eggs available for oviposition (Iwasa *et al*, 1984).

The real nature of whether egg-limitation or time -limitation could be much complicated in parasitoids in the field (Casas *et al.*, 2000). There is still debate on the theoretical argument over egg-limitation and time -limitation in parasitoid reproduction strategies (Rosenheim,1996; Sevenster *et al.*,1998; Rosenheim, 1999). The results of this study highlighted the importance of the debate and reinforce the view of time- vs. egg-limitation for pre-ovigenic species (Roitberg, 1989; Rosenheim, 1996; Heimpel *et al*., 1998; Mangel & Heimpel, 1998; Casas *et al.*, 2000; Papaj, 2000).

Chapter 4 Patch time allocation by *D. semiclausum*: effects of inter-patch distance

4.1 Introduction

Since the resources used by many foragers have a patchy distribution, they face decisions on how to allocate foraging time over patches of varying profitability in order to maximize resource harvest rate (MacArthur & Pianka, 1966). Optimal patch time allocation involves two basic decisions: patch selection and patch leaving (Charnov, 1976b). In particular, a number of patch-leaving models in animals have been proposed in the last three decades (reviewed in Stephens & Krebs, 1986; Godfray, 1994; Driessen *et al.*, 1995).

The best-known model is the Marginal Value Theorem (Charnov, 1976b), which assumes that a forager has complete information about the foraging habitat and can assess patch quality instantaneously while foraging. The model thus predicts that an optimal forager should leave each patch when the instantaneous resource harvest rate falls to the average rate expected for the habitat, given the forager searches for resource items randomly and thus experiences a decreased resource availability with every successful harvest. This produces two general predictions that: (1) forager should spend more time in better quality patches when travel time between patches is constant; and (2) stay longer when travel times between patches become longer in presence of patches of equal quality but with varying travel times among patches (see Chapter 1; Figure 1.1).

These general predictions often qualitatively agree with empirical observations (e.g., Roitberg & Prokopy, 1982; Alonoso *et al.*, 1995; Bonser *et al.*, 1998; Lei & Camard, 1999; Chapter 2), although the assumptions are frequently violated (McNamara, 1982; Green, 1984; Godfray, 1994). In real species, for example, insect parasitoids, it is not known how precisely the parasitoids can assess patch quality. Many parasitoids of phytophagous insects use chemicals emitted from host plants due to the feeding activities of their hosts as a cue for patch location (Waage, 1978; Strand & Vinson, 1982; van Alphen & Galis, 1983; Vet & Dicke, 1992; Godfray, 1994; Shaltiel & Ayal, 1998; Chapter 2) and patch quality assessment (Waage, 1979; Driessen *et al.*, 1995; Geervliet *et al.*, 1998). Such an estimate of kairomone concentration could be an unreliable measure of actual patch quality, because effective host density in the patch depends on the number of available *unparasitised* hosts. Furthermore, such estimation depends largely on the ability of the parasitoids to detect the chemicals. For example, increasing inter-patch distance could be a factor influencing the estimation of patch quality due to the constraint of information decay. Therefore, many studies on patch leaving in animals have sought to determine how foragers process information about the distribution and abundance of patch resources (see Stephens & Krebs, 1986).

In insect parasitoids, given their limited prior information on patch quality, two opposite behavioral mechanisms have been proposed based on empirical studies of the parasitoid *Venturia canescens* by Waage (1979) and Driessen *et al.* (1995), respectively. Both assume that the parasitoid has a basic tendency to remain in a given patch as a response to the patch odor (kairomone concentration), which decays with time. While Waage's model predicts that an oviposition increases this responsiveness by a set amount that is assumed to be a linearly increasing function of unsuccessful search time since last oviposition with an asymptotic cut-off, Driessen's model predicts a decreased responsiveness to oviposition, and the effect of each subsequent oviposition decreases with increasing oviposition number. The results of some empirical studies are in general agreement with the predictions of either of the above models (reviewed in Driessen & Bernstein, 1999; Wajnberg *et al.*, 1999).

No doubt, limited prior information about patch quality could limit a parasitoid's ability to behave in a theoretically optimal way as predicted by the Marginal Value Theorem (Keller & Tenhumberg, 2000). The behavioral mechanism models could be too simple to consider the complexity of environmental cues that a parasitoid might use in patch-leaving decisions, because many factors could influence a parasitoid's behaviour (van Alphen & Visser, 1990;

van Lenteren, 1991; Rosenheim & Mangel, 1994; Nelson & Roitberg, 1995; van Alphen & Jervis, 1996).

Haccou *et al.* (1991) first used the Cox's proportional hazards model (Cox, 1972) to analyze the effects of various factors on the patch-leaving tendency of the parasitoid *Leptopilina heterotoma.* The statistical analysis of patch-leaving tendency by means of the proportional hazards model enables estimation of the influence of various factors on the patch-leaving tendency directly from the data, with few *a priori* assumptions (Haccou *et al.*, 1991; Hemerik *et al.,* 1993; van Roermund *et al.*,1994; van Steenis *et al.*, 1996; Vos *et al.,* 1998; Driessen & Bernstein, 1999; Wajnberg *et al.*, 1999).

This chapter reports the effect of inter-patch distance and within-patch foraging experience on the patch-leaving tendency of *D. semiclausum.* The foraging behaviour of the parasitoid was observed in a wind tunnel, where a single wasp was released onto an experimental host plant, and freely allowed to leave the experimental plant, by providing a plant bearing an equal host density at varying distances upwind of the experimental plant. The general prediction of the Marginal Value Theorem was tested and the effects of within patch experience such as oviposition on the patch-leaving tendency of the parasitoid were analyzed by means of the proportional hazards model.

4.2 Materials and methods

4.2.1 *Insects and plant*

For detailed rearing procedures of *P. xylostella* and *D. semiclausum*, see Appendix.

All experiments used 2nd and 3rd instar *P. xylostella* as host, and 2-3 day-old, mated female *D. semiclausum.* Parasitoid cocoons were taken from the culture and incubated separately in glass vials of 7 x 2 cm. A droplet of honey on the wall of the vial served as food for the parasitoids. One to two days prior to the experiments, individual female wasps were caged with 5 males in a cage (20 x 20 x 20 cm) until the female was mated. Mated females were then individually held in vials for the experiment the next day.

The potted cabbage plants with 5-6 fully extended leaves (*Brassica oleracea* var. *capitata*, cv. Green Coronet) were used in all experiments.

4.2.2 *Experimental set-up*

The experiments were conducted in a wind tunnel (for wind tunnel design see Keller, 1990), located in a room with controlled conditions of 24-25°C and 50-70% RH. The wind speed was set at 32.5 cm/s.

One day before the experiments, each leaf of both the experimental and extra plants was first numbered sequentially from bottom to top to permit rapid identification during the experiment, and then the plants were infested with three unparasitised larval *P. xylostella* by placing one larva on each of three randomly selected leaves. In the morning immediately prior to the experiments, each leaf was examined again to check the presence of the hosts and their positions in relation to the feeding damage site. Very few larvae moved away from the selected leaves, but when this happened, these plants were discarded.

A single female wasp was released from a 7 x 2 cm vial on a stand at equal height to the odor source (Figure 4.1). Immediately before release, the parasitoid was held for 5 minutes in an 80 mm Petri dish containing a piece of cabbage leaf with fresh feeding damage produced by one larval *P. xylostella* overnight, which increased the parasitoid's orientation towards host-related cues (Chapter 2). Once the wasp arrived on the experimental plant, its behaviors (attack, sting and waiting for hosts; for the behavioral catalogue, see Chapter 2) and location on each leaf were recorded continuously with the event recorder 'The Observer for Windows 3.0' (Noldus, 1991). When the parasitoid left the experimental plant and landed on the extra plant, an observation was terminated. Elapsed time was recorded to the nearest 1s. All the larvae that were stung were collected individually and dissected to determine the number of ovipositions immediately after the experiments.

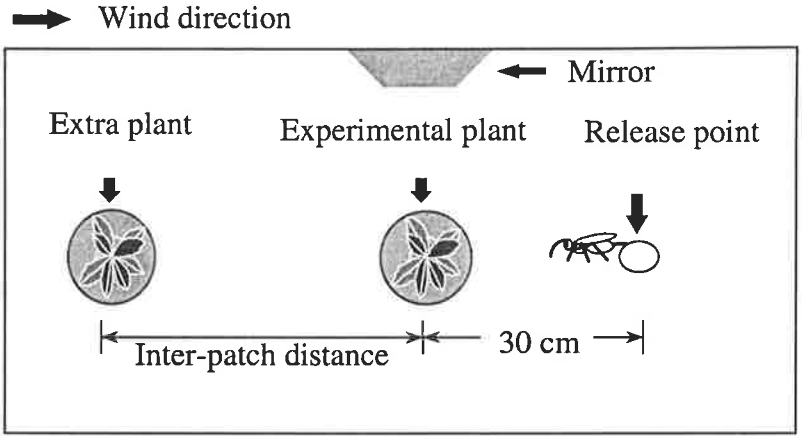

Figure 4.1 The experimental layout in the test section of a wind tunnel (160 x 65 cm). An extra plant was placed upwind of the experimental plant with varying inter-plant distance of 30, 60 or 90 cm. A female *D. semiclausum* was released 30 cm downwind of the experimental plant. A mirror was placed behind the experimental plant to allow observation of the searching behaviors of the wasp on the leaves opposite to the observer.

The experiments consisted of three treatments of different distances between the experimental and extra plant: 30, 60 and 90 cm. All experiments were conducted between 9:00 to 16:00. On each day, one or two female wasps were observed in a random order for each of the three treatments. When the inter-patch distance was 30 cm, in a high percentage of replicates the wasps left the experimental plant without oviposition. In order to obtain comparable replicates in analyzing the effect of oviposition on patch time, the number of replicates was increased in this treatment. On occasions, observations were terminated because the wasp stopped searching and remained motionless for more than 5 minutes. In this case, the censored data were included in the analysis of patch-leaving tendency. The data were excluded if a parasitoid directly landed on the extra plant after being released (only one case in the 30 cm inter-plant distance treatment). In total, 20, 24 and 29 wasps were released for the three different inter-patch distance treatments of 30, 60 and 90 cm, and finally 20, 24, and 28 complete replicates were collected, respectively.

All values are given as mean ± SE. Comparisons of mean values on various statistics among treatments were analyzed using Kruskal-Wallis tests. All the time-related measures such as patch residence time, were compared using log-rank test on survival analysis (Kaplan-Meier procedure, JMP, SAS, see below). The patch residence time defined as the total time between arriving on the experimental plant for the first time and leaving the experimental plant for the last time before flying to the extra plant, including all excursion flights around the experimental plants. The effects of inter-patch distance and within-patch foraging experience on the patch-leaving tendency were analyzed using Cox's proportional hazards model (Cox, 1972).

4.2.3 *Survival analysis*

The Kaplan-Meier survivor function $S(t)$ for each time-related measure in this study was calculated and the means of the distributions of the time variable among the inter-patch treatments were compared, using a non-parametric log rank test (Kalbfleisch & Prentice, 1980).

One important feature of survival analysis is its ability to handle data censoring, which inevitably occurs in behavioral research. For example, when one measures the giving-up time (GUT) of a searching parasitoid in a given patch, an encounter with a host can be a censoring observation, because the wasp might have left the patch at a different time if no encounter had occurred. For further discussion of data censoring, see Haccou & Meelis (1994) and Allison (1997).

When there are no censored data, $S\ (t)$ is intuitively the probability that an event time is greater than t; i.e. the sample proportion of observations with event times greater than t.

$$S\ (t) = Pr\ (T > t) = 1 - F\ (t)$$

Where $F\ (t)$ represents the cumulative distribution function of a random event time variable T.

When there are censored data, $S\ (t)$ is calculated as follows. Suppose there are k distinct event times, $t_1 < t_2 \ldots < t_k$. At each time t_j, there are n_j individuals who are said to be at risk of an event. At risk means that they have not experienced an event nor have they been censored prior to time t_j. If any cases are censored at exactly t_j, they are also considered to be at risk at t_j. Let d_j be the number of individuals who left the population at time t_j because the event of interest took place. The Kaplan-Meier estimator is then defined as

$$\hat{S}(t) = \prod_{j:t_j \le t} \left(1 - \frac{d_j}{n_j} \right)$$

Taking patch time for an example, the Kaplan-Meier estimate can be interpreted as the conditional probability that the individual leaves the patch, given that the individual has not yet left at the start of the time interval.

4.2.4 *The proportional hazards models*

Cox's proportional hazards model estimates the probability or hazard rate per unit time that a certain event occurs, given that it has not occurred yet (Collett, 1994). When it is used to analyze the patch-leaving tendency in insect parasitoids, the hazard rate can be considered as the tendency of a parasitoid to leave a given patch (Haccou *et al.*, 1991). In a similar fashion to Waage's model (Waage, 1979), the parasitoid is assumed to have a basic tendency to leave a given patch once she arrives, probably as a response to patch odor. The basic leaving tendency (baseline hazard) is then altered by the joint effects of covariates such as oviposition experience and other environmental information. The general form of the model with p covariates is

$$h(t;\, z)=h_o(t\,)\ exp\left\{\sum_{i=1}^{p}\beta_i Z_i\right\}$$

Where $h\ (t;\ z)$ denotes the probability per unit time (in this case per second) of leaving a patch. $h_o\,(t\,)$ is the basic leaving tendency when there is no effect of any covariates. Z_i are a set of p covariates selected for testing the effects on the patch-leaving tendency. β_i represents the coefficients of each of the covariates, which are estimated by means of partial likelihood maximization. The procedure is a Newton-Raphson iteration, and gives also the estimates of the variance of the coefficients (for further details, see Haccou *et al.*, 1991; Collett, 1994). One important feature of the proportional hazards model analysis is also its handling of data censoring. The model only considers the covariates which are set at the start of the foraging period when the wasp enters the patch or when the wasp self-censors the observation by performing some act such as an encounter with a host. The basic leaving tendency is reset after such renewal points. In this study, the renewal points were when the wasp firstly arrived onto the experimental plant and each subsequent encounter with a host including unsuccessful and successful encounters, i.e. oviposition.

The relationship between $S\ (t)$ and $h\ (t)$ can be described as:

$$h(t) = -d \log S(t)\ /\ dt$$

$$S\ (t) = Exp\left\{-\int_0^t h(u)du\right\}$$

If the survivor curves are plotted on a log scale, then inspection of the shape of the curve provides a visual test for time-dependent changes in this probability. If the survivor curves appear to be concave, the implication is an increasing departure tendency, and steeper decline of the curves indicates an increased tendency to leave the patch. If, instead, they are linear, i.e. the leaving tendency does not increase with the time (constant hazards over time) or the observed event has an exponential distribution (occurred randomly):

$$-\ \mathrm{Log}\ S\ (t) = \alpha\, t \quad (h\ (u) = \text{a constant})$$

The proportional hazards model implicitly assumes that for different values of any discrete-valued covariates the hazards rates are proportional. The proportionality assumption of the proportional hazards model can be examined by data stratification of the covariate under test, and then plotting estimates of cumulative hazard rates (- Log S (t)) at different levels of the covariate in such a way that deviations from proportionality can be detected visually (see, Kalbflesich & Prentice 1980; Hemerik *et al.,* 1993).

The goodness-of -fit of the model could be checked by a residual plot. The residual value should be distributed randomly around zero.

The critical steps in the analyzing the patch-leaving tendency of insect parasitoids by means of the proportional hazard model are covariate selection, coding of covariate and analysis procedures involved in significance tests of the selected covariates. In previous studies factors generally found to be important in the patch-leaving decisions of other parasitoids were selected as preliminary covariates (e.g. Vos *et al.*, 1998). Many factors involved in within-patch experience such as an encounter with a parasitised host, oviposition and oviposition rate are considered to be potentially important in patch-leaving decisions of parasitoids (Waage, 1979; van Lenteren, 1991; Hemerik *et al.,* 1993; Driessen *et al.*, 1995; Nelson & Roitberg, 1995; van Alphen & Jervis, 1996). In this study, the two patches were identical in host density. The only difference between individual wasps foraging was the inter-patch distance, and within-patch experience. Based on the behavioral observation of the parasitoid in this and previous studies (Chapter 2), inter-patch distance, each oviposition and its sequence, the cumulative number of ovipositions, and oviposition rate were considered as the potential covariates. The parasitoid usually waits for a host near to the silk to climb up to the leaf and attacks it again, if the first attack is not successful (Chapter 2). Thus, additionally each unsuccessful encounter and cumulative number of unsuccessful encounters were included as two extra covariates. Potentially important factors found in other studies, such as encounter with parasitised host, superparasitism, and rejection of parasitised host, were not considered in this analysis, as in this experiment superparasitism was observed only in 4 of 82 ovipositions (see results). In total, 10 covariates were selected to test. The detailed description of each covariate is given in Table 4.1. For covariate coding, see an example in Figure 4.2.

Table 4.1 Explanatory covariates selected and tested in the analysis of patch-leaving tendency of *D. semiclausum* by means of the proportional hazards model

Covariates	Coding *
Is the inter-patch distance 60 (D60) or 90 cm (D90)?	Yes = 1, No = 0
Was the previous encounter unsuccessful (ENT)?	Yes = 1, No = 0
Did the previous encounter involve an oviposition (OVI)?	Yes =1 , No = 0
Number of ovipositions since entering patch (COVI)	Observed value
Number of unsuccessful encounters since entering patch (CENT)	Observed value
Recent oviposition rate (= 1/ time since last oviposition) (RATE)	Observed value
Was the previous oviposition the first one since on the patch (O1)?	Yes = 1, No = 0
Was the previous oviposition the second one since on the patch (O2)?	Yes = 1, No = 0
Was the previous oviposition the third one since on the patch (O3)?	Yes = 1, No = 0

*For covariate coding, see an example in Figure. 4.2.

Two different analysis procedures for the Cox's proportional hazard model have been used in previous studies. Haccou *et al.* (1991) first proposed a multiple comparison method. Their method includes three steps: (1) First test if the whole set of selected covariates has a significant joint effect on the hazard rate (joint test). If this is found to be significant, at least one or a combination of the covariates is significant. (2) Second test each of the covariates separately, to see if any single covariate has a significant effect on its own. (3) Third test whether any combinations of covariates that are not significant by themselves, have a significant effect by testing them in pair-wise combinations. Finally the entire statistical test (Wald test) is compared to the chi-squared critical value with maximum degrees of freedom.

Many studies have followed theses procedures (Hemerik *et al.,* 1993; van Roermund *et al.*, 1994; van Steenis *et al.*, 1996; Vos *et al.,* 1998; Driessen & Bernstein, 1999).

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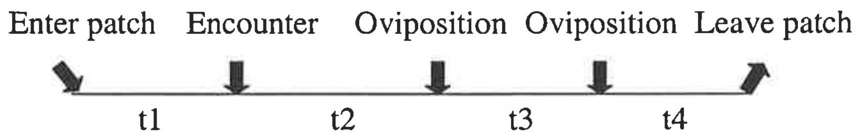


Patch residence time = t1+t2+t3+t4

Covariates and coding

GUT	CEN	D60	D90	ENT?	OVI?	RATE	CENT	COVI	O1?	O2?	O3?
t1	0	0	0	0	0	0	0	0	0	0	0
t2	0	0	0	1	0	0	1	0	0	0	0
t3	0	0	0	0	1	1/(t1+t2)	1	1	1	0	0
t4	1	0	0	0	1	1/t3	1	2	0	1	0

Figure 4.2 A fictitious example of covariate coding in this analysis. In this example, the inter-patch distance was 30 cm. GUT represents the period between two subsequent renewal points, i.e. from entering patch to the first encounter, between successive encounters, and from last encounter until leaving the patch. CEN indicates a censored data, the value is zero if it is a censored observation). For other abbreviations see Table 4.1. ENT, OVI, O1, O2, and O3, were binary covariates of either 1 (yes) or 0 (no), respectively. In a few replicates, the wasp had more than 3 ovipositions before leaving the patch, in these cases, O3 was coded as 1. CENT, COVI and RATE are observed values.

Recently, Wajnberg *et al.* (1999) used an iterative regression method, which is routinely used for identifying significant variables in any kind of generalized linear model, including Cox's model (Collett, 1994), to analyze the patch-leaving tendency of the parasitoid *Telenomus busseola.* The iterative regression method includes 4 steps. (1) First test if each covariate on its own has a significant effect on the patch-leaving tendency. (2) Second test if the combination of the seemingly important covariates from step 1 is significant. In the presence of certain covariates, others may cease to be important. Consequently, the change in the test statistic (-2 log L) when each covariate is omitted from the set is evaluated, and only those covariates that lead to a significant increase in the value of (-2 log L) are retained in the model. (3) Third re-consider the covariates which are not under consideration in step 2, by adding each one to the model, and retaining those that reduce -2 log L significantly. (4) A final check of the model is to make sure that no covariates can be omitted or included without significantly changing -2 log L. For the detailed procedures, see Collett (1994) and Wajnberg *et al.* (1999).

Which procedure should be followed depends on the aims of the study. The multiple comparison method intends to identify significant covariates or their combinations, rather than to fit the final model with the least possible number of covariates (P., Haccou; personal communication). The iterative regression method aims to fit all significant variables into a final model (E, Wajnberg; personal communication), and such a model could then be used to predict quantitatively the effects of a group of covariates on the patch-leaving tendency of the parasitoid. Here both methods were used to analyze the data, and to compare the results of different analysis procedures. All analyses were conducted with the PHREG procedure of the SAS software (Version 6.0, Allison, 1997).

4.3 Results

4.3.1 *Patch time allocation*

With increasing inter-patch distance, *D. semiclausum* stayed and searched longer, visited the infested leaves more often, attacked and parasitised more hosts (Table 4.2). In total 154 encounters with hosts were observed in all the experiments. 80 of the 154 encounters led to successful ovipositions, of which only 4 were superparasitism. The unsuccessful encounter resulted from the host defense by dropping off the plant, usually on a silk thread or otherwise directly onto the floor. In reaction, the parasitoids waited for the host to climb back and attacked it again. The mean waiting period was 354.2 ± 53.6 s (n = 25) when the waiting led to a re-attack, and was 357.2 ± 71.5 s (n = 27) when the parasitoid finally gave up waiting. Survival analysis on the waiting time distribution found no significant difference in mean period between these two kinds of waiting (Long-rank test, Kaplan-Meier procedure, $P > 0.7100$). The length of an individual waiting period was highly variable, ranging from 13.8 s to 1415.8 s, which in part depended on the suspension time of the hanging larvae. The tendency to give up waiting increased over time (Figure 4.3).

Table 4.2 Comparison of within-patch search parameters by *D. semiclausum* females as affected by the inter-patch distance

Search efforts	Inter-patch distance #			
	30	60	90	*P*
N	28	24	16	
Patch residence time (s)	709 ± 105 a	1015 ± 153 a	1560 ± 184 b	0.0027
Search time (s) *	593 ± 96 a	820 ± 115 a	1328 ± 147 b	0.0030
No. of infested leaves visited	4.3 ± 0.72 a	6.9 ± 0.77 a b	10.8 ± 0.97 b c	0.0001
No. of clean leaves visited	0.79 ± 0.32	1.43 ± 0.55	1.88 ± 0.63	0.0542
No. of attacks	1.54 ± 0.23 a	2.38 ± 0.32 a b	3.25 ± 0.49 b c	0.0060
No. of ovipositions	0.79 ± 0.15 a	1.25 ± 0.19 a b	1.75 ± 0.23 b c	0.0021

* Patch time in which the waiting period was subtracted.

Within a row, the values followed by the same letter were not significantly different (Kruskal-Wallis test). All time-related parameters were compared using long-rank tests of survival analysis (Kaplan-Meier procedure, JMP, SAS).

Table 4.3 Giving-up time without oviposition or since last oviposition by female *D. semiclausum* when leaving the experimental host plant

Inter-patch distance (cm)	Giving-up time without oviposition (s) *	Giving-up time since last oviposition (s)	*P*
30	543 ± 135 (12)	316 ± 85 (16)	0.0350
60	533 ± 151 (4)	532 ± 151 (20)	0.5240
90	834 ± 303 (3)	493 ± 100 (13)	0.2012

* Figures in brackets are the number of replicates. Long-rank test of survival analysis (Kaplan-Meier procedure, JMP, SAS).

Table 4.4 Mean duration from entering the experimental plant to first oviposition and between first and second oviposition of female *D. semiclausum* when searching within the experimental host plant

Inter-patch distance (cm)	Time to the first oviposition (s) *	Time between first and second oviposition (s)	*P*
30	226 ± 69 (16)	268 ± 74 (5)	0.4047
60	213 ± 57 (20)	368 ± 90 (6)	0.0679
90	338 ± 96 (13)	484 ± 77 (13)	0.1473

*Figures in brackets are the observed replicates. Long-rank test of survival function analysis (Kaplan-Meier procedure, JMP, SAS).

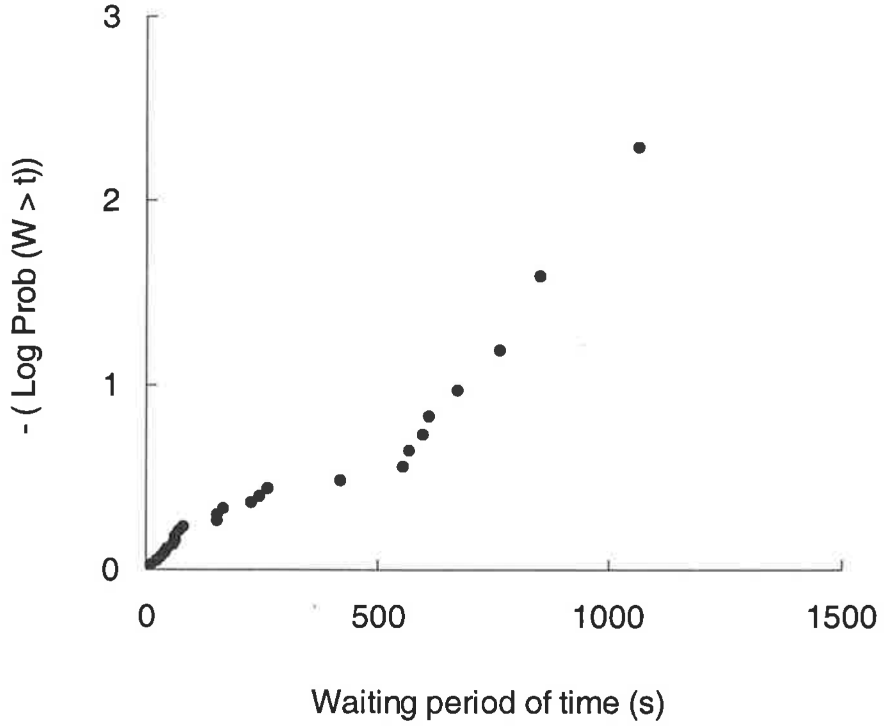


Figure 4.3 Logarithm of the probability distribution of waiting time (*W*) across all treatments by *D. semiclausum* following an unsuccessful encounter with its host *P. xylostella*. A waiting period leading to a re-attack of the host was treated as a censored observation (Kaplan-Meier procedure, JMP, SAS).

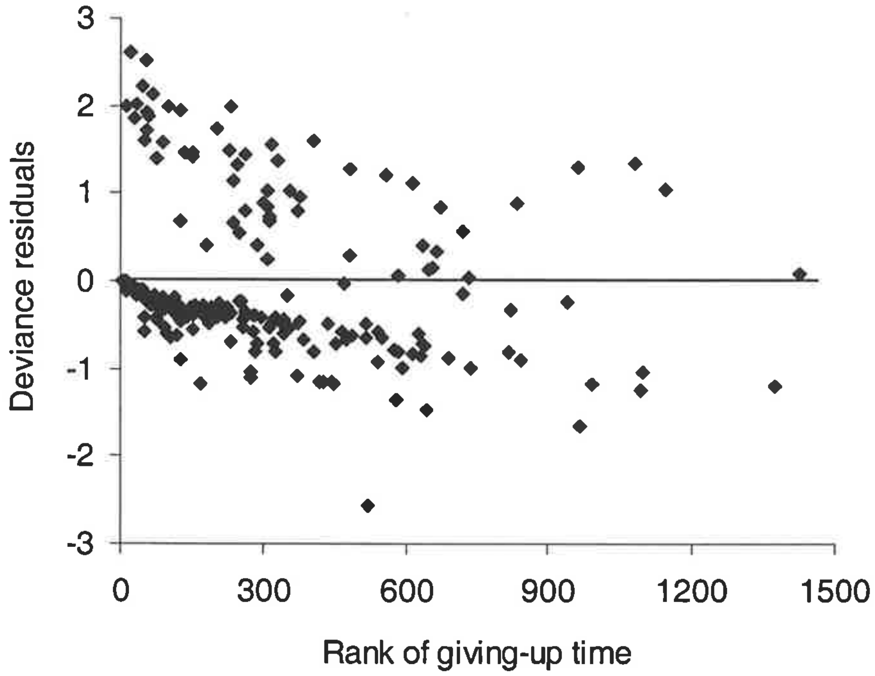


Figure 4.4 Plot of the deviance residuals of the final fitted model with four covariates against the rank order of GUT

When the inter-patch distance was 30 cm, 12 of 28 wasps left the experimental plant without oviposition (Table 4.3), 11 wasps had one oviposition, and five wasps had two ovipositions. Most of the wasps left the patch after at least one oviposition (20 of 24) when the inter-patch distance was 60 cm, and at least two ovipositions (13 of 16) when the inter-patch distance was 90 cm (Table 4.3). Five wasps oviposited three or four times in the experiments. The giving-up times of wasps were quite variable. On average, there was no significant difference between the giving-up time without oviposition and the giving-up time since last oviposition, except that in the 30 cm inter-patch distance treatment, oviposition significantly decreased the giving-up time (Table 4.3). There was also no significant difference between the time to first oviposition and the interval between the first and second oviposition, indicating that the cumulative number of ovipositions increased linearly over time in this experiment (Table 4.4).

4.3.2 *Patch-leaving tendency*

The patch leaving tendency analysis initially considered 10 covariates (Table 4.5). When the data were analyzed with the multiple comparison method (Haccou *et al.*,1991), all test statistics were compared to the chi-squared critical value of a χ^2 distribution with 9 degrees of freedom. The joint effect of all 10 covariates was found to be significant compared to the null-hypothesis $\beta_1 = ... = \beta_{10} = 0$, indicating that at least one of the covariates had a significant effect on the patch-leaving tendency of the parasitoid. When each of the covariates was tested one at a time, only oviposition (OVI) and the cumulative number of ovipositions (COVI) were significant covariates. No pair-wise covariates that were not significant by themselves had a significant interaction effect on the patch-leaving tendency. Thus only OVI and COVI were included in the final model when the multiple comparison method was used.

Table 4.5 Significance tests and the estimated coefficients of the covariates

Covariates	β	SE	Wald test statistics	P (d.f)
A. Joint test (all)			54.8	0.0001 (9)
B. Single tests				
D60 (1)	0.1096	0.2640	0.17	0.6788 (1)
D90 (2)	-0.6928	0.2897	5.72	0.0168 (1) **
ENT (3)	-0.7834	0.2824	7.70	0.0055 (1)**
OVI (4)	1.0733	0.2558	17.61*	0.0001 (1)**
COVI (5)	0.6055	0.1346	20.23*	0.0001 (1)**
CENT (6)	-0.0399	0.1125	0.13	0.7225 (1)
RATE (7)	6.5452	2.5869	6.40	0.0114 (1)**
O1 (8)	0.1837	0.2517	0.53	0.4656 (1)
O2 (9)	0.5451	0.2890	3.56	0.0593 (1)
O3 (10)	1.5098	0.4757	10.08	0.0015 (1) **
C. Combination test				
(2) + (3)			15.98	
(2) + (9)			16.90	
(2) + (10)			15.17	
(3) + (7)			15.73	
(3) + (10)			16.28	
(7) + (10)			16.17	
(2) + (3) + (7)			22.89 *	
D. Final model				
(2)+(3)+(5)+(7)			45.134*	

* Multiple comparison (Haccou *et al.*, 1991). All test statistics were compared to the chi-squared critical value of a χ^2 distribution with 9 degrees of freedom, i.e. 16.92.

** Covariates that are significant on their own test.

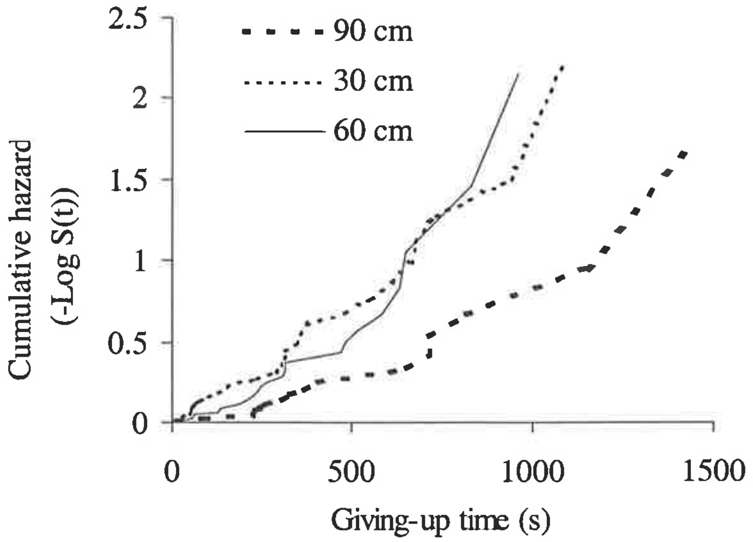


Figure 4.5 Negative Log-Survivor curves for patch leaving at different inter-patch distance treatment, i.e. graphical test on proportional assumption

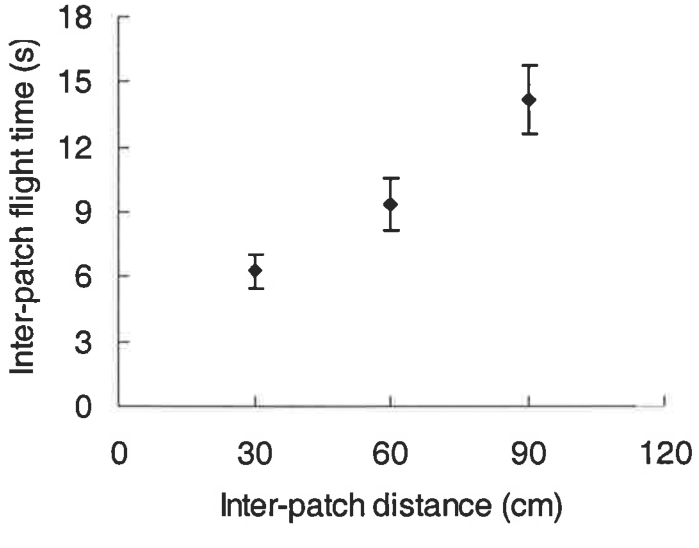


Figure 4.6 Flight time (s) (± SE) when *D. semiclausum* females finally left the experimental plant and landed on the extra plant (Kruskal-Wallis test, $P < 0.0003$).

However, when the data were analyzed with the iterative regression method, six covariates on their own appeared to be significant. Finally, 4 covariates were fitted into the model (Table 4.6). Residual analysis showed that the final model seemed to describe the patch-leaving tendency of *D. semiclausum* well (Figure 4.4). Thus, the iterative regression method showed that the patch-leaving tendency of *D. semiclausum* decreased with increased inter-patch distance (D90) and unsuccessful encounter with host (ENT), and increased with the number of successful ovipositions (COVI) and the unsuccessful search time since last oviposition (RATE). The effects of inter-patch distance on the patch-leaving tendency could further be viewed graphically and the proportionality assumption can be justified (Figure 4.5).

4.4 Discussion

4.4.1 *The proportional hazards model*

The proportional hazards model has been used to analyze the patch leaving tendency in a number of insect parasitoids: *L. heterotoma* (Haccou *et al.*, 1991), *L. clavipes* (Hemerik *at el.*, 1993), *Encarsia formosa* (Roermund *et al.*, 1994), *Aphidius colemani* (van Steenis *et al.*, 1996), *Cotesia rubecula* (Vos *et al.*, 1998; Keller & Tenhumberg, 2000), *C. glomerata* (Vos *et al.*, 1998), *V. canescens* (Driessen & Bernstein, 1999); and *Telenomus busseolae* (Wajnberg *et al.*, 1999). The statistical analysis of behavioral rules is based on fewer *a priori* assumptions about the information available to parasitoids and their ability to utilize such information. The advantage of this approach over other optimization models, as pointed by Haccou *et al.* (1991), is in its ability to analyze quantitatively the effects of several variables on the patch-leaving tendency of a parasitoid in a more complicated environment. Furthermore, this statistical model can be used to test the predictions or hypotheses of many optimal foraging models, and the statistically derived behavioral rules could provide useful insights leading to the refinement of the evolutionary models (Haccou *et al.*, 1991; Godfray, 1994). Thus optimal foraging models can guide empirical research, while the proportional hazards model is an important tool to identify factors that affect the decision-making processes of searching wasps, which could expose the deficiencies of optimal foraging models when applied to real species. The interplay between foraging theory and analysis of observations of foraging wasps should lead to a better understanding of the mechanisms that govern foraging behaviour (Keller & Tenhumberg, 2000).

Table 4.6 Estimated regression coefficients (β), standard errors (SE) and hazard ratios[exp(β)] for the final fitted model that included all the significant covariates affecting the patch-leaving tendency of *D. semiclausum*. χ^2 corresponds to the likelihood ratio tests ($P < 0.05$). All of them were estimated with all other significant terms present in the model.

Covariates	β	SE	exp(β)]	χ^2 (d.f)	*P*
Inter-patch distance (90 cm)	-1.2508	0.3048	0.286	16.84 (1)	0.0001
Unsuccessful encounter	-0.8619	0.2981	0.422	8.36 (1)	0.0038
Number of ovipositions	0.6089	0.1379	1.838	19.50 (1)	0.0001
Rate of oviposition	6.1267	2.7438	457.9	4.99 (1)	0.0256

However, this study highlighted an important methodological problem in dealing with covariate selection and analysis procedures of the proportional hazards model. Different methods would result in different results. With the iterative regression method (Collett, 1994; Wajnberg *et al.*, 1999), a group of four statistically significant covariates were fitted in the final model. In contrast, with the multiple comparison method (Haccou *et al.*, 1991), only two covariates, successful oviposition and the cumulative number of ovipositions, were identified as the significant covariates influencing the patch-leaving tendency of *D. semiclausum*. Correlation analysis showed that these two covariates were positively correlated (r = 0.6347, n = 222, $P < 0.0001$). Thus the effects of these covariates were consistent. Clearly, the inter-patch distance and unsuccessful encounter with hosts were important factors influencing the patch time (Table 4.2; Figure 4.3 & 4.5). With the multiple comparison method, the significance test of covariates based on the Wald test statistics was determined by a critical value of the chi-squared distribution with maximum degrees of freedom (Haccou *et al.*, 1991). Thus, the initial number of covariates included in the analysis would be very important, the more the covariates included the higher the degrees of freedom. In this study, the number of selected covariates was reduced initially from 10 to 6, by omitting the other four non-significant covariates, D60, CENT, O1, and O2. As a result, the critical value of the chi-squared distribution was reduced from 16.9 to 11.7, and all the pair-wise combination tests were significant (Table 4.5). With the iterative regression method, the results did not depend on the number of covariates selected as long as the significant covariates were included, and the interactive procedure used (Collett, 1994).

It is necessary to be extremely cautious with discarding variables in each subsequent step in the analysis, although some covariates that are not significant statistically in one circumstance may turn out to be important under different circumstances. An important characteristic of the proportional hazards model is the correlation between the coefficients of covariates. Variables which appear to be important on their own in the separate tests may cease to be important in presence of other variables when they are fitted together. For example, when the six significant covariates: D90, ENT, OVI, COVI, RATE, and O3 were fitted together, OVI and O3 ceased to be significant (Table 4.5). Thus, the multiple comparison method could be useful in the initial attempts to identify the potentially important covariates for further tests, and the iterative regression method could be used to fit a final model that will quantitatively predict the patch-leaving tendency under various conditions. When many potential factors are under consideration, the iterative regression method should be used.

4.4.2 *The adaptive significance of patch-leaving rules*

Four covariates: D90, ENT, COVI, and RATE, significantly influenced the patch-leaving tendency of *D. semiclausum*. In this experiment, host density of the patches was relatively low and uniformly distributed over the leaves (0 or 1 larva per leaf). In such an environment, an increased tendency to leave the patch after one successful oviposition could be adaptive (Iwasa *et al.*, 1981, Driessen *et al.*, 1995). The higher the rate of oviposition, the sooner the patch should have been given up, indicating that successful oviposition resulted in more rapid movement between patches to reduce self-superparasitism, as the parasitoid discriminates parasitised hosts by internal examination (Chapter 3).

In this experiment, 34.5 percent of the host larvae were observed resting away from the feeding sites while the wasp was visiting the patch. Among those larvae that were on the feeding damage, 63.5 percent of them were attacked by the parasitoid, while among those that had moved from the feeding sites, only 33.5 percent were attacked by the parasitoids before they left the patch. As reported in Chapter 2, larval *P. xylostella* is very defensive, and only half of the first attacks were found to be successful in this study. The parasitoid cannot discriminate a parasitised host without ovipositor probing (Chapter 3), so the parasitoid often waits for a discovered host whether it is parasitised or not following an unsuccessful encounter (Chapter 2 & 3). Although oviposition often took only a few seconds, the handling time for a host including the waiting time lasted up to 1405s (Figure 4.3). Thus, locating and recognizing a host is time-consuming for *D. semiclausum,* so there was no need to reject a discovered host.

The pattern of patch time allocation agreed with the general predictions of the Marginal Value Theorem. The parasitoid stayed longer and parasitised more hosts with increasing inter-patch distance. However, the results did not agree with the central assumption that the cumulative number of ovipositions should be an increasing and negatively accelerated gain function over time. It was found that the patch was depleted without having an increasing and negatively accelerated gain function, as the mean oviposition interval was nearly consistent (Table 4.4). The model assumption is only met when a parasitoid searches for hosts randomly, and thus experiences decreased host availability with every successful oviposition (Charnov, 1976b). In the fields, the host densities of *P. xylostella* varied extremely, from none to up to 30 larvae per plant (Wang, XG., unpublished data). It is possible that in an extremely high host density patch, the cumulative number of ovipositions could meet the assumption of Marginal Value Theorem. In comparison, the treatment of 9 larvae per plant with clustered distribution, the rate of oviposition was significantly higher than that of other 4 low host density treatments (See Chapter 5). However, in this experiment, many random events such as unsuccessful encounter, waiting period of time, and whether or not the host was off the damage site, would have influenced encounter rates and thus the cumulative gain functions of particular wasps.

The Marginal Value Theorem, based on the rate maximization rules, also assumes that travel between patches involves costs in time. This could be true, as many parasitoids are time-limited. In this experiment, although the inter-patch flight time increased with increasing inter-patch distance (Figure 4.6), the duration of flight time was virtually negligible, compared with the patch residence time. When the inter-patch distance was increased to 90 cm, in 5 of 21 observations, the wasp did not emigrate from the patch, and made more repeated visits to the infested leaves. Thus, a possible mechanism underlying the decreased effects of inter-patch distance on the patch-leaving tendency could be that the longer inter-patch distance influenced the detectability of the extra patch, which would lead to similar predictions to the Marginal Value Theorem. Patch location in many parasitoids of phytophagous insects depends on the sensitivity of the parasitoid to host-related cues (Vet & Dicke, 1992, Godfray, 1994; Shaltiel & Ayal, 1998). *D. semiclausum* responds to the local variation of patches infested by different host densities by preferentially landing on highly infested plants (Chapter 2). However, quantitative responses of parasitoids to kairomone levels could be influenced by the inter-patch distance. This suggested that if a parasitoid could not estimate patch quality at a distance due to its physiological constraint, patch-leaving decisions may be more important than patch selection in optimal patch time allocation.

In this experiment, the host density was relatively low and hosts were evenly distributed. It is also possible that under different environmental conditions such as varying host density and distribution, the parasitoid would use different patch-leaving rules. Many other factors could also influence the patch-leaving tendency, including varying host density and distribution, host type, and the complexity of the patch environment. Some of these remaining factors are investigated in the latter chapters.

Chapter 5 Patch time allocation by *D. semiclausum*: effects of host density and distribution

5.1 Introduction

Because of time limitation in many parasitoids, optimal allocation of foraging time over patches of different profitability should be under strong natural selection (van Alphen & Vet, 1986; Godfray, 1994). A number of patch-leaving models in animals, particularly in insect parasitoids, have been proposed (Charnov; 1976b; Waage, 1979; Stephens & Krebs, 1986; Godfray, 1994; Driessen *et al.*, 1995, Vos *et al.*, 1998). The key is to understand how a forager gathers information about the abundance and distribution of resources in making decisions about patch time allocation (see Stephens & Krebs, 1986; Krebs & Kacelnik, 1991). In the preceding chapter, the effect of inter-patch distance on the patch time allocation of *D. semiclausum* was investigated. The experiment discussed in this chapter investigates the effects of host density and distribution on the patch time allocation and patch-leaving decisions of the parasitoid.

In insect parasitoids, two opposite behavioral mechanism models predict different influences of oviposition experience on the patch time allocation of the parasitoid *Venturia canescens* (Waage, 1979; Driessen *et al.*, 1995). In general, some empirical studies agreed with the predictions of Waage's model, while others agreed with the predictions of the Count-down mechanism (see Driessen & Bernstein, 1999). From an evolutionary point of view, which mechanism a parasitoid should use could depend on the host density and distribution (Iwasa *et al.*, 1981; van Alphen & Vet, 1986; Driessen *et al.*, 1995; Driessen & Bernstein, 1999). In a patchy environment with low and evenly distributed host resource, each oviposition provides information regarding the loss of the future value of the patch. Increasing tendency to leave the patch with each successful oviposition should be an adaptive strategy

(Iwasa *et al.*, 1981). While in a rich environment with cluster host resources, Waage's mechanism should be an adaptive strategy (Driessen *et al.*, 1995; Vos *et al.*, 1998). Under natural conditions, patches of many insect parasitoids often vary considerably in host abundance and distribution. If host density as well as distribution could influence a parasitoid's patch-leaving decisions, a parasitoid would switch to use different mechanisms in different environments; any fixed mechanism would not do better. However, in *V. canescens* oviposition consistently decreased the patch-leaving tendency even in high host density patches (Driessen & Bernstein, 1999). It is argued that a Count-down mechanism should be advantageous and therefore be selected for, irrespective of host distribution as long as the parasitoid has good enough information on the initial quality of the patch it is foraging in (Shaltiel & Ayal, 1998; Driessen & Bernstein, 1999).

The question is how a parasitoid access patch quality? Kairomone is only a guide to possible host presence rather than a reliable indicator of available host density and host distribution (Chapter 4). Parasitoids may not necessarily have complete information on the estimation of initial patch quality, and other cues such as actual encounters may provide more reliable estimates for parasitoids than would kairomones (Morrison & Lewis, 1981; Vet & Dicke, 1992). In real species, the patch-leaving behaviour could be complicated. If a parasitoid has complete information on the initial patch quality, an incremental mechanism would make a parasitoid trapped in a patch with high kairomone concentration but low host density available to parasitoids if most of the hosts have left the patch or are not available. If the initial assessment of patch quality is not perfect, 'Count-down' parasitoids will sometimes leave too early from a patch in a heterogeneous and rich environment (Driessen & Bernstein, 1999).

In this chapter parasitoid foraging behavior was directly observed in a wind tunnel, where a single wasp was released onto an experimental plant with varying host density and distribution. The female wasp was allowed to freely leave the 'patch', by providing her with an extra plant upwind of the experimental plant. The data were analyzed by means of the proportional hazards model, which can be used to address the complexity of behavioral factors influence patch-leaving tendency of the parasitoid, and the results can also be used to compare with the predictions of optimization models (Chapter 4).

5.2 Materials and methods

5.2.1 *Insects, plant and experimental set up*

The insect culture, parasitoid handling, host plant and experimental set up used in this experiment were same as in Chapter 4. The difference was in this experiment, the distance between the experimental plant and extra plant was fixed as 60 cm (see Chapter 4; Figure 4.1), but the experimental plants were infested with varying host densities and distributions. There were three host density treatments (1, 3, 9 larvae per plant), and two host distribution treatments for each of the two high host densities (uniform and clustered, respectively).

One day before the experiments, each leaf of the experimental plants was first numbered sequentially from bottom to top to permit rapid identification during the experiment and then infested with host larvae as required. With the uniform distribution, the host larvae were evenly distributed over the leaves of the experimental plant, while in the clustered treatment, all the host larvae were placed on one selected leaf. In order to restrict larval movement, a paper collar was wrapped around the stem of the infested leaf. In all the experiments, the extra plants were infested with three unparasitised host larvae.

The experimental procedures and data analysis methods were the same as given in Chapter 4. 16 to 24 replicates were conducted for each of the treatments.

5.2.2 *The proportional hazards model*

Covariate selection is a critical step in the analysis of the patch-leaving tendency of insect parasitoids by means of the proportional hazards model (Chapter 4). In the previous chapter, four covariates were found to be important in the patch-leaving behaviors of *D. semiclausum*: Inter-patch distance, unsuccessful encounter, number of ovipositions, and rate of oviposition. Many aspects of the within-patch foraging experience in this study, such as unsuccessful encounters with hosts, oviposition, and oviposition rate, were found to be similar to the previous study and thus were considered as potentially significant covariates. In addition, the host density, host distribution, each oviposition and its sequence were considered as additional covariates, as these factors were found to be important in the studies of other parasitoid species (Waage, 1979; Hemerik *et al.*, 1993; Driessen *et al.*, 1995; Nelson & Roitberg, 1995; van Alphen & Jervis, 1996). In considering the effects of host density and distribution, host density 3 with uniformly distribution was taken as a reference, thus 4 covariates representing the other host densities and distributions of the experimental treatments were included. Encounters with parasitised hosts and superparasitism were also considered as covariates since superparasitism and rejection of self-parasitised hosts were observed in this experiment. In total, 18 covariates were selected for the analysis (Table 5.1).

As in Chapter 4, in this study, the renewal points were when the wasp first arrived on the experimental plant and each subsequent encounter with a host including unsuccessful and successful encounters, i.e. oviposition. The basic leaving tendency is reset after such renewal points. The iterative regression method was applied to the analysis of the influence of the selected covariates on the patch-leaving tendency of the parasitoid (Chapter 4). The analysis was conducted with the PHREG procedure of the SAS software package (Version 6.0, Allison, 1997).

5.3 Results

5.3.1 *Patch time allocation and foraging efficiency*

The residence time of the parasitoid on the experimental plant increased with host density and the wasps stayed significantly longer when the hosts were aggregated than uniformly distributed over the plant (Log rank test on the survival functions of patch time, JMP, SAS, Figure 5.1a). There was no significant difference in patch residence time between densities of 1 and 3 per plant with uniform distribution.

Table 5.1 Covariates selected in the analysis of the patch-leaving tendency of *D. semiclausum* by means of the proportional hazards model

Description of the covariates	Coding
Is the host density 1? (DEN1)	Yes = 0, No = 1
Is the host density 3 with clustered distribution? (DEN3C)	Yes = 0, No = 1
Is the host density 9 with uniform distribution? (DEN9E)	Yes = 0, No = 1
Is the host density 9 with clustered distribution? (DEN9C)	Yes = 0, No = 1
Was the previous encounter unsuccessful? (ENT)	Yes = 0, No = 1
Did the previous encounter involve an oviposition? (OVI)	Yes = 0, No = 1
Did the previous encounter involve superparasitism? (SUP)	Yes = 0, No = 1
Was a parasitised host rejected at the last encounter? (REJ)	Yes = 0, No = 1
Cumulative number of unsuccessful encounters (CENT)	Observed value
Cumulative number of ovipositions (COVI)	Observed value
Rate of last oviposition (RATE)	Observed value
Oviposition (O1-O7) *	Yes = 0, No = 1

* O1- O7 represents the number of ovipositions in sequence. All ovipositions after the seventh were coded as O7 = 1.

Mean number of attacks on hosts increased with host density and was significantly higher when the hosts were clustered on the plants (Table 5.2). The attack rate also increased with host density but not in proportion to host density (Table 5.2). When the host density was 9 per plant, the attack rate significantly increased when the hosts were aggregated. Although the mean number of ovipositions also increased with host density and clustered distributions of the hosts over the plant (Table 5.2), the mean oviposition rate was not significantly different among the treatments, except for the treatment of 9 larvae per plant with clustered distribution, in which the rate of oviposition was significantly higher than that of the others (Table 5.2).

There were large variations in oviposition intervals among individuals, but there were no increasing trends in the oviposition interval with patch time (Figure 5.2).

The giving-up time since the last encounter did not differ between host density 1 and 3, but was shorter when the host density was 9 (Figure 5.3). Within the same host density, the giving-up time tended to be shorter when hosts were aggregated (Log rank test on the survival functions of giving-up time, JMP, SAS, $p < 0.05$).

Table 5.2 Mean ± SE number of attacks and oviposition, attack rate and oviposition rate of *D. semiclausum* on patch infested with different densities and distributions by larval *P. xylostella.* Values followed by different letter in the same column were significantly different ($P < 0.05$, Kruskal-Wallis test).

Host density (distribution)	N	No. attacks	No. ovipositions	Attack rate	Oviposition rate
1	17	1.59 ± 0.47a	1.01 ± 0.31a	6.59 ± 1.93a	4.35 ± 2.52a
3 (uniform)	24	2.54 ± 0.33b	1.29 ± 0.20a	9.01 ± 1.17b	6.43 ± 1.28a
3 (clustered)	22	3.64 ± 0.52b	2.02 ± 0.27b	9.16 ± 1.31b	7.18 ± 1.42a
9 (uniform)	16	7.44 ± 1.01c	4.50 ± 0.68c	11.9 ± 1.62c	7.72 ± 1.13a
9 (clustered)	17	13.8 ± 1.07d	7.47 ± 0.45d	18.6 ± 1.44d	12.0 ± 1.55b

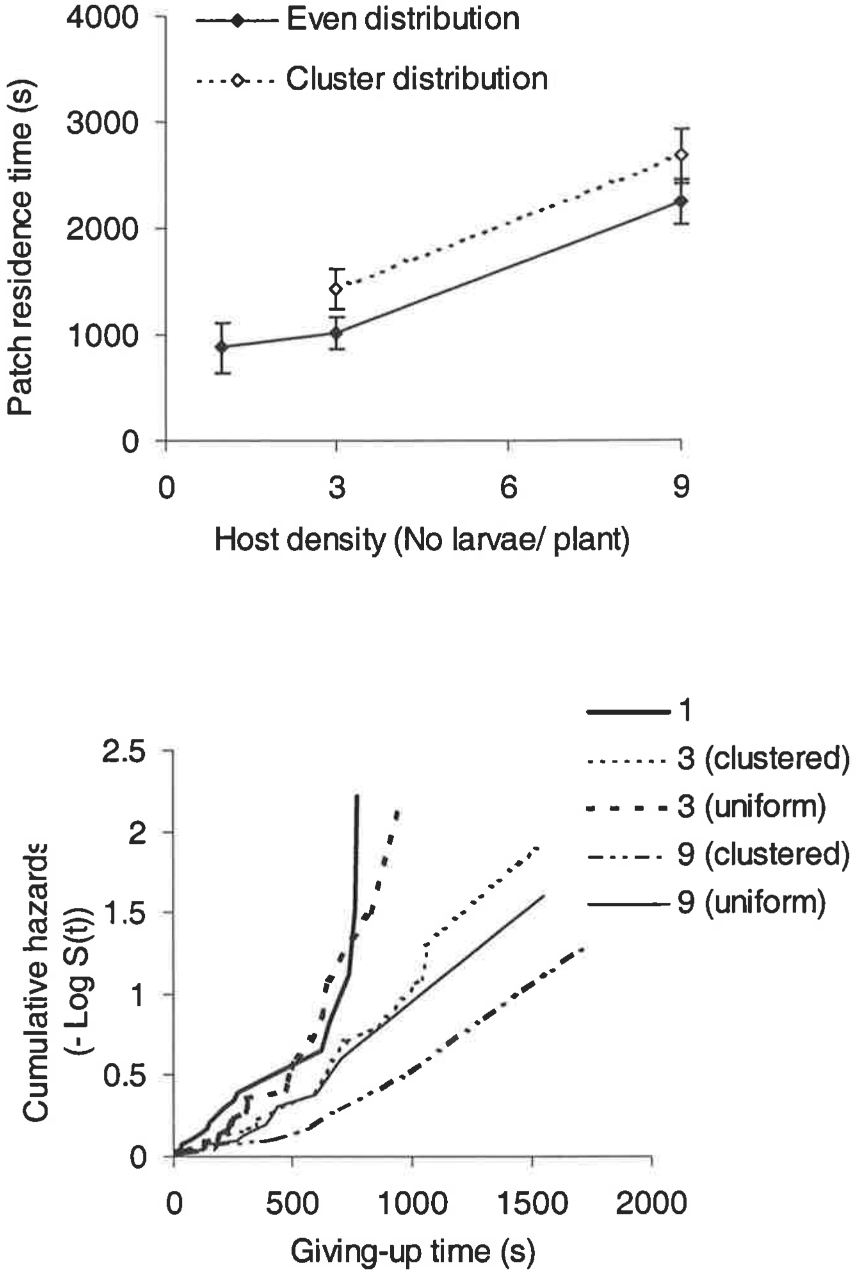


Figure 5.1 (a) Patch residence time (± SE) as a function of the host density and distribution. (b) Negative Log-Survivor curves of giving-up time for each host density and distribution.

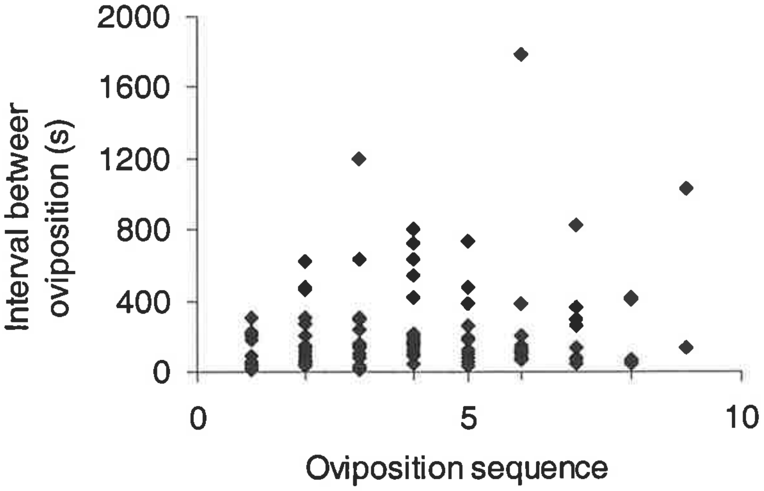


Figure 5.2 Interval between ovipositions, i.e. the time from first oviposition to second oviposition, and between each subsequent oviposition. Host density = 9/ plant (clustered distribution).

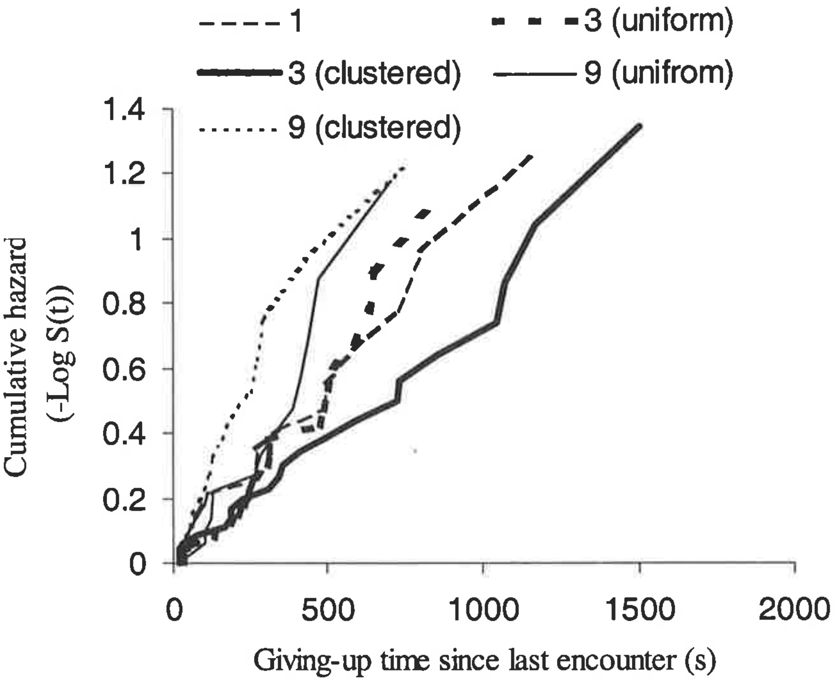


Figure 5.3 Kaplan-Meier survivor functions of giving-up time since the last encounter with host.

The number of parasitised hosts was a function of host density and distribution (Table 5.3). The percent superparasitism was low irrespective of the host density and distribution (Table 5.3).

Table 5.3 Mean ± SE number of *P. xylostella* larvae parasitised by *D. semiclausum* and percentage of superparasitism on patch of different host densities and distributions. Values followed by different letters in the same column were significantly different ($P<0.05$, Kruskal-Wallis test).

Host density (distribution)	N	No. parasitised	% Superparasitism
1	17	0.59 ± 0.12a	0.235
3 (uniform)	24	1.13 ± 0.16b	0.161
3 (clustered)	22	1.68 ± 0.19c	0.136
9 (uniform)	16	3.88 ± 0.49d	0.125
9 (clustered)	17	6.53 ± 0.33e	0.126

5.3.2 *Patch-leaving tendency*

In order to verify the hypothesis that the effect of oviposition on the patch-leaving tendency could depend on the host density and distribution ('switching rules', see introduction), a preliminary analysis was performed to test the data for each different host density and distribution separately. The number of ovipositions consistently increased the patch-leaving tendency irrespective of host density and distribution (Table 5.4). Therefore, in the final model analysis, all the data from different host densities and distributions were pooled, and all the 18 covariates (see Table 5.1) were tested.

Five covariates were fitted into the final model (Table 5.6). The deviance residuals are symmetrically distributed around zero (Figure 5.5), indicating that the final model seems to properly describe the patch-leaving tendency of *D. semiclausum*.

Table 5.4 Estimated effects of number of ovipositions on the patch-leaving tendency of *D. semiclausum* at different host densities and distributions. Single covariate tests while all other covariates were not included in the model.

Host density (distribution)	$\beta \pm SE$	χ^2	*P*
1	0.1293 ± 0.2349	0.3029	0.5820
3 (uniform)	0.6817 ± 0.0987	11.772	0.0006
3 (clustered)	0.5070 ± 0.1707	8.825	0.0030
9 (uniform)	0.2547 ± 0.0990	6.160	0.0131
9 (clustered)	0.6346 ± 0.1593	15.864	0.0001

Table 5.5 Estimated regression coefficients (β), standard errors (SE) and hazard ratios [exp(β)] for the final fitted model that included all the significant covariates affecting the patch-leaving tendency of *D. semiclausum*. χ^2 corresponds to the likelihood ratio tests ($P<0.05$). All of them were estimated with all other significant terms present in the model.

Covariates	β	SE	χ^2	*P*	exp(β)
DEN3C	-0.9810	0.4235	5.366	0.0205	0.375
DEN9E	-1.4426	0.5007	8.299	0.0040	0.236
DEN9C	-1.7180	0.4599	13.957	0.0002	0.179
ENT	-0.9311	0.3549	6.881	0.0087	0.394
COVI	0.0400	0.0149	7.272	0.0071	1.041

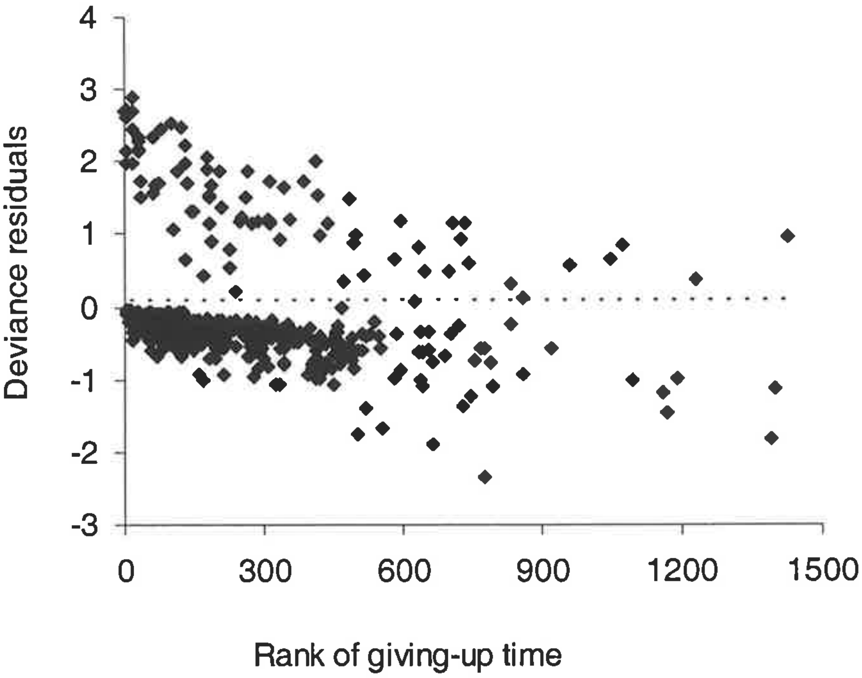


Figure 5.4 Plot of the deviance residuals of the final fitted model with six covariates against the rank order of giving-up time.

The number of ovipositions increased the patch-leaving tendency, while the unsuccessful encounters with hosts decreased the patch-leaving tendency. These results were consistent as reported in Chapter 4. In this analysis, it was found that increasing host density and clustered distribution of the hosts over the plants decreased the patch-leaving tendency (Table 5.4). This was clearly shown when plotting the cumulative hazards (negative Log-survivor) against the giving-up time for each host density and distribution (Figure 5.1b), the curves of host density 1 and 3 (even) were intertwined, suggesting that the parasitoids did not distinguish between the two relatively low host densities. All the curves had a more or less concave shape, implying that the leaving tendency was an increasing function of the time already spent in the patch.

5.4 Discussion

Host density and distribution affected the patch residence time and searching efficiency of *D. semiclausum*. The observed patch residence time of *D. semiclausum* agreed with the general predictions of Charnov's Marginal Value Theorem model. The parasitoid spent more time in better quality patches when inter-patch distance was constant under this experimental conditions (Charnov, 1976b). However, there were no trends showing that oviposition interval increased with patch time elapsed (Figure 5.2), which did not meet the assumption of the Marginal Value Theorem that the oviposition rate should be a diminishing function of continuing past residence time. Thus, the results generally agreed with the predictions of Charnov's model, but for different reasons. Due to host defense and the random occurrence of waiting resulting from unsuccessful encounters (Chapter 2 & 4), there was large variation in the oviposition interval. The cumulative gain function is unlikely to be a good indicator of patch depletion.

None of the simple rules such as fixed patch time, fixed number of ovipositions or fixed giving-up time could explain the observed pattern of patch residence time (Table 5.2, Figure. 5.1 &. 5. 3). The Marginal Value Theorem and these simple rules of thumb do not include the effects of many within-patch foraging experiences such as encounters with hosts, and clearly are not valid in this parasitoid. The host density and distribution, unsuccessful encounters with hosts and numbers of ovipositions significantly influenced the patch-leaving tendency.

The patch-leaving tendency decreased with host density and degree of aggregation. A similar effect of host density on patch time allocation has been also found for other parasitoids searching for patchily distributed hosts (van Lenteren & Bakker, 1978; Waage, 1979; van Alpine & Galis, 1983; van Steenis *et al.*, 1996; Keller & Tenhumberg, 2000). However, the mechanism is unknown. The decreased leaving tendency in patches of abundant and clustered hosts could be the result of the high density-dependent presence of kairomones per unit area (Haccou *et al.*, 1991; Hemerik *et al.*, 1993; Nelason & Roitberg, 1995; Driessen & Bernstein, 1999), or host-associated cues. Thus, a separate test of the effect of kairomone sources on the patch-leaving tendency of *D. semiclausum* is needed. To accomplish this, an additional experiment was conducted to see if the presence of host kairomone alone would affect the patch-leaving tendency in *D. semiclausum* (see Chapter 6).

The effects of unsuccessful encounter and oviposition and oviposition rate on the patch-leaving tendency were consistent with previous findings (Chapter 4). The decrease in the patch-leaving tendency following unsuccessful host encounters could be an adaptive strategy for *D. semiclausum* to overcome the host's defensive behavior (Chapter 2-4). Oviposition consistently increased the patch-leaving tendency, irrespective of the inter-patch distance (Chapter 4), host density and distribution. This confirms that the behaviour of *D. semiclausum* is consistent with a decremental mechanism similar to the 'Count-down' mechanism proposed by Driessen *et al.* (1995) for the parasitoid *V. canescens*. Such a mechanism has been observed in other parasitoid species such as *Cardiochiles nigriceps* (Strand & Vinson, 1982), and *Diaeretiella rape* (Shaltiel & Ayal, 1998), although the results were not obtained through the Cox regression model. However, the proportional hazards model has been used to demonstrate the occurrence of such a Count-down mechanism in the parasitoid species *Encarsia formosa* (van Roermund *et al.*, 1994), *Aphidius colemani* (van Steenis *et al.*, 1996), *V. canescens* (Driessen *et al.*, 1995; Driessen & Bernstein, 1999) and *Telenomus busseolae* (Wajnberg *et al.*, 1999).

From a functional point of view, a Count-down mechanism is often explained as an adaptive strategy to uniform host distribution across patches in the environment (Iwasa *et al.*, 1981) or when host density is low (Driessen *et al.*, 1995). The effects of oviposition on the patch leaving a parasitoid could depend on the host density and distribution. However, Driessen and Bernstein (1999) recently confirmed that the parasitoid *V. canescens* employs the Count-down mechanism irrespective of host density. They argued that the Count-down mechanism should be employed as long as a parasitoid can assess initial patch quality. *D. semiclausum* clearly has the ability to assess patch quality, as demonstrated in this study; the basic leaving tendency decreased with increased host density (Figure 5.1 b). Also it was observed that the parasitoid was more likely to land on the infested plant with the highest host density when it first encounters a group of host plants (Chapter 2). However, it is not known how well a parasitoid can initially distinguish the quality of patches. The kairomone concentration may not be a reliable indicator of host availability, so relying on a Count-down mechanism could make a parasitoid inefficient if there was no strong linkage between kairomone concentration and host availability. The best strategy for a parasitoid that can assess initial patch quality may be to set an expected patch residence time as assumed by Waage's model (1979), but then to use other cues to adjust the 'Count-down' mechanism according to the within-patch foraging experience.

Factors such as an encounter with a host parasitised by other species and superparasitism may also influence the patch time allocation and patch-leaving tendency in *D. semiclausum*. These factors are considered in the next chapter.

Chapter 6 Patch time allocation by *D. semiclausum*: effects of kairomone sources and previous parasitism

6.1 Introduction

Patch time allocation is of utmost importance in determining parasitoid foraging success, particularly for time-limited parasitoids (Godfray, 1994). In the preceding chapters, the effects of inter-patch distance, host density and distribution on the patch residence time and patch-leaving tendency of *D. semiclausum* were investigated. The results showed that successful oviposition, unsuccessful encounters with hosts, host density and distribution, inter-patch distance and recent oviposition rate all influenced the patch-leaving tendency (Chapter 4 & 5). This chapter focuses on investigation of the possible effects of kairomone sources and previous parasitism on patch residence time and the patch-leaving tendency of the parasitoid.

Of the factors influencing the patch-leaving decisions in many parasitoids, the presence of kairomones and intra-experience such as encounters with parasitised hosts might be used to assess patch profitability (e.g. Waage, 1979; Hemerik *et al.*, 1993; Driessen *et al.*, 1995). Kairomone concentration is assumed to be used by some parasitoids to set up a basic tendency to leave a patch (Waage, 1979; Driessen *et al.*, 1995). Many parasitoids do respond to patch odor (e.g. Waage, 1978; Strand & Vinson, 1982; van Alphen & Galis, 1983), and patch residence time is influenced by the presence of host-associated kairomones in some species (Nelson & Roitberg, 1995; Shaltiel & Ayal, 1998; Driessen & Bernstein, 1999).

Kairomone concentration might not be a reliable measure of host quality. Parasitoids with limited information on patch quality might use the other cues such as intra-patch experience to adjust their patch-leaving tendency. Host encounters may play an additional role as they gradually provide the parasitoids with information on patch quality. Parasitoids foraging within a patch may re-encounter hosts parasitised by the female herself, a conspecific or other species. The risk of superparasitism could increase with patch residence time. Many parasitoids demonstrated increased probabilities of leaving a patch upon detection of previously parasitised hosts (van Lenteren, 1981, 1991; van Alphen & Vet, 1986; Hemerik *et al.*, 1993; Rosenheim & Mangle, 1994; Wajnberg *et al.*, 1999).

In this chapter, the foraging behaviour of *D. semiclausum* was directly observed in a wind tunnel, where a single wasp was released onto an experimental plant without host and host damage, with host damage only, or with hosts previously parasitised by the female herself or a female of *C. plutellae*. The female wasp was allowed to freely leave the 'patch', by placing an extra plant upwind of the experimental plant. The effects of kairomone presence, previous parasitism and within patch foraging experience on the patch-leaving tendency of *D. semiclausum* were investigated by means of the proportional hazards model. The results, together with those in the previous two chapters, give a detailed insight into the decision-making processes of *D. semiclausum* in patch leaving decisions.

6.2 Materials and methods

6.2.1 *Insects, plant and experimental set up*

The insect culture, parasitoid handling, host plant and experimental set-up used in this experiment were same as in Chapter 4. In this experiment, the extra plant was placed 60 cm upwind of the experimental plant. Two types of experiments were conducted. Experiment 1 was designed to test the effects of kairomone resources on the patch residence time, and consisted of following three treatments:

(1) The experimental plant was clean, without host and host damage.

(2) The experimental plant was infested by three larval *P. xylostella*, the diamondback moth (DBM), one day before the experiments by placing one larva on each of three randomly selected leaves. In order to restrict the larval movement, a paper collar was

wrapped around the stem of the infested leaf. Just prior to the experiment, the larvae were removed. Thus, the experimental plant only contained host damage.

(3) The experimental plant was prepared as (2), but the larvae were not removed. Thus, the experimental plant contained both hosts and their damage.

Experiment 2 was designed to test the effects of previous parasitism on the patch residence time and patch-leaving tendency, and consisted of following three treatments:

(1) The experimental plant was infested by three unparasitised larval DBM one day before the experiments by placing one larva on each of three randomly selected leaves. In order to restrict the larvae movement, a paper collar was also wrapped around the stem of the infested leaf.

(2) As (1), but the three larval DBM were parasitised by the female herself just before being placed on the experimental plant.

(3) As (1), but the three larval DBM were parasitised by a *C. plutellae* female just before being placed on the experimental plant.

In both experiments, the extra plants were also infested one day before the experiments by three unparasitised larval DBM. In the morning immediately prior to the experiments, each leaf was examined to make sure the required number of hosts was present.

The experimental procedures were the same as given in Chapter 4. Each treatment of Experiment 1 was replicated for 19-24 times and of Experiment 20-25 times.

6.2.2 *The proportional hazards model*

Covariate selection is a critical step in the analysis of patch-leaving tendency of insect parasitoids by means of the proportional hazard model (Chapter 4). In the previous studies, six factors were found important in patch-leaving decision of *D. semiclausum*: inter-patch distance, unsuccessful encounter, number of ovipositions, rate of oviposition, host density and host distribution. This study was similar to the previous studies (Chapter 4-5). The difference was that the experimental patch was presented with hosts of different quality. Other aspects of within-patch experience such as encounter with parasitised hosts and presence of kairomones were considered to be potentially significant in patch-leaving decisions of parasitoids (Waage, 1979; van Lenteren, 1991; Hemerik *et al.,* 1993; Driessen *et al.*, 1995; Nelson & Roitberg, 1995; van Alphen & Jervis, 1996). Thus, in addition, the presence of damage and presence of hosts and damage were considered as new covariates in Experiment 1 (Table 6.1). In experiment 2, three potentially important factors found in other studies such as encounters with parasitised hosts, superparasitism and rejection of parasitised hosts (van Lenteren, 1991; Nelson & Roitberg, 1995; van Alphen & Jervis, 1996; Wajnberg *et al.*, 1999) were tested (Table 6.2).

Table 6.1 Explanatory covariates selected for analyzing the patch-leaving tendency of *D. semiclausum* by means of the proportional hazards model (Experiment 1)

Description of the covariates	Code
Is the plant presented with hosts and damage? (HOST)	Yes = 0, No = 1
Does the plant only contain host damage? (DAM)	Yes = 0, No = 1
Was the previous encounter unsuccessful? (ENT)	Yes = 0, No = 1
Did the previous encounter involve an oviposition? (OVI)	Yes = 0, No = 1
Cumulative number of ovipositions (COVI)	Observed value
Rate of last oviposition (RATE)	Observed value

As in Chapter 4, in this study, the renewal points were when the wasp firstly arrived on the experimental plant and each subsequent encounter with a host including unsuccessful and successful encounters, i.e. oviposition. The basic leaving tendency is reset after such renewal points. The iterative regression method was applied to the analysis of the influence of the selected covariates on the patch-leaving tendency of the parasitoid (Chapter 4). All analysis was conducted with PHREG program of SAS software package (Version 6.0, Allison, 1997).

Table 6.2 Explanatory covariates selected for analyzing the patch-leaving tendency of *D. semiclausum* by means of the proportional hazards model (Experiment 2)

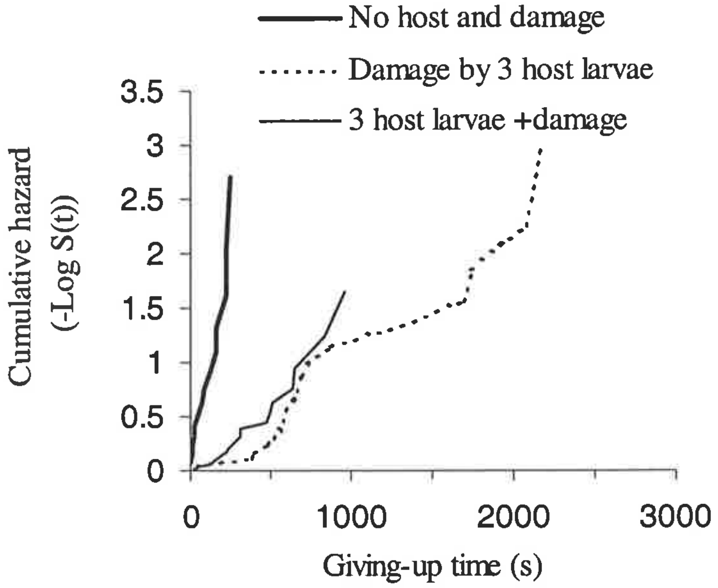
Description of the covariates	Code
Was the previous encounter unsuccessful? (ENT)	Yes = 0, No = 1
Did the previous encounter involve an oviposition? (OVI)	Yes = 0, No = 1
Did the previous encounter involve superparasitism? (SSP)	Yes = 0, No = 1
Did the previous encounter involve multiparasitism? (CSP)	Yes = 0, No = 1
Was a parasitised rejected at the last encounter? (REJ)	Yes = 0, No = 1
Cumulative number of ovipositions (COVI)	Observed value
Rate of last oviposition (RATE)	Observed value

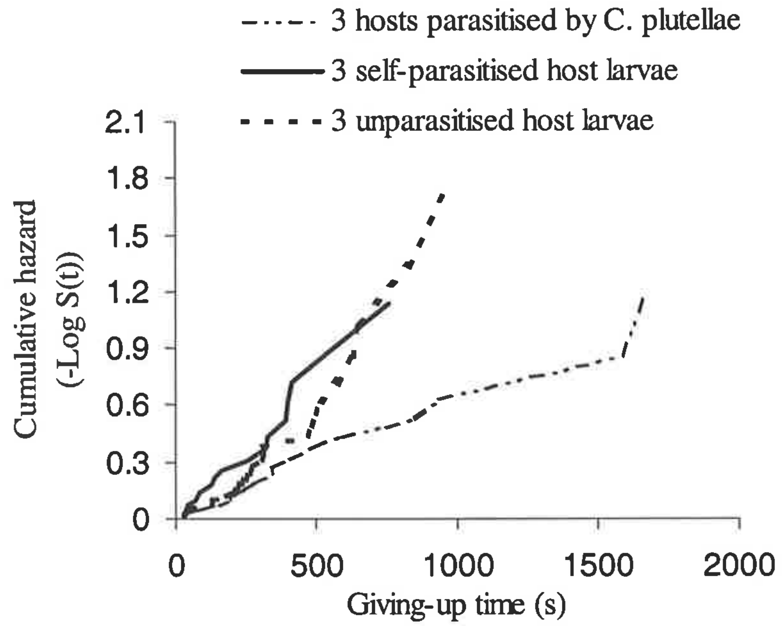
6.3 Results

6.3.1 *Patch time allocation*

In Experiment 1, the mean (± SE) residence times on plants without hosts and damage, containing host damage only, and presence of both hosts and their damage were 103 ± 29 s (n = 20), 1015 ± 153s (n = 24) and 977 ± 161 s (n = 19), respectively. The presence of host damage, or both hosts and damage significantly increased the patch residence time (long rank test on the survival function, JMP, SAS, $P < 0.001$). When the wasp landed on the patches without hosts and damage, they left quickly. In five cases, the parasitoids flew directly to the extra plant when presented with patches without hosts and damage. The cumulative hazards were approximately a straight line over time on the empty patch (Figure 6.1). This suggested that the parasitoids left such empty patches randomly. There was no significant difference in the patch residence time between the patches from which the hosts were removed just before

Figure 6.1 Negative Log-Survivor curves of the giving-up time by *D. semiclausum* on patches infested with larval *P. xylostella*. Top: Experiment 1. Bottom: Experiment 2.





the experiments and the patches containing hosts (long rank test on the survival function, JMP, SAS, $P > 0.05$).

In Experiment 2, the patch residence time significantly increased when the parasitoid searched on the patch containing hosts all previously parasitised by *C. plutellae* (1656 ± 291, n = 20), compared with the patches containing unparasitised hosts (978 ± 151, n = 25) or hosts parasitised previously by the female herself (841 ± 100, n = 21) (long rank test on the survival function, JMP, SAS, $P < 0.001$). However, there was no significant difference in the patch residence time between the patches containing unparasitised hosts and containing hosts parasitised previously by the female herself (long rank test on the survival function, JMP, SAS, $P > 0.05$).

The parasitoids readily superparasitised after being released on the patches that contained only parasitised hosts (Table 6.3). Multiparasitism (28 / 29) and self-superparasitism (18 / 22) were all common. The mean (± SE) number of attacks, stings and ovipositions were higher on the patches containing hosts previously parasitised by *C. plutellae* than the patches infested either with unparasitised hosts or hosts previously parasitised by the female herself (Table 6.3). However, the oviposition rate was not significantly different among treatments.

Table 6.3 Mean (± 1 SE) number of attacks and ovipositions by *D. semiclausum* on patches infested with different types of host (Experiment 2). Values followed by the same letter in a column were not significantly different (Kruskal-Wallis test, $p > 0.05$).

Host type	N	No. attacks	No. stings	No. ovipositions	Oviposition rate
Unparasitised	25	2.48 ± 0.32a	1.40 ±0.22a	1.28 ±0.19a	8.14 ± .75a
Self-parasitised	21	2.53 ± 0.35a	1.38 ±0.16a	1.19 ±0.15a	10.5 ± .03a
Parasitised by *C. plutellae*	20	3.40 ± 0.39b	2.0 ± 0.27 b	1.83 ± 0.24b	6.34 ±1.18a

6.3.2 *Patch-leaving tendency*

The initial leaving tendency was random when the patch was empty (Figure 6.1). The parasitoids had a basic tendency to remain on the patches with host damage as the first derivatives of the cumulative hazards curves at $t = 0$ were different from zero, and the cumulative leaving tendency increased exponentially with the time spent in the patch (Figure 6.1). As expected, in both experiments the effects of the oviposition and unsuccessful encounter with host on the patch leaving tendency of *D. semiclausum* were consistent with the experiments reported in Chapter 4 & 5 (Table 6.4). In addition, the presence of host damage in Experiment 1 significantly decreased the patch leaving tendency (Figure 6.1), and 3 covariates were significant when the final model was fitted (Table 6.6; Figure 6.2).

In Experiment 2, the effects of the oviposition, unsuccessful encounter and recent oviposition rate on the patch leaving tendency of *D. semiclausum* were consistent with the experiments reported in Chapter 4 & 5 (Table 6.5). In addition, rejection of parasitised hosts and self-superparasitism increased the patch leaving tendency, and 5 covariates were significant when the final model was fitted (Table 6.7; Figure 6.2) The leaving tendency significantly decreased on the patch containing hosts all previously parasitised by *C. plutellae* (Figure 6.1).

6.4 Discussion

This study confirmed that the presence of host damage strongly decrease the patch-leaving tendency of *D. semiclausum*. Thus, the decreased leaving tendency on patches of high host density, as shown in last chapter, could be associated with the density-dependent presence of kairomones. Similar effects of the presence of kairomones on patch-leaving tendency have been extensively demonstrated in other parasitoids (Haccou *et al.*, 1991; Hemerik *et al.*, 1993;

van Roermund, 1994; Nelason & Roitberg, 1995; van Steenis *et al.*, 1996; Shaltiel & Ayal, 1998; Driessen & Bernstein, 1999; Vos *et al.*, 1998).

Table 6.4 Significance tests and the estimated coefficients of the covariates (Experiment 1)

Covariates	β	SE	Wald test	*P* (d.f.)
A. Joint test (all)			46.07	0.0001 (6)
B. Single test				
HOST	-0.5398	0.2787	3.7507	0.0528
DAM	-2.0007	0.3221	38.573	0.0001
ENT	-0.8334	0.3826	4.7445	0.0294
OVI	0.0810	0.3229	0.0629	0.8019
COVI	0.2376	0.1594	2.2235	0.1359
RATE	6.6887	4.7091	2.0174	0.1555

Table 6.5 Significance tests and the estimated coefficients of the covariates (Experiment 2)

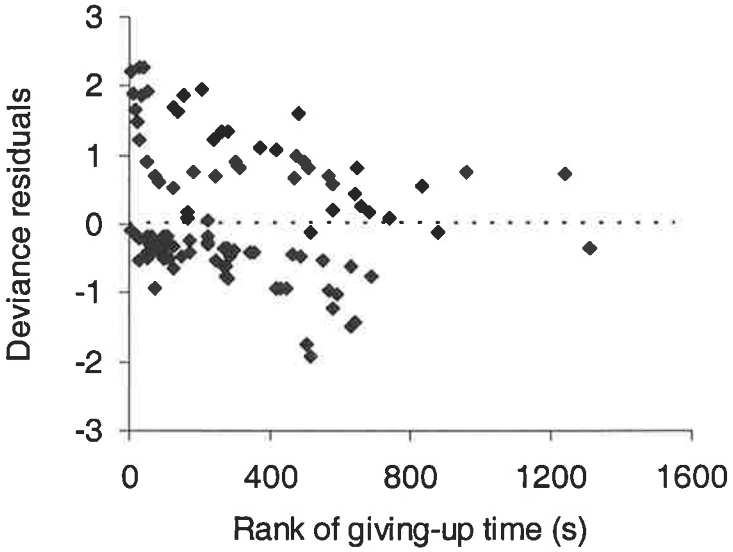
Covariates	β	SE	Wald test	*P* (d.f.)
A. Joint test (all)			45.90	0.0001 (7)
B. Single test				
ENT	-0.9344	0.2905	10.3437	0.0013
OVI	0.5003	0.2856	3.0689	0.0798
SSP	1.0995	0.2924	14.1394	0.0002
CSP	-0.0329	0.3602	0.0084	0.9272
REJ	1.6442	0.4770	11.8779	0.0006
COVI	0.4774	0.1393	11.7505	0.0006
RATE	8.9244	3.6584	5.9508	0.0147

Table 6.6 Estimated regression coefficients (β), standard errors (SE) and hazard ratios [exp(β)] for the final fitted model that included all the significant covariates affecting the patch-leaving tendency of *D. semiclausum*. χ^2 corresponds to the likelihood ratio tests ($P < 0.05$). All of them were estimated with all other significant terms present in the model.

Covariates	β	SE	χ^2	p	exp(β)
DAM	-2.3386	0.3581	42.65	0.0001	0.096
ENT	-1.0735	0.4019	7.13	0.0079	0.342
COVI	0.4877	0.1639	8.85	0.0029	1.629

Table 6.7 Estimated regression coefficients (β), standard errors (SE) and hazard ratios [exp(β)] for the final fitted model that included all the significant covariates affecting the patch-leaving tendency of *D. semiclausum*. χ^2 corresponds to the likelihood ratio tests ($P < 0.05$). All of them were estimated with all other significant terms present in the model.

Covariates	β	SE	χ^2	p	exp(β)
ENT	-0.5450	0.3104	3.0819	0.0791	0.580
SSP	1.0796	0.3189	11.459	0.0007	2.943
REJ	1.7908	0.4915	13.278	0.0003	5.995
COVI	0.3896	0.1335	8.4004	0.0038	1.472
RATE	9.4976	3.9141	5.8878	0.0152	13327



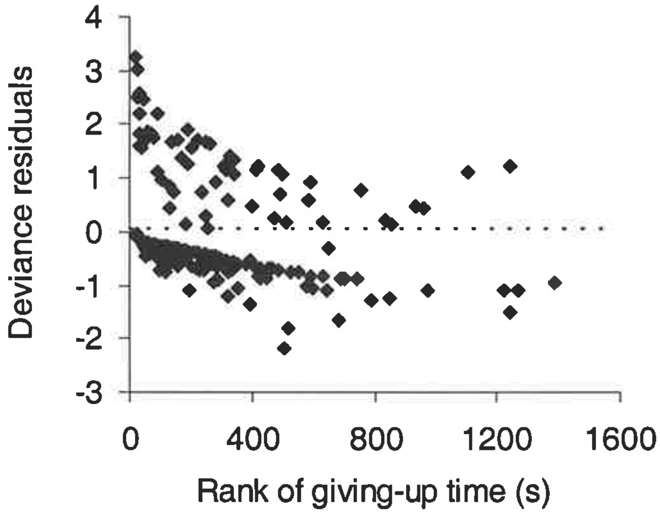


Figure 6.2 Plot of the deviance residuals of the final fitted model with six covariates against the rank order of GUT. Top: Experiment 1; Bottom: Experiment 2.

Again, the effects of oviposition, unsuccessful encounters with hosts and oviposition rate on the patch-leaving tendency were consistent with previous findings (Chapter 4-5). Because the effects of oviposition and unsuccessful encounters on the patch-leaving tendency in *D. semiclausum* are opposite, the presence of hosts did not increase patch time overall, compared with that on the patches with host damage only. About half of the attacks on unparasitised hosts were unsuccessful (Table 6.3). Thus, the two opposing mechanisms balanced out the effects of host presence on the patch residence time.

Both self-superparasitism and multiparasitism were common in Experiment 2. In this experimental set-up, all the hosts were previously parasitised. Superparasitism may be favored by the parasitoid, as there is a finite chance of the superparasitism progeny out-competing the other progeny (Van Alphen & Visser, 1990; Sirot, 1996; Scott *et al.*, 1997; Yamada & Miyamoto, 1998, see chapter 3). However, self-superparasitism is associated with a lower fitness gain compared to multiparasitism (van Alphen & Visser, 1990), and, therefore, should increase the patch-leaving tendency (Rosenheim &Mangel, 1994). Such an increase was observed in this experiment (Table 6.7).

Each host rejection also led to a significant increase in the patch-leaving tendency. The rejection of a parasitised host provides the female with some information regarding the decreasing value of the patch she is currently exploiting (van Alphen & Vet, 1986; van Lenteren, 1991; van Alphen, 1993). The adaptive value of such a mechanism is self-evident. This was also observed in *T. busseloae* (Wajnberg *et al.*, 1999). Recent oviposition rate also increased the patch-leaving tendency. This would allow the 'Count-down' mechanism to better track the host availability. With parasitoids of larval *Drosophila*, a similar result was obtained with *Leptopilina heterotoma* (Haccou *et al.*, 1991), whereas in *L. clavipes*, the instantaneous oviposition rate did not have a significant effect on the patch-leaving tendency. Clearly, selection may drive species to adopt different strategies to exploit the same host, perhaps as a consequence of competition interactions.

The patch-leaving tendency has been reported in eight different parasitoids by means of the proportional hazards model: *Leptopilina heterotoma* (Haccou *et al.*, 1991), *L. clavipes* (Hemerik *et al.*, 1993), *Encarsia formosa* (van Roermund *et al.*, 1994), *Aphidius colemani* (van Steenis *et al.*, 1996), *Cotesia rubecula* (Vos *et al.*, 1998; Keller & Tenhumberg, 2000), *C. glomerata* (Vos *et al.*, 1998), *Venturia canescens* (Driessen & Bernstein, 1999), and *Telenomus busseolae* (Wajnberg *et al.*, 1999). However, none of previous studies has comprehensively investigated the effects of various covariates on the patch-leaving tendency of a parasitoid. This chapter, together with the previous two chapters, provides a closer examination of the factors that could influence the patch-leaving tendency in *D. semiclausum.*

Based on current studies of *D. semiclausum*, two points should be highlighted. First, patch residence time of parasitoids is determined in a more complex way than described by simple models. Many factors could affect the patch-leaving decisions and it may impossible with some experiments to separate the effects of all the different factors. The experiments to evaluate a *priori* rules of 'thumb' or behavioral mechanism models (Wage, 1979; Stephens & Krebs, 1986; Driessen *et al.*, 1991) have been performed under closely defined environmental settings, and cannot be used to match the observed data to behavior in more realistic setting. Due to the stochastic occurrence of some behavioral events and the complexity of patch behaviour, statistical modeling should be used to derive behavioral rules (Haccou *et al.*, 1991; van Alpine, 1993, Hemerik *et al.*, 1993; Keller & Tenhumberg, 2000; Chapter 4-5). Second, it should be noted that the statistical method should be considered as an extension to the usual *a priori* modeling. On the one hand, *a prior* consideration is used to determine which covariates are potentially relevant. On the other hand, the results of such an analysis give important directions for further testing or the development of more realistic behavioral models. The statistical method can only provide a test of the relative importance of factors influencing the patch-leaving tendency under particular conditions. While the importance of some covariates and their effects may be consistent under different conditions, some factors could cease to be important when the environment has changed. Thus, the general context of this work is the understanding of decision-making by a foraging parasitoid from both the functional and mechanistic point of view, rather than absolute predictions of parasitoid behaviour.

A sequential conceptual model of patch exploitation by *D. semiclausum* can be derived from the results of all the proportional hazards analyses.

1. Upon arriving at an empty patch without hosts and host damage, a parasitoid should leave randomly, *i.e.*, the observed patch time follows an exponential distribution.
2. Upon arriving at a patch containing host kairomones, the parasitoid has a basic leaving tendency', which is an increasing function of the time already spent on the patch. The parasitoid has the ability to estimate the initial patch quality in relation to the kairomone concentration. However, such estimation is obviously not reliable, as the kairomone concentration is only a relative estimate of host density rather than host availability or quality. Subsequent foraging experience within the patch would indicate host availability more reliably, and enable the parasitoid to track the degree of patch exploitation.
3. The basic leaving tendency decreases with increasing host density or clustered distribution of hosts over a patch.
4. The leaving tendency further decreases with increasing inter-patch distance
5. There is a strong decrease in leaving tendency when the encounter with a host is not successful.
6. A successful oviposition experience increases the patch-leaving tendency.

7. The leaving tendency further increases when the ovipositions occur in rapid succession.
8. There is an increased leaving tendency when a parasitoid rejects a parasitised host or self-superparasitism.

Obviously, the patch-leaving decisions in parasitoids adjust continually according to 'good' and 'bad' experiences (Vos et al., 1998). Potential effects of experience or other factors on the patch-leaving tendency of the parasitoid such as egg load, age and encounters with conspecific females, could be included into the above model.

This model can be used to compare the performance of complex and simple decision rules under different conditions. In general, the model agrees with the predictions of Charnov's Marginal Value Theorem in terms of the effects of host density and inter-patch distance on the patch time allocation, but for different reasons (Chapter 4-5). It also agrees with the predictions of Driessen's model regarding the effect of oviposition on the patch-leaving tendency, and Waage's model with respect to the effect of kairomone concentration on the basic leaving tendency. The Count-down mechanism is used by *D. semiclausum*, irrespective of the host density and distribution (Driessen & Bernstein, 1999). There was no switching in the effects of the covariates under varying experimental conditions.

So far, the patch-leaving tendency of *D. semiclausum* has been investigated within relatively simple patches (Chapters 4-5 and this chapter). The solitary wasps were foraging on a single plant that had not been visited before. As the experimental plant and extra plant were reasonably separate, the 'patch boundary' was presumed to be distinctive. Thus, the single plant was presumed to be a 'patch'. In this situation, patch-leaving decisions are determined mainly by initial patch quality assessment and intra-patch experience. It is likely that many other factors such as inter-patch experience may also influence the leaving tendency on current patch (Waage, 1979; van Steenis *et al.*, 1996; Bernstein & Driessen, 1996; Vos *et al.*, 1998; Keller & Tenhumberg, 2000). It is also possible that in a more complicated environment such as a multi-plant environment, the parasitoid may 'perceive' the environment as a spatially hierarchical patch structure, and use different rules to leave at particular patch levels. These questions are addressed in the next chapter.

Chapter 7 Patch leaving decisions by *D. semiclausum* in a hierarchical patch system

7.1 Introduction

Foraging theory began with a recognition of the importance of the patchy distribution of resources (MacArthur & Pianka, 1966), and the development of theoretical models that addressed the optimal exploitation of patches by foragers (Fretwell & Lucas, 1970; Charnov, 1976b). One central question is how does a forager decide to leave a given patch, in order to maximize its lifetime resource harvest rate? A number of patch-leaving models in animals have been proposed (see Stephens & Krebs, 1986; Godfray, 1994; for a review). Previous studies on patch-leaving decisions in insect parasitoids have been carried out in simple patch-level experiments where an experimental 'patch boundary' is usually distinctive from the surrounding environment (Waage, 1978; Haccou *et al.*, 1991; Hemerick *et al.*, 1993; Driessen *et al.*, 1995; Driessen & Bernstein, 1999). However, when experiments were conducted in a multi-patch environment, some studies have been based on simple assumptions about the parasitoid's perception of 'patchiness' (Waage, 1979; van Steenis *et al.*, 1996; Vos *et al.*, 1998), without paying much attention to the scale at which the 'patch boundary' could really occur (Ayal, 1987; Rosenheim *et al.*, 1989; Kotliar & Wiens, 1990; Keller & Tenhumberg, 2000). Studies restricted to different spatial scales may yield different results, depending on which scale is selected for studies (Kotliar & Wiens, 1990; Keller & Tenhumberg, 2000). Thus, it is necessary to describe how foraging decisions may change at different spatial scales within the foraging environment. This Chapter addresses the importance of spatial scales for elucidating the patch-leaving decisions of *D. semiclausum*.

The foraging environment of insect parasitoids is usually divided into three hierarchical levels: the habitat, the patch, and the host (Hassell & Southwood, 1978). While the borders of the habitat and the host can be clearly defined, a general difficulty is to determine the boundary of the 'patch' that reflects the parasitoid's rather than the investigator's own perception of 'patchiness' (Rosenheim *et al.*, 1989). Hassell and Southwood (1978) defined an aggregation of hosts as a 'patch'. Their definition emphasized the existence of the hierarchical levels of a foraging environment, and patch is a spatial sub-unit of the foraging environment in which an aggregation of hosts occurs. Waage (1978) defined a host patch as a physical area that contains an arresting stimulus to parasitoids, and elicits a unique behavioural change in the parasitoids when the border of the 'patch' is crossed. His definition emphasized the hierarchy of patchiness. Movement between the levels of patchiness requires a different type of orientation than movement within a level.

Ayal (1987) proposed the concept of 'elementary unit of foraging' (EUF) based on empirical observation of the foraging behaviour of the aphid parasitoid *Diaeretiella rapae* on a cabbage plant. While foraging within the EUF, in this case a cabbage plant, the parasitoid performs a directed and predetermined foraging behavior. Searching behavior follows an evolutionarily predetermined strategy within the EUF. From an evolutionary point of view, it is expected that many parasitoids have been selected to use the particular architecture of their host plants to direct their search for hosts. Thus, the EUF is considered as a behavioural functional unit on which the different behavioral responses can be commonly observed and are combined into a foraging strategy. The EUF resembles the basic features of the patch concept of Hassell & Southwood (1978) and the lower patch level of Waage (1979).

The patch definition in many previous studies obviously took on a somewhat arbitrary meaning. Indeed, many studies have generally resorted to a arbitrary choice of patch boundary (Waage, 1979; Heads & Lawton, 1983; Rosenheim *et al.*, 1989; Nealis, 1990; Wiskerke & Vet, 1994; Vos *et al.*, 1998). In such simple patch-level experiments, a foraging arena such as a host plant or a Petri dish containing a host substrate provided a discrete and internally homogeneous surface area differing from its surroundings, and thus was reasonably presumed to be a 'patch' , albeit an artificial patch (Haccou *et al.*, 1991; Hemerik *et al.*, 1993; van Roermund *et al.*, 1994; Driessen *et al.,* 1995; van Steenis *et al*, 1996; Driessen & Bernstein, 1999; Wajnberg *et al.*, 1999, Chapter 4-6). Furthermore, these empirical studies aimed to investigate the effects of the initial 'patch' quality and intra-patch foraging experience on the tendency of the parasitoids to leave the closely defined foraging area. Undoubtedly, these studies have been very useful in exploring parasitoid's responses to the particular patch systems. Thus, the simple assumptions about patchiness, although arbitrary, may not be a major issue.

However, in a natural environment, individual parasitoids may forage on many plants and leaves in their lifetime. In such an environment, a simple assumption about the parasitoid's perception of 'patchiness' may not be true. It may change with the pattern of resource aggregation within the foraging environment, the topography of the host plants, or the parasitoids' ability to assess host quality and spatial distribution. Simple definitions of patch levels may not exist. Instead, heterogeneity among individual leaves, plants, or groups of plants in the field often occurs. One may readily identify the field as a habitat, but at least two levels of 'patchiness' occur within this. At one level, there is the plant and at the other the individual leaves upon which the hosts aggregate. Any discussion of the patch-leaving decisions could be complicated by the distinction of the patch boundary, because a parasitoid's patch-leaving decision at a particular level changes with her experience obtained from the surrounding environment (Vos *et al.,* 1998; Keller & Tenhumberg, 2000). As predicted by the Marginal Value Theorem model, the current patch-leaving decision depends on the mean host availability in the surrounding environment (Charnov, 1976b). Further, a greater understanding of the foraging behaviour of a parasitoid may need to be derived from studies of how the parasitoid responds to multiple scales of spatial heterogeneity in its environment (Kotliar & Wiens, 1990;

Fauchald, 1999; Keller & Tenhumberg, 2000). A forager may adopt different foraging strategies at different spatial levels (Ives *et al.*, 1993).

In this chapter, the leaving tendency of *D. semiclausum* is examined at three different spatial levels: a leaf, a plant and a group of plants, by means of the proportional hazards model. Observations of the foraging behaviour of *D. semiclausum* in a group of host-infested plants were presented in Chapter 2.

Optimal foraging models and simple behavioral mechanism models are based on a few assumptions about a parasitoids' ability to assess initial patch quality and the information available to the foraging parasitoid (Charnov, 1976b; Waage, 1979; Driessen *et al.*; 1995). The application of these models to complex environments is limited in the light of the complexity of behavioural cues (van Alphen & Visser, 1990; van Lenteren, 1991; Rosenheim & Mangel, 1994; Nelson & Roitberg, 1995; van Alphen & Jervis, 1996, Chapter 4-6). In order to compare the results of the statistical analysis with the general predictions of the Marginal Value Theorem (Charnov, 1976b), and Waage's (1979) and Driessen's (1995) models, data collected on the first leaf or plant on which the parasitoid landed were analyzed separately.

7.2 Materials and methods

7.2.1 *Experiment*

This analysis used the behavioral data collected in Chapter 2. Here the methods are summarized briefly. The behaviour of *D. semiclausum* was observed in an open wind tunnel as individual wasps foraged among four plants infested with 0, 1, 2 or 4 larval DBM that were placed randomly into 2 x 2 grid (see Chapter 2, Figure. 2.1). Upwind of the experimental plants, two extra plants each infested with 4 larval DBM was placed to provide alternative landing sites when the wasps left the experimental plants. Wasps were released downwind of the experimental plot. Once the wasp flew off the release vial, the foraging behaviour including each encounter with host and sting, and the location and residence time on each leaf were continuously recorded with the event recorder. An observation was terminated when the wasp left the experimental plot and landed on the outside of experimental arena or on the extra plants. All the larvae that were stung were collected individually and dissected to determine the number of ovipositions. The patch-leaving tendency was analyzed by mean of the Cox's proportional hazards model (Cox, 1972).

7.2.2 *'Patch' definition, covariate selection and coding*

Although the parasitoid itself defines the patch, it is probably impossible to determine if the parasitoid perceives a leaf, a plant or a group of plants as a 'patch' in this experimental set-up. Therefore, it is important to examine the parasitoid's behaviour at each spatial level. Hence, the data were analyzed to see how the three different spatial scales might influence the leaving tendency of wasps, rather than to define arbitrarily the real patch boundaries.

Covariate selection is a critical step in the proportional hazards model analysis (Chapter 4). From the previous simple patch-level experiments, factors found to have a significant effect on the patch-leaving tendency of *D. semiclausum* were the presence of host damage or hosts, host density and distribution, unsuccessful encounters with hosts, oviposition, the recent oviposition rate, rejection of a parasitised host, self-superparasitism and inter-patch distance (Chapter 4-6). These factors associated with intra-patch foraging experience were included in the analysis of the leaving tendency at the different spatial levels, except that rejection of parasitised hosts and superparasitism were not considered, because superparasitism rarely occurred in this experimental set-up (Chapter 2). Thus, the basic features of the selected covariates associated with intra-patch experiences were similar across the spatial levels. Additionally, inter-patch experience during previous patch visits and the information obtained from other levels were also considered. These covariates changed according to the spatial level (Table 7.1.

Table 7.1 Covariates selected and tested for the effects on the patch-leaving tendency on *D. semiclausum* at 3 different spatial levels in the multi host plant environment

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At leaf level :

1. Host density of current leaf
2. Is this the first-landed leaf?
3. Presence of damage on the current leaf?
4. Ovipositions during the current leaf visit?
5. Recent oviposition rate
6. Unsuccessful encounters with host during the current leaf visit?
7. Cumulative number of previous visits to the current leaf
8. Host density of the plant
9. Cumulative number of clean leaves visited
10. Cumulative number of infested leaves visited
11. Cumulative number of ovipositions

At a plant level:

1. Host density of current plant
2. Is this the first-landed plant?
3. Cumulative number of ovipositions during the current plant visit
4. Recent oviposition rate
5. Cumulative number of unsuccessful encounters with hosts during the current plant visit
6. Cumulative number of previous visits to the current plant
7. Cumulative number of ovipositions on the current plant during previous visits
8. Cumulative number of unsuccessful encounters with hosts on the current plant during previous visits
9. Total number of unsuccessful encounters
10. Total number of ovipositions

At the level of a group of plants:

1. Host density of the first-landed plant
2. Cumulative number of ovipositions
3. Cumulative number of unsuccessful encounter with hosts
4. Recent oviposition rate
5. Cumulative number of clean leaves visited
6 Cumulative number of infested leaves visited
7. Cumulative number of plant visited

Binary covariates were coded as 1 (yes) or 0 (no). For those covariates with an observed value, first the frequency distribution of the observed value was analyzed, and then the covariates were coded based on their relative frequency distribution. For example, the cumulative number of infested leaves visited ranged from 0 to 14 (see Table 7.2). The observed frequencies of visits to infested leaves decreased as the overall number increased; observations of more than 5 visits infested leaves were coded as 5 to reduce the possible effects caused by inappropriate coding due to small sample sizes.

Table 7.2 An example of covariate coding: the cumulative number of infested leaves visited (see Table 7.1)

Number of. infested leaves visited	Frequency	Coding value	Frequency
0	61	0	61
1	40	1	40
2	22	2	22
3	21	3	21
4	19	4	19
5	7	5 (>=5	38
6	5		
7	5		
8	4		
9	3		
10	6		
11	2		
12	2		
13	1		
14	1		

An encounter with a host including unsuccessful and successful encounter (i.e. oviposition) was treated as censored observation, and the basic leaving-tendency was reset after such a renewal point.

7.2.3 *Test of optimal foraging models*

The data collected from the first leaf and first plant visited were analyzed separately to test the predictions of optimal foraging models of the effects of patch quality and oviposition experience on the patch residence time and giving-up time since the last encounter.

7.3 Results

7.3.1 *Leaving tendency on a leaf*

Seven covariates had a significant influence on the patch-leaving tendency of *D. semiclausum* from leaves in single tests (Table 7.3). In the final analysis, four covariates were fitted in the model. The presence of damage and unsuccessful encounters with hosts significantly decreased the leaving tendency, while oviposition and the cumulative number of infested leaves visited increased the leaving tendency.

7.3.2 *Leaving tendency on a plant*

The parasitoid's leaving tendency from single plants was influenced by host density, unsuccessful encounters with hosts, cumulative numbers of ovipositions on the current plant and the cumulative number of visits to the present plant (Table, 7.4). The qualitative effects of these covariates on the leaving tendency were consistent with the results from leaves. Additionally, the leaving tendency was lower on the first plant the wasp visited during the experiment.

Table 7.3 Estimated effects (β) of covariates on the leaving tendency of *D. semiclausum* from a single leaf of the first plant visited

Covariates	β	Wald test	*P*
Tests of single covariates			
Host density of the current leaf	-0.2932	6.323	0.0119
Presence of damage on the current leaf?	-0.6381	9.9293	0.0016
Oviposition during the current visit?	1.0175	16.952	0.0001
Recent oviposition rate	9.5745	3.7757	0.0520
Unsuccessful encounters with host during the current visit?	-1.5348	28.754	0.0001
Cumulative number of previous visits to the current leaf	0.0769	1.4065	0.2350
Host density of the first plant visited	-0.0203	0.0685	0.7936
Cumulative number of clean leaves visited	0.0559	0.3498	0.5542
Cumulative number of infested leaves visited	0.0995	13.925	0.0002
Cumulative number of oviposition	0.3125	3.1484	0.0760
Is this the first-landed leaf?	-0.4032	5.2786	0.0216
Final model			
Presence of damage on the current leaf?	-0.6599	8.4710	0.0036
Oviposition during the current visit?	1.1437	19.918	0.0001
Unsuccessful encounters with host during the current visit?	-1.2770	18.201	0.0001
Cumulative number of infested leaves visited	0.0934	10.308	0.0013

7.3.3 *Leaving tendency from a group of plants*

Five covariates had a significant influence on the patch-leaving tendency of *D. semiclausum* from leaves in single tests (Table 7.5). But only two covariates had been fitted in a final model. Both the cumulative number of ovipositions during previous visits and the cumulative number of infested plants visited increased the leaving tendency. These results suggest that the wasp may employ a simple foraging strategy at highest spatial level of the foraging environment.

Consistently, oviposition had an incremental influence on the leaving tendency from all levels tested (Table 7.3-3.5).

Table 7.4 Estimated effects (β) of the covariates on the leaving tendency of *D. semiclausum* from a single plant.

Covariates	β	Wald test	P
Tests of single covariates			
Host density of the current plant	-0.2943	44.931	0.0001
Cumulative number of ovipositions during the current visit	0.0423	0.1664	0.6833
Recent oviposition rate	1.3528	0.2668	0.6055
Cumulative number of unsuccessful encounters during the current visit	-0.6432	33.819	0.0001
Cumulative number of visits to the current plant	0.3570	74.054	0.0001
Cumulative number of ovipositions on the current plant during previous visits	0.3840	30.061	0.0001
Cumulative number of encounters on the current plant during previous visits	0.0467	0.4853	0.4860
Cumulative number of ovipositions during previous visits	0.4061	101.518	0.0001
Cumulative number of unsuccessful encounter with hosts			
Is this the first plant visited?	-0.6405	23.680	0.0001
Final model			
Host density of the current plant	-0.2981	31.428	0.0001
Cumulative number of unsuccessful encounters during the current visit	-0.3688	10.350	0.0001
Cumulative number of visits to the current plant	0.3064	42.771	0.0001
Cumulative number of ovipositions during previous visits	0.3125	12.711	0.0004
Is this the first plant visited?	-0.2927	4.0433	0.0443

Table 7.5 Estimated effect (*β*) of the covariates on the leaving tendency of *D. semiclausum* in a group of four plants.

Covariates	β	Wald test	P-value
Tests of single covariates			
Host density of first-landed plant	-0.1374	0.2317	0.6303
Cumulative number of ovipositions	0.8357	42.199	0.0001
Recent rate of oviposition	73.679	13.492	0.0002
Cumulative number of unsuccessful encounter	0.1438	2.9281	0.0871
Cumulative number of clean leaves visited	0.2382	22.370	0.0001
Cumulative number of infested leaves visited	0.1424	37.587	0.0001
Cumulative number of plants visited	0.2613	34.936	0.0001
Final model			
Cumulative number of ovipositions	0.5999	21.333	0.0001
Cumulative number of plants visited	0.2181	19.752	0.0001

7.3.4 *Test of optimal foraging model*

Because in most of the releases, wasps first landed on the plant infested with 4 larvae (20 of 28), there was insufficient data to make comparisons among the remaining three densities. Only the observed patch time and giving-up time were compared with the predictions of Waage's (1979) incremental model and Driessen's (1995) Count down model. Oviposition had no significant effects on patch residence time of *D. semiclausum* either on the first-landed leaf or the first-landed plant, but significantly influenced the giving-up time (Figure 7.2).

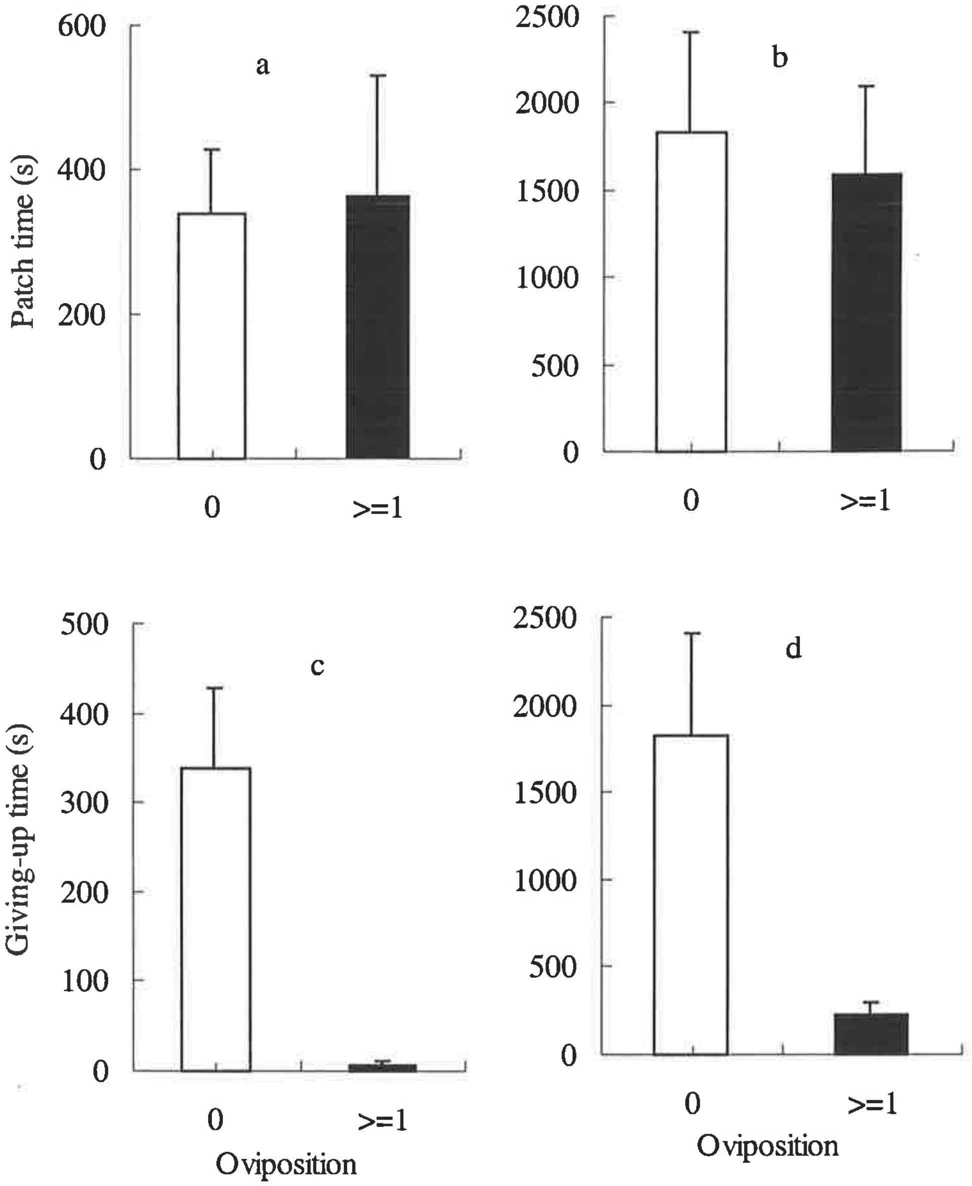


Figure 7.2 Effect of oviposition on the patch residence time and giving-up time of *D. semiclausum*. (a) Patch residence time on the first leaf visited that was infested by 1 DBM larva (n = 23, p>0.05). (b) Patch residence time in the first plant visited that was infested by 4 DBM larvae (n = 5, p>0.05). (c) Giving-up time in the first leaf visited that was infested by 1DBM larva (n = 10, $p < 0.001$), (d) Giving-up time in the first plant visited that was infested by 4 DBM larvae (n =10, $p < 0.001$). Statistical significance was determined by Kruskal-Wallis Tests.

7.4 Discussion

7.4.1 *Hierarchical patch structure*

This study showed that the tendency of *D. semiclausum* to move within a patchy environment is influenced by different factors depending on the spatial scales of reference. At the leaf level, the presence of host damage, oviposition, unsuccessful encounters with hosts and cumulative visits to the infested leaves influenced the leaving tendency of the parasitoid. At the plant level, a similar range of information as at the leaf level influenced the leaving tendency. The effects of those foraging experiences on the leaving tendency at both the leaf and plant level were consistent with previous results of simple patch-level experiments (Chapter 4-6). However, at the highest level of the foraging arena, only the cumulative number of ovipositions and cumulative number of visits to plants influenced the leaving tendency. One possible explanation of the results is that the parasitoid may respond to the local variation in spatial heterogeneity, and perceives the foraging arena as a hierarchical patch structure.

D. semiclausum seems use kairomones to assess initial patch quality (Chapter 5). At the smallest spatial scale, the parasitoid may be able to more accurately assess the kairomone levels. The higher the spatial level, the more problematic such an assessment becomes due to the constraints of perceptual ability (Vos *et al.*, 1998). *D. semiclausum* probably can effectively assess kairomone presence at the leaf scale, as the wasp often searched a whole leaf repeatedly before leaving if no encounters with hosts occurred (Chapter 2). The larger scale introduced additional sources of variability due to spatial heterogeneity. Fine scale cues such as kairomones may not be tracked effectively by the parasitoid at the higher spatial levels. Instead, rewarding oviposition experience could be used to assess patch depletion and the wasp could change its patch-leaving decisions accordingly. Thus, *D. semiclausum* seems to use different foraging strategies according to the spatial scale of reference. However, it is possible that the different results at three spatial scales of reference were artifacts of the statistical method used. The effects of some covariates many be masked by others at different spatial scales due to their concurrent occurrence. Also, since sample sizes necessarily were smaller at the larger spatial scales of reference, there was less statistical power to detect the influence of factors which might have had less significant effects on wasp behaviour.

After one successful oviposition, the parasitoid often immediately left a leaf or plant (Chapter 2). The straight flight was also observed when the parasitoid left the experimental plant to the extra plant in the single plant experiments (Chapter 4-6). In this multi-plant experiment, the parasitoids were also observed to make a straight flight from the experimental plants to one of the extra plants. In previous studies the patch boundary was identified by a characteristic change in behavior as the wasp left the 'patch' (Waage, 1979; van Roermund *et al.*, 1994; Vos *et al.*, 1998). Such a behavioral definition of the patch is consistent with the EFU concept (Ayal, 1987) and Waage's lower patch level concept (1979). Obviously, such a definition depends largely on the foraging environment. In the experimental multiple plant environment no unique behavioral change occurred when the borders of between leaves and plants were crossed.

It is possible that *D. semiclausum* functionally perceives the environment as a spatially hierarchical patch structure. The wasp may perceive a leaf as first-order patch, a plant as a second-order patch, and a group of plants as a third-order patch. In nature, the structure of the foraging environment may change over time and space for many parasitoids. At the early stages of a crop, the plants are widely separated. With growth, the leaves of different plants begin to touch and may eventually closely overlap each another. Thus, the change of the architecture of the host plants and plant boundaries cause changes in the travel costs involved in movement between patches. Thus a wasp's perception of patches may vary over time due to changes in the architecture and spatial arrangement of plants and host insects.

This study considers the likely existence of a perceptual hierarchy of spatial levels in the environment of a forager. Classification of the environmental level is central, not only to the understanding of foraging strategies or testing of current foraging theories, but also to an understanding of the population dynamics of host-parasitoid interactions. Many studies have considered changes in forager behaviour as a result of changes in resource density at a particular spatial level (Heads & Lawton, 1983; Walde & Murdoch, 1988; Rosenheim *et al.*, 1989; Ives *et al.*, 1993; Vos *et al.*, 1998). At the highest level, the parasitoid appears relatively unresponsive to the host-associated information, whereas at lower levels the same parasitoid appears to be tightly linked to the host-associated information. Thus, the choice of patch levels can influence patterns of movement and parasitism. This is a source of behavioral variation that should not be ignored when testing the optimal decisions of parasitoids in multiple patch environments. Studies of foraging behaviour should be conducted at different spatial scales in order to fully understand the factors that influence decisions by foraging wasps (Keller & Tenhumberg, 2000).

7.4.2 *Adaptive significance of the patch-leaving behaviour*

At all levels, the act of oviposition consistently increased the leaving tendency. In this experiment, the overall host density per leaf was relatively low. 74.7 % of the leaves were left uninfested. Among the infested leaves, most of them contained one larva (80.8%), and only a few leaves contained two larvae. An increase in the parasitoid's tendency to leave after one successful oviposition could be explained by the adaptive advantage of avoidance of self-superparasitism, as host discrimination involves a substantial time cost for this parasitoid (Chapter 3). Therefore, the cost of self-superparasitism is minimized by an increased leaving tendency after ovipositing (Strand & Vinson, 1982; Rosenheim & Mangel, 1994). Waiting for hosts after an unsuccessful encounter could also be an adaptation to the host's defensive behaviour (Chapters 2,4,5), because locating larval DBM is very time-consuming. Thus there was no need to reject a discovered host, if there was a low probability that it was parasitised.

As the number of visits to a patch increases the wasp may be able to perceive that the patch has been exploited before, as one of the possibilities is that wasp might leave a chemical mark on the leaf (Bernstein & Driessen, 1996). If this is so, then the leaving tendency should increase with the perception of the increasing concentration of the chemical mark deposited by itself during previous searching on the leaf. Another possible explanation is that, due to patch depletion with increasing visits, the probability of encountering suitable hosts decreases with increasing visits.

Some covariates ware significantly correlated. At the leaf level, the presence of host damage was positively correlated with presence of host density ($r = 0.6282$, $n = 199$). At a plant level, the number of previous ovipositions was positively correlated with the first-landed plant ($r = 0.4271$, $n = 403$), as the parasitoid paid more visits to the highest host density plant and laid more eggs when on it (Chapter 2). At the level of groups of plants, the cumulative number of ovipositions was positively correlated with the cumulative number of infested leaves visited ($r = 0.6490$, $n = 168$). Therefore, in the analysis of patch-leaving decisions, some covariates that had a statistically significant effect in separate tests ceased to be significant when fitted together in a more comprehensive model (Chapter 4). For example, at the leaf level, three covariates ceased to be important in the complete model: host density of the leaf, recent oviposition rate and first landing of the leaf (Table 7.3).

7.4.3 *Optimal foraging models*

The parasitoid spent more time on the highly infested areas of leaves and plants in the foraging arena, ie the patch time allocation of *D. semiclausum* over leaves and plants bearing different host densities agreed with the general predictions of Charnov's Marginal Value Theorem (see Chapter 2). However, the Marginal Value Theorem does not consider the effects of within-patch foraging experience, such as successful encounters with hosts, on patch residence time. It seems that the foraging decisions of *D. semiclausum* are driven by its changing perception of the local environment rather than more global assessments of patch profitability and spatial structure.

The decrease of the patch-leaving tendency or giving-up time after one or more successful ovipositions agreed with the predictions of the Count-down model (Driessen *et al.*, 1995).

Chapter 8 Patch exploitation by *D. semiclausum*: a test of optimal foraging predictions

8.1 Introduction

The host resources of many parasitoids are patchily distributed in the environment, and parasitoids face decisions on how to allocate their foraging time over patches of different profitability (Godfray, 1994). The question of patch time allocation has long been an active issue in optimal foraging theory and host-parasitoid interactions (Godfray & Shimada, 1999).

Optimal foraging theory assumes that natural selection has shaped parasitoid foraging behaviour in such a manner that they maximize their oviposition rates (Charnov, 1976b). Thus an optimally foraging parasitoid should be expected to concentrate searching within patches of high profitability, which will lead to an aggregation response of foraging time or parasitoid distribution on patches of high host density (e.g. Hassell & May, 1974). Three different optimal foraging models associated with patch exploitation describe the aggregation response in parasitoids or the distribution of a group of parasitoids over patches: (1) patches should be exploited in order of profitability. The parasitoids first occupy the best patches, and then sequentially disperse to the lesser-ranked patches, until all patches are reduced to the same rate of encounter with healthy hosts (Cook & Hubbard, 1977; Comins & Hassell; 1979); (2) patches are selected randomly, but patch residence time is determined by optimal patch-leaving rules. Individual parasitoids should leave each patch when the instantaneous encounter rate with hosts falls to the average rate expected for the habitat (Charnov, 1976b); and (3) the aggregation response to high host density patches is balanced by the rising interference among individual parasitoids simultaneously exploiting the patch, eventually the parasitoids should distribute themselves among patches such that each obtains the same encounter rate with hosts, i.e. the ideal free distribution (Fretwell & Lucas, 1970; Sutherland, 1983).

The three models are interrelated. The former models primarily describe the process of parasitoid aggregation, while the ideal free distribution describes the equilibrium distribution of a group of parasitoid among patches. At equilibrium, each individual parasitoid experiences the same encounter rate with hosts. When the interference is nil or the travailing time between patches was zero, the former two models converge to the ideal free distribution (Sutherland, 1983; Bernstein *et al.*, 1991). These three models produce the following general predictions: (1) the degree of aggregation to high host density patches decreases with increasing parasitoid densities or patch exploitation; and (2) at equilibrium all patches are reduced to the same rate of oviposition, thus the proportion of parasitoids in the *i*th patch is equal to the proportion of available hosts in that patch (i.e., proportional aggregation). These optimal foraging models predict a changing pattern of aggregation response. The degree of aggregation will depend largely on the time that is available for patch exploitation, parasitoid density and the prevailing host density (Cook & Hubbard, 1977). The principle of understanding the parasitoid distribution over patches is the ideal free distribution, however, many factors such as traveling time, interference and the structure and scale of the patch environment may result in the parasitoid population not distributed according to the ideal free distribution (Sutherland, 1983; Bernstein *et al.*, 1988; 1991).

The active aggregative response of insect parasitoids has been demonstrated in many laboratory studies (e.g. Waage, 1979; van Alphen & Galis, 1983), and field observations (Stamp, 1982; Waage, 1983; Smith & Maelzer, 1986; Thompson, 1986; Jones & Hassell, 1988; Casas, 1989; Ives *et al.*, 1999). However, the dynamic pattern of patch exploitation has rarely been quantified in previous studies (Hubbard & Cook, 1978; Hassell, 1980). Optimal foraging models have made specific assumptions about the parasitoids' ability to assess host spatial distribution; real predictions may need a thorough understanding of the parasitoid's patch use rules (Godfray, 1994). Empirical studies, that seek to determine the particular patch use rules of parasitoids, and that test the dynamic predictions of optimal patch exploitation are lacking.

The aggregative response of parasitoids has been considered to have important stabilizing effects on host-parasitoid interactions that contribute to host regulation (Hassell & May, 1973, 1974). A huge amount of theoretical work has been devoted to explaining the important population consequences of parasitoid aggregation (Beddington *et al.*, 1978; May, 1978; Hassell *et al.*, 1980; Murdoch *et al.*, 1985; Reeve & Murdoch, 1985; Chesson & Murdoch, 1986; Strong, 1988; Murdoch & Stewart-Oaten, 1989; Pacala *et al.*, 1990; Godfray & Pacala, 1992; Ives, 1995; Murdoch & Briggs, 1996). The positive aggregation response by parasitoids should generate a direct density-dependent parasitism if the parasitoids are not strongly egg-limited (Comins & Hassell, 1979; Hassell, 1980; 1982; Waage, 1983; Lessells, 1985), or if the interference between individuals is not strong enough (Sutherland, 1983). However, empirical studies often fail to detect density-dependent parasitism, although the parasitoids were observed more frequently on high-density patches (e.g Morrison & Strong, 1980; Waage, 1983; Smith & Maelzer, 1986). Insect parasitoids display highly variable patterns of parasitism from direct and inverse density-dependent to density-independent (reviewed in Lessells, 1985; Stiling, 1987; Walde & Murdoch, 1988).

Theoretical explanations for non density-dependence in parasitism include behavioral or physiological limitations of the foraging parasitoids, such as the limited availability of eggs and handling time when foraging in high host density patches (e.g. Hassell, 1982; Waage, 1983; Lessells, 1985), stochastic variations in patch time allocation (Morrison, 1986), or other forms of aggregation response (e.g. Walde & Murdoch, 1989; Reeve *et al.*, 1989). Although the modeling approaches have sorted out some general ideas, it seems impossible to produce a universal explanation. For example, an analytical model based on the ' random searching equation' (Rogers, 1972) and incorporating a fixed aggregative response, predicts that any form of aggregation will produce direct density-dependent parasitism if the handling time is small (Hassell, 1978, 1982). If there is no egg limitation and superparasitism, then an aggregative response of parasitoids, and thus an attack rate proportional to host density, is required to generate direct density-dependent parasitism. Any conflicting or interacting mechanisms that influence the attack rate may outweigh the aggregative effect on parasitism. Thus sufficient strength of the aggregation may be necessary to generate directly density-dependent parasitism.

Many studies have attempted to infer statistically the aggregation response of parasitoids indirectly based on the observed levels of parasitism (e.g. Heads & Lawton, 1983). It could be very difficult to distinguish the behaviorally mediated aggregation from demographically mediated aggregation (Rosenheim *et al.*, 1989). Unfortunately, as is often acknowledged (e.g. Hassell, 1980; Waage, 1983; Walde & Murdoch, 1988; Rosenheim *et al.*, 1989; Ives *et al.*, 1999), empirical studies based on direct observation of the foraging behaviour of the parasitoids are less commonly used to test the direct density-dependence of parasitism. Thus, laboratory work is needed to critically assess these hypotheses of parasitoid limitation, and to identify the possible conflicting or interacting mechanisms that govern the relationship between aggregation response and parasitism. At the same time, experimentally orientated field work would be particularly valuable to look at the aggregative response of parasitoids to local variation in host density over time and space. The aggregation response of a parasitoid or predator and thus parasitism is often sensitive to the spatial scale (Morrison & Strong, 1980; Walde & Murdoch, 1988; Rosenheim *et al.*, 1989; Sheehan & Shelton, 1989; Ives *et al.*, 1993). We also need to understand the real parasitoid 's perception of ‘patchiness’ or spatial scale at which the parasitoids recognize and respond to differences in host density and distribution (Walde & Murdoch, 1988; Rosenheim *et al.*, 1989).

This chapter provides a test of the optimal foraging predictions of patch exploitation and the population consequences of the aggregation response using *D. semiclausum* under laboratory and field conditions. The work is based on the studies presented in previous chapters on foraging behavior and a thorough understanding of the patch use rules of the parasitoid (Chapters 2-7). The laboratory experiments aimed to quantify the dynamic pattern of patch exploitation by using a group of wasps in patches of varying host densities, and identify the mechanisms that potentially govern the pattern of parasitism. The field experiments measured the response of a field population of *D. semiclausum* to local variation in host densities over time at two different spatial scales.

8.2 Materials and methods

8.2.1 *The host-parasitoid system*

The *P. xylostella-D. semiclausum* system is suitable for the study of foraging behaviour of parasitoids in the field. *D. semiclausum* is a specialist on *P. xylostella*, the most destructive insect pest of crucifer crops worldwide. The basic biological and ecological characteristics of both the host and the parasitoid are well-documented (Talekar & Shelton, 1993). In Southern Queensland, Australia, both *P. xylostella* and *D. semiclausum* dominate in crucifer crop fields during winter when other crucifer pests and parasitoids of *P. xylostella* occur at very low levels. Another morphologically similar parasitoid, *D. rapi* attacks larval *P. xylostella* in Queensland but is rare (Heisswolf, *et al.*, unpublished data). During the experiments, independent sampling in the fields did not detect *D. rapi*. Thus the winter provides an ideal season for field observations on the foraging behaviour of *D. semiclausum* in South Queensland, when the possible interference effects of other insect species are minimized.

The previous chapters detailed the foraging behaviour and patch use rules of *D. semiclausum* (Chapters 2-7). Larval *P. xylostella* avoid parasitoids by moving away from damaged plant parts after short feeding bouts and by vigorously wriggling or dropping off the plants along a silk thread when encountered. The parasitoid often waits for the larva hanging on the silk thread to climb back to the plant and then attacks it again. This causes a variable handling time of the host (from several seconds up to 25 min). *D. semiclausum* displays both random and wide-area search on host-infested leaves which increases the probability of detecting hosts resting away from the damage site, and re-encountering the hosts climbing back to the plants. The parasitoid tends to superparasitise hosts even when searching alone. This might confer an adaptive advantage due to the relatively high search time costs associated with overcoming the host defense. The patch-leaving tendency decreases with increasing host density and successful oviposition, but increases with unsuccessful encounter with hosts. At different levels of 'patchiness', the parasitoid employs different patch-leaving rules. At the higher spatial levels in a hierarchical patch system, the parasitoids use simpler patch leaving rules.

Waage (1983) pioneered the study of parasitoid foraging behaviour in the field. He investigated the foraging time allocation of a field population of *Diadegma* spp. to plants artificially infested with different densities of *P. xylostella*. He found a significant aggregation response of the parasitoids to high host density plants, but the resultant pattern of parasitism was density-independent, which was explained as a result of handling time required to parasitise hosts on high host density plants (Waage, 1983).

The field experiments reported here were similar to those of Waage (1983). The present study looked at the parasitoid's response at two spatial scales: (1) response to variation in host density at the level of individual plants; and (2) response to variation in host density at the level of a group of plants. In view of the hierarchical structure of the crop system (Chapter 7), it could be important to look at the relative aggregation response of the parasitoid to host density and distribution at different spatial scales.

8.2.2 *Laboratory experiments*

Laboratory experiments were conducted in an open wind tunnel, designed to provide a free-flight environment for the parasitoids and located in a windowless room (1.8 x 2.2 x 2.8 m) with controlled temperature of 24-25 °C. Wind was produced by an electric fan. In this experiment the wind speed was set at approximately airflow of 30-35 cm s-1. The experimental arena was lit by 4 cool white 40 W fluorescent tubes hanging from the ceiling. See Chapter 2 for a detailed description of the open wind tunnel.

For the detailed rearing procedures of both the parasitoid and host, see the Appendix. The parasitoids used in this experiment were 2 to 3 day-old mated females. Parasitoid cocoons were collected and maintained individually in glass vials (7 x 2 cm). Upon emergence a drop of honey was added to the vial wall serving as food for the adult parasitoid. One day prior to thc experiments, individual female wasps were caged (15 x 15 x 15 cm) with 5 males until each mated. The mated females were then returned to the vials for experiments the next day. In all experiments, second to third instar *P. xylostella* from the culture were used.

Common cabbage (*Brassica oleracea var. capitata*, cv. Green Coronet) was grown in pots (14 cm diameter) in a greenhouse for culture. Young potted cabbage plants with 5-6 fully extended leaves grown in plastic cups (8 cm diameter) were used for the experiments.

To facilitate observation of multi-wasp foraging, one day prior to the experiments, all leaves of the experimental plants but the middle ones were cut off, and the experimental wasps were carefully marked under a microscope on the thorax with a unique dot of colored enamel paint. Preliminary observation showed that there was no difference in the searching abilities between marked and unmarked parasitoids. One day before the experiments, the single leaf 'plants' were infested with 5 different densities: 0,1, 2, 4, or 8 host larvae, 2 plants at each density. The 10 plants were arranged in two parallel rows (Figure 8.1). The densities were ascending in one row and descending in other. Four wasps were released, each at one randomly selected release point (Figure 8.1). Immediately before release, each wasp was primed for 5 min in a 80 mm glass Petri dish, containing a piece of cabbage leaf with fresh feeding damage produced by one 3rd instar host over night. This ensured the experimental wasps were experienced with host-related cues.

Immediately before the experiments, each 'plant' was checked for the number of host larvae, their positions in relation to the feeding damage (distinguished as on damage or off damage; during the observation the larvae rarely moved much). Upon opening each of the Petri dishes holding the experimental wasps, the behaviors (encounter, sting, and direct contact with each another) and positions (Petri dishes, 'plant', and elsewhere outside) of each wasp were continually recorded using an event-recording computer (The Observer 3.0 for Windows). Host larvae often dropped onto the floor after being stung. These larvae were put back onto the basc of the plant, so that they could be recovered after the experiments.

To decide upon a suitable experimental time, a preliminary experiment was set up for one hour. It was observed that after one hour foraging some individuals started to leave the experimental arena and rested for a long period, possibly to replenish egg supply. Thus the experimental time was set to about 1 hour if the wasps started to leave the experimental arena, or up to 1.5 hours if no emigration was observed. To investigate if the wasps were suffering from depletion of their egg supply, all the experimental wasps were immediately killed and dissected to determine their mature egg-load after the experiments. At the end of each experiment, all larvae were dissected to determine the presence of parasitoid eggs.

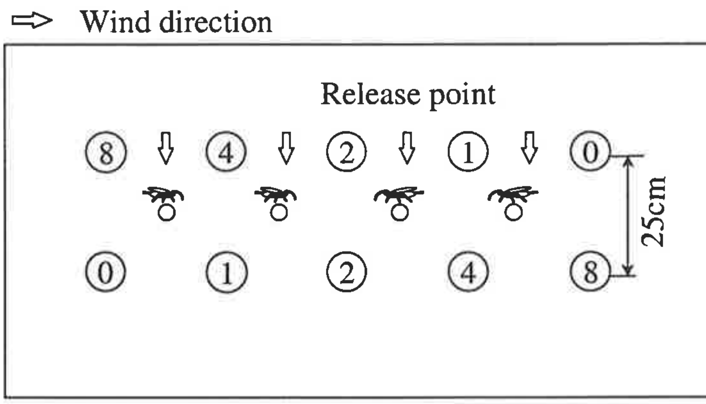


Figure 8.1 The laboratory experimental layout in an open wind tunnel: four *D. semiclausum* females were released into patches of 10 host plants infested with five different densities of larval *P. xylostella*. The four wasps were each released randomly at one of the four release points. Circles represent the potted host plants (patches), and the figures inside the circles refer to the host densities.

All experiments were conducted between 09:00 and 16:00. On each day, one or two observations were conducted. The experiment was replicated 26 times. In a few cases, one of the four wasps was not successfully released, in total 92 wasps were followed through the experiments.

To determine the dynamic pattern of patch exploitation, the data were analyzed for the four different periods of the experiments: from release to 15 minutes (D1), 16-30 minutes (D2), 31-45 minutes (D3), and 46 minutes to the end of the experiment (D4).

8.2.3 *Field experiments*

The field experiments were carried out from 30 July to 2 September 1999 at Gatton Vegetable Crops Research Farm, Queensland Department of Primary Industries, South Queensland, Australia. Two very different fields of host plants were created. In one field (50 x 15 m, referred as Field 1 below), common cabbage plants transplanted in early April had been heavily infested by *P. xylostella* before the experiments. By the time of the experiments, the plants were at the end of harvest. Independent sampling showed that over 95 percent of larval *P. xylostella* in Field 1 were parasitised by *D. semiclausum*, and the parasitoids were at peak emergence. Thus host resource of Field 1 was seriously depleted. In another field (50 x 20 m, referred to as Field 2 below), the broccoli plants transplanted in early June were still young at the beginning of the experiments. Weekly monitoring from early June to the end of August showed that there were two peaks in the abundance of host larvae on 20 July and 18 August, and one peak of *D. semiclausum* cocoons on 12 August. At the beginning of the crop, the host population density increased quickly up to about 5 larvae per plant, and then decreased with increasing *D. semiclausum* population. Thus the parasitoid population density in Field 2 was very low at the beginning of the experiments and gradually increased. The two fields were about 150 meters apart. There were no other cruciferous crops within several km around the farm during the experiments.

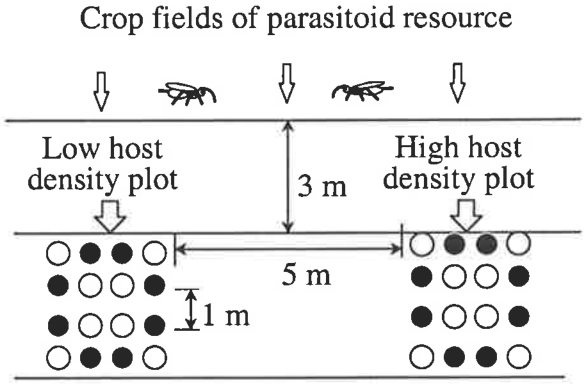


Figure 8.2 The field experimental layout: two different overall host density plots each consisting of 16 potted broccoli plants either infested with larval *P. xylostella* (black circles) or without infestation (white circles) were set up downwind of a resource field of a naturally occurring *D. semiclausum* population. The host density of the infested plants (number of plants per density in parentheses) was 2 (4) and 4 (4) larvae in the low host density plot, and 4(4) and 16 (4) larvae in the high host density plot. On each experimental date, the plants were assigned by a random block design to 4 x 4 grids (i.e. each row or column contains two different host density plants, respectively).

Ten sets of observations were made on sunny or partly sunny and calm days (Table 8.1). On each observation date, two different host density plots, each consisting of 16 potted broccoli plants were set up on the bare soil about 3 meters downwind from the resource fields to intercept naturally occurring, dispersing wasps (Figure 8.2). The host densities per plant were 4 x 4, 4 x 2, and 8 x 0 hosts / plant in the low host density plot, and 4 x 16, 4 x 4 and 8 x 0 hosts / plant in the high host density plot, respectively. A yellow mesh cloth was spread over the bare soil of each plot to enhance observation of the wasps. A 4 x 4 one meter grid was marked on the cloth, and the plants were assigned in a random block design to the 4 x 4 grids (each row or column containing two different host density plants).

The potted broccoli plants were grown in a glasshouse until 4-5 fully expanded leaves were present. The experimental plants were infested by 2nd and 3rd instar *P. xylostella* at the densities required in the laboratory one day prior to the experiments, and were moved into the field on the morning of the observational date. Immediately before setting up the experimental plot, all infested plants were checked to ensure the exact number of hosts were present as required. A tray was placed under each of the plants bearing host larvae to catch the dropped larvae following an attack by the parasitoid, so that these larvae were likely to be recovered after the experiments. A 1.5 meter-high mesh fence was set up 5 meters downwind of the experimental arena to protect from occasional strong wind gusts. Wind direction was nearly consistent during the period of the experiment.

The observations usually started at 08:30 to 10:30 and finished at 15:30 to 16:30 (Table 8.1). The starting and ending time of each observation depended on the field conditions, with the exception of the last three observations, which ended at 12:30. When the plant surface was still wet early in the morning, or when the temperature started to drop in the late afternoon, there was no obvious wasp activity. At 10 min intervals, the observer walked around the plots and checked the plants from all sides, and recorded the number of *D. semiclausum* on each plant. With practice it was possible to census the plants quickly and to record virtually all the wasps on the experimental plants. In most cases one to four parasitoids were observed during a recording interval. If a parasitoid was observed, the observer then walked close to the plant, and checked the sex of the parasitoid with minimal disturbance. On 4th August, the parasitoid density was quite high in Field 1, so it was impossible to record the sex of all the parasitoids. The sex ratio was estimated based on the sex ratio observed on one plant. At the end of each observation, all larvae were collected and dissected to determine the presence of eggs.

Table 8.1 A summary of the field experiments and daily temperature.

Date	Resource	Exposure time		Daily temperature (ºC)	
(day-month)	field	From	To	Max	Min
30-July (1)*	Field 1	9:30	15:30	20.2	6
30-July (2)*	Field 1	9:30	15:30	20.2	6
30-July (3)	Field 1	9:30	15:30	20.2	6
8-August	Field 1	10:30	16:30	20.2	5.3
19-August	Field 2	8:30	16:30	22.3	5.6
25-August	Field 2	8:30	15:30	21.4	9.8
28-August	Field 2	9:30	15:30	21.6	14.9
29-August #	Field 2	8:30	12:30	22.3	11.8
1-September	Field 2	8:30	12:30	21.6	10.2
2-September	Field 2	8:30	12:30	21.6	10.2

* No low host density plot treatment.

\# No wasp observation.

The number of parasitoids observed per plant gave an indication of the relative amount of time spent by the population at different host densities (Waage, 1983). Parasitism was calculated based on the recovered hosts. Mean percentage recovery of the experimental larvae was 84 to 96 %.

To test the effects of spatial scale or ' patch size' at which parasitoids may respond differently to local variation in host density and distribution, the data were analyzed at two spatial scales: plant and plot.

In South Queensland the daily temperatures varied dramatically during the winter, but the temperature varied little during the sunshine time during the experimental days (Table 8.1).

8.3 Results

8.3.1 *Laboratory experiments*

The percentage of time allocated to foraging among patches by *D. semiclausum* females increased with host density in a similar manner in all four periods (Figure 8.3a). Overall, the parasitoid spent little time (0.54 %) in the empty patches without hosts, and the overall percentage time allocation increased non-linearly with increasing host density per patch (Figure 8.3b). The parasitoid spent proportionally more time relative to the density on the patches of 1-4 larvae, and proportionally less time on the highest host density patches.

Patch time allocation by parasitoids has be described using a fixed aggregative response of parasitoid numbers to host density per patch (Hassell & May, 1973):

$$\beta_i = c\alpha_i^{\ u} \ (\Sigma\beta_i = \Sigma\alpha_i = 1) \quad (1)$$

Where β_i and α_i are the corresponding proportions of the parasitoids and hosts in *i*th patch, and *c* is normalization constant and *u* is an 'aggregation index '. $u > 0$ indicates aggregation in patches of high host density, and $u = 1$ gives linear aggregation, which means the parasitoids distribute themselves in proportion to hosts. The model does not describe the time allocated to the empty patches by the parasitoids. Within the host density range of 1-8 larvae per patch, the relationship between percentage time allocation by *D. semiclausum* and host density (Figure 8.3b) can be described (the curve fitted to means) as:

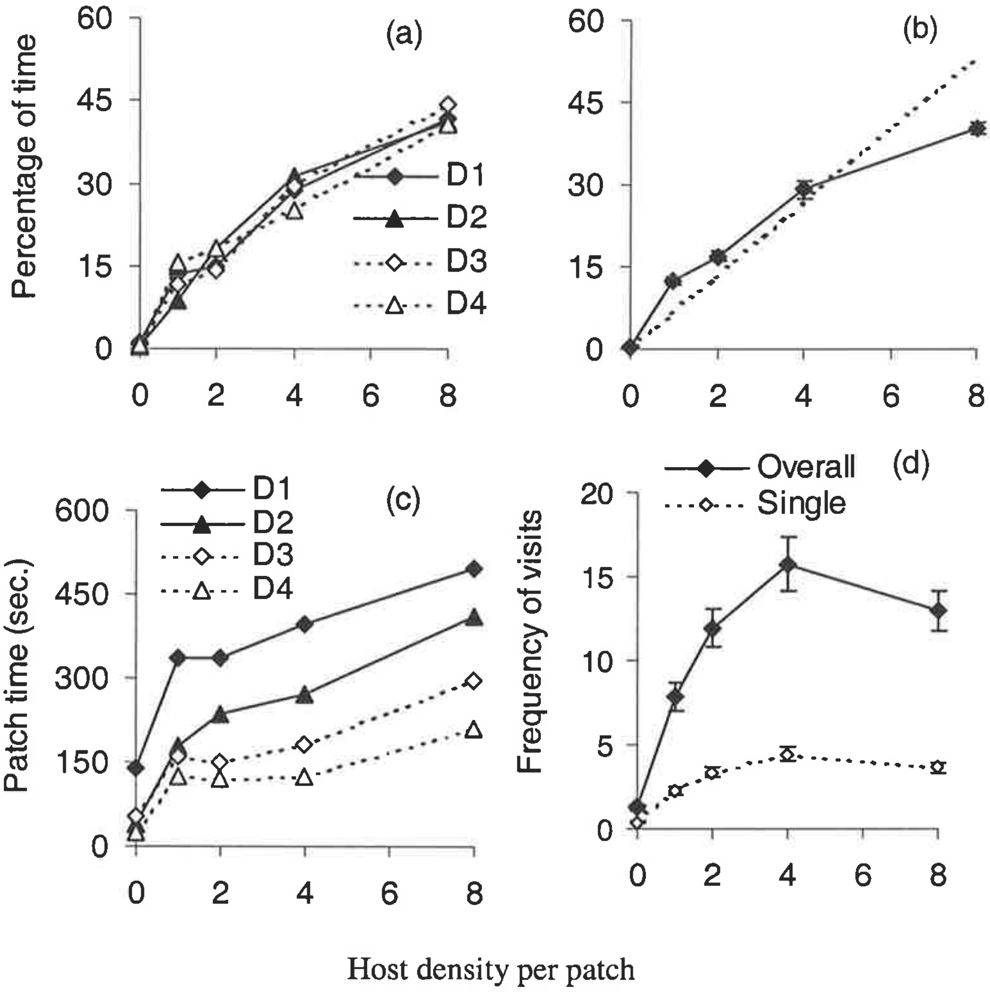


Figure 8.3 Patch time allocation by a group of *D. semiclausum* females exploiting patches of varying density of larval *P. xylostella* (laboratory experiment, n = 26 replicates). (a) Mean percentage time allocation at the four different stages of patch exploitation (D1 = 0-15 min., D2 = 16-30 min., D3 = 31-45, D4 = 46 to end of the experiments). (b) Overall pattern of percentage time allocation (mean ± 1 SE), the dotted line represents a predicted patch time allocation given the parasitoids aggregate in proportion to host density. (c) Mean residence time per patch visit at the four different stages of patch exploitation. (d) Frequency distribution of the overall visits to different patches by single vs. a group of wasps.

$$\beta_i = 59.74 \times \alpha_i^{\,0.592} \quad (r^2 = 0.9788,\ \mathrm{n} = 4,\ p < 0.01) \quad (2)$$

Here, β_i represent the proportion of time, which should be a better indicator than the proportion of the parasitoids as the wasps repeatedly re-visit the same patches during the experiment.

Mean patch residence time increased with host density, but decreased per patch visit at each density with patch exploitation (Figure 8.3c). The parasitoid stayed longest during each patch visit at the first stage of the patch exploitation. The high host density patches were visited more often where the parasitoids were first released (Figure 8.4). Patches with 4 hosts received the largest number of visits overall (Figure 8.3d). Thus the aggregation of foraging time allocation arose from non-random patch selection and density-dependent patch leaving at the density range of 0-4 larvae per patch. In the highest host density patches, aggregation mainly arose from non-random patch leaving.

The sting rates per patch visit increased with host density during patch exploitation, and was approximately constant over time, except that in the last time period when the sting rate in the 1-4 host patches decreased (Figure 8.5a). Overall, the mean sting rate (Y) increased approximately linearly with host density (X) (Figure 8.5b), a typical Type 1 variable time functional response ($Y = 2.4121 + 0.8511\,X$ ($r^2 = 0.980, p < 0.01$).

The rate of unsuccessful encounters with hosts increased with host density (Figure 8.5c), which substantially decreased the sting rate with increased host density. However, the mean percentage of larvae resting away from the damage site per patch (Y) decreased with increasing host density (X) ($Y = 0.697 - 0.05\,X$ ($r^2 = 0.803$, $p < 0.013$). This should have increased the encounter rate at the high host density patch, and balanced out the effect of density-dependent increases in handling time as a result of unsuccessful encounters with host. Overall, the relative foraging efficiency, i.e. sting rate per host density, decreased with increased host density (Figure 8.5b).

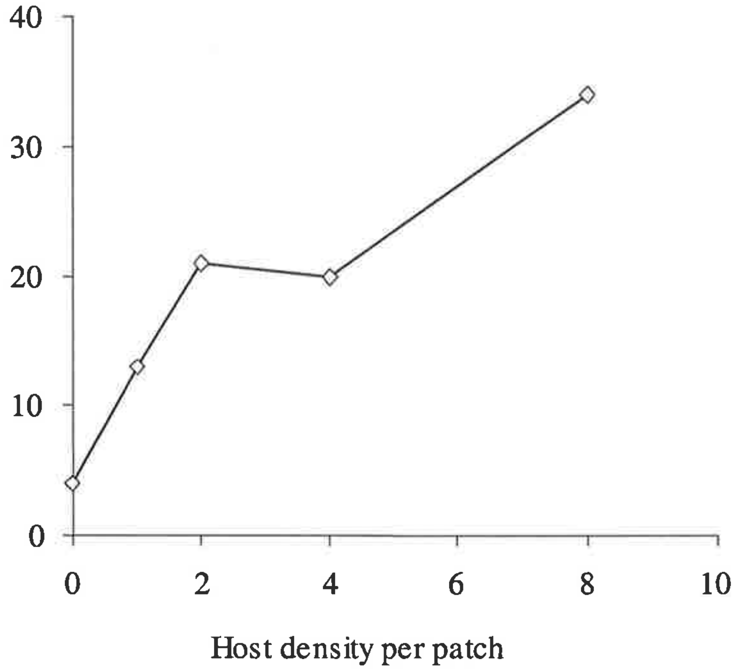


Figure 8.4 Overall frequency of first visits by *D. semiclausum* to patches infested with different density of larval *P. xylostella* in the laboratory experiment.

Table 8.2 Searching efforts by individual female *D. semiclausum* during the laboratory experiments

Parameters	N	Mean ± SE	Range
Foraging time (s)	92	3463 ± 98.8	2400-4800
No. of patch visits	92	14.9 ± 1.06	2-37
No. of stings	92	6.3 ± 0.48	0-18
No. of unsuccessful encounter with hosts	92	2.9 ± 0.25	0-8
No. of direct contact with another wasp	92	0.96 ± 0.13	0-5
Mature egg-load after the experiments	69	21 ± 2.09	2-64

The frequency of direct contacts between wasps was low in the experiments (Table 8. 2), and was density-independent per unit foraging time (Figure 8.5d).

Correlation analysis of the relationship between mature egg-load of the individual wasps after the experiments and the frequency of patch visit ($r = 0.056$, $n = 69$, $p > 0.182$) or the number of stings during the experiments ($r = 0.042$, $n = 69$, $p > 0.025$) did not show significant relationships. Thus, egg limitation probably did not influence the behaviour observed during the experiments.

The overall pattern of parasitism was density-independent (Figure 8.6a; Kruskal-Wallis test on percentage parasitism transformed by arcsin square root, $n = 52$, $p > 0.47$). Because the parasitoids randomly encounter hosts (Chapter 3), and avoidance of superparasitism was low in these experiments, a final dissection found that the observed total number of stings (Y) roughly matched the actual number of eggs (X) per host density ($Y = 0.09 + 1.08\,X$, $r^2 = 0.99$, $p < 0.01$). The Nicholson & Bailey model (Nicholson & Bailey, 1935) and random search equation (Rogers, 1972) could therefore be used to describe the relationship between parasitism and host density:

$$N_{ai} = N_i(1 - Exp(^{-Enc/\,N_i}) \quad (3)$$

Where N_{ai} = the number of host parasitised in ith patch, N_i = the total number of hosts present in ith patch, and Enc = total number of stings that the parasitoid made in ith patch. Enc / N_i is the average number of eggs per hosts:

$$Enc = r_i \,.\, PTi \quad (4)$$

Where PTi = the total foraging time the parasitoids allocated to the ith patch, and r_i = the mean sting rate in the ith patch (Figure 8.5b). Substituting for the total number of stings (Enc) in equation (4) gives

$$N_{ai} = N_i(1 - Exp(-r_i PT_i / N_i) \quad (5)$$

Substituting equation (1) into (5), the percent parasitism can be rewritten as:

$$P_i = 1 - Exp(-r_i c\alpha_i^{\,u} T / N_i) \quad (6)$$

Here, P_i = percentage parasitism, T = total foraging time. Here, r_i / N_i represents the relative foraging efficiency per host density, which decreased with increasing host density (Figure 8.5b; the dotted line). Here, $\alpha_i = N_i / N$, N = total number of hosts in the patches. Thus, equation (6) can be rewritten as:

$$P_i = 1 - Exp(-(r_i / N_i \,.\, A.\, N_i^{\,u})) \; (A = c.T / N, \text{ constant}) \quad (7)$$

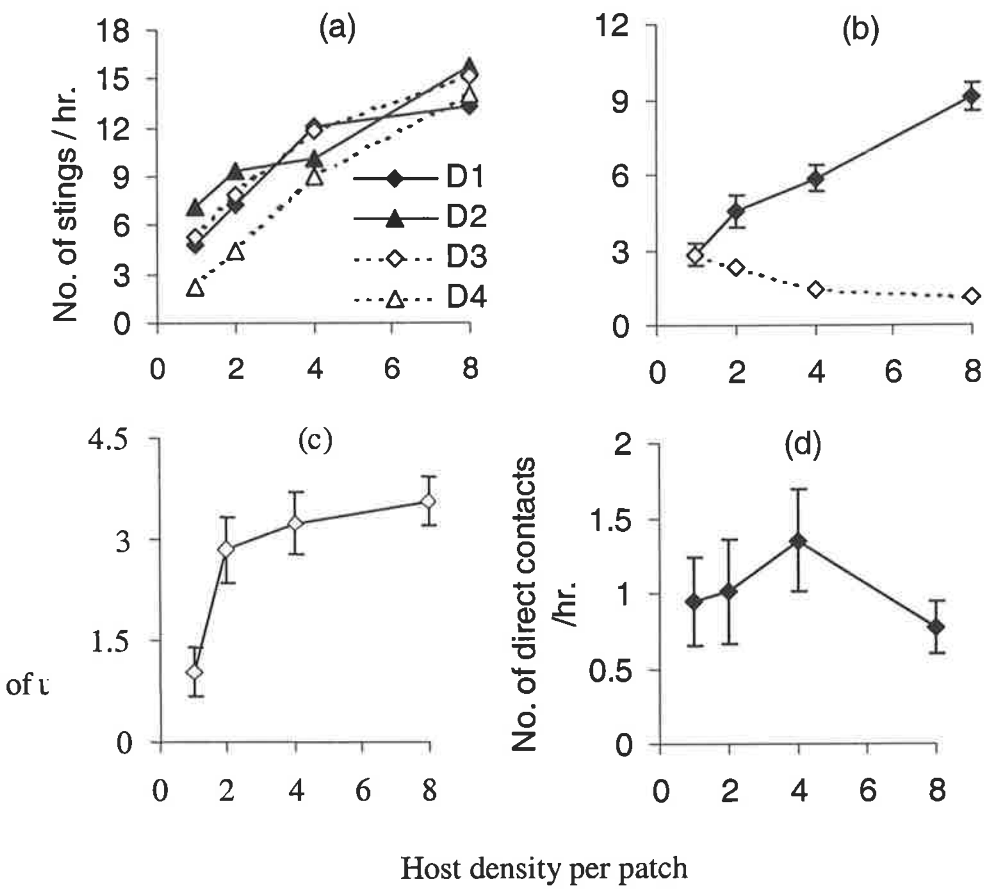


Figure 8.5 The relationship between density of larval *P. xylostella* per patch and (a) sting rate at the four different stages of the patch exploitation; (b) overall sting rate (the dotted line represents the relative foraging efficiency, i.e. sting rate per host density); (c) the rate of unsuccessful encounters; and (d) the number of direct contact with other individuals per unit time, by a group of *D. semiclausum* females (laboratory test, n = 26 replicates). All values are expressed as mean ± SE.

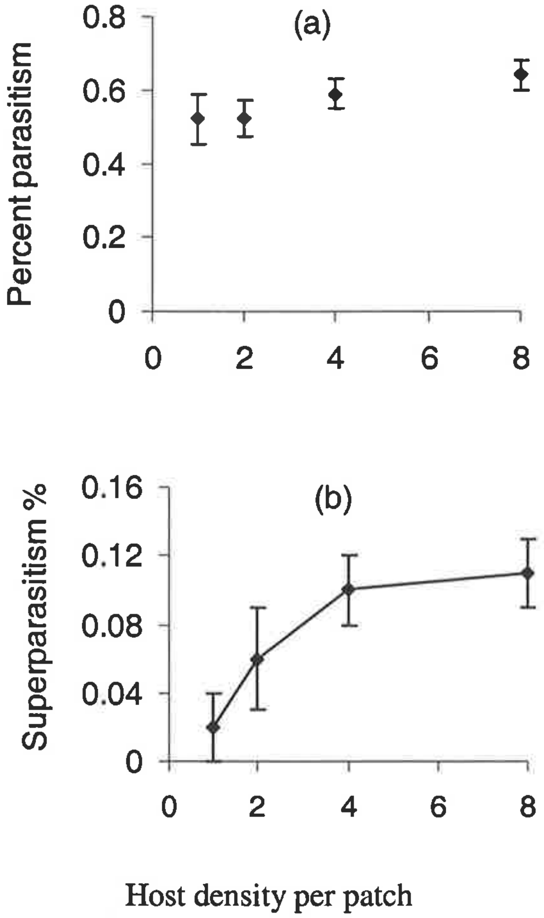


Figure 8.6 The relationship between host density of larval *P. xylostella* per patch and (a) percentage parasitism and (b) superparasitism (laboratory experiment, n = 26 replicates).

Clearly, the relationship between percentage parasitism and host density depends on the interplay between the degree of aggregation (*u*) and the factors that determine the relative foraging efficiency. If the relative foraging efficiency is assumed to be constant as in many analytical models (e.g. Rogers, 1972; Morrison, 1986), any form of the aggregative response will lead to density dependent parasitism. Otherwise, a sufficient degree of aggregation is needed to outweigh the decreasing foraging efficiency at the high host density patches.

Two conflicting mechanisms that influenced the relative foraging efficiency were identified. The density-dependent host defense (resting away from the feeding damage site) increased the rate of encounter with hosts per host density at the high host density patches, while increasing handling time per unit foraging time, which resulted from unsuccessful encounters with hosts (distinguished from other mechanisms causing an increase in handling time), decreased the sting rate. In this case, the interplay finally resulted in the overall foraging efficiency decreasing with increasing host density (Figure 8.5b). Not surprisingly, the resultant pattern of parasitism was density-independent (Figure 8.6a). If the interplay of the two main factors changed during patch exploitation, it would change the pattern of parasitism. With exploitation, the relative foraging efficiency would decrease in low host density patches (Figure 8.5a), and this would lead to direct density dependency if the exploitation continued.

The percentage superparasitism increased with host density (Figure 8.6b), which would further outweigh the effects of an aggregative response on the density-dependency in parasitism.

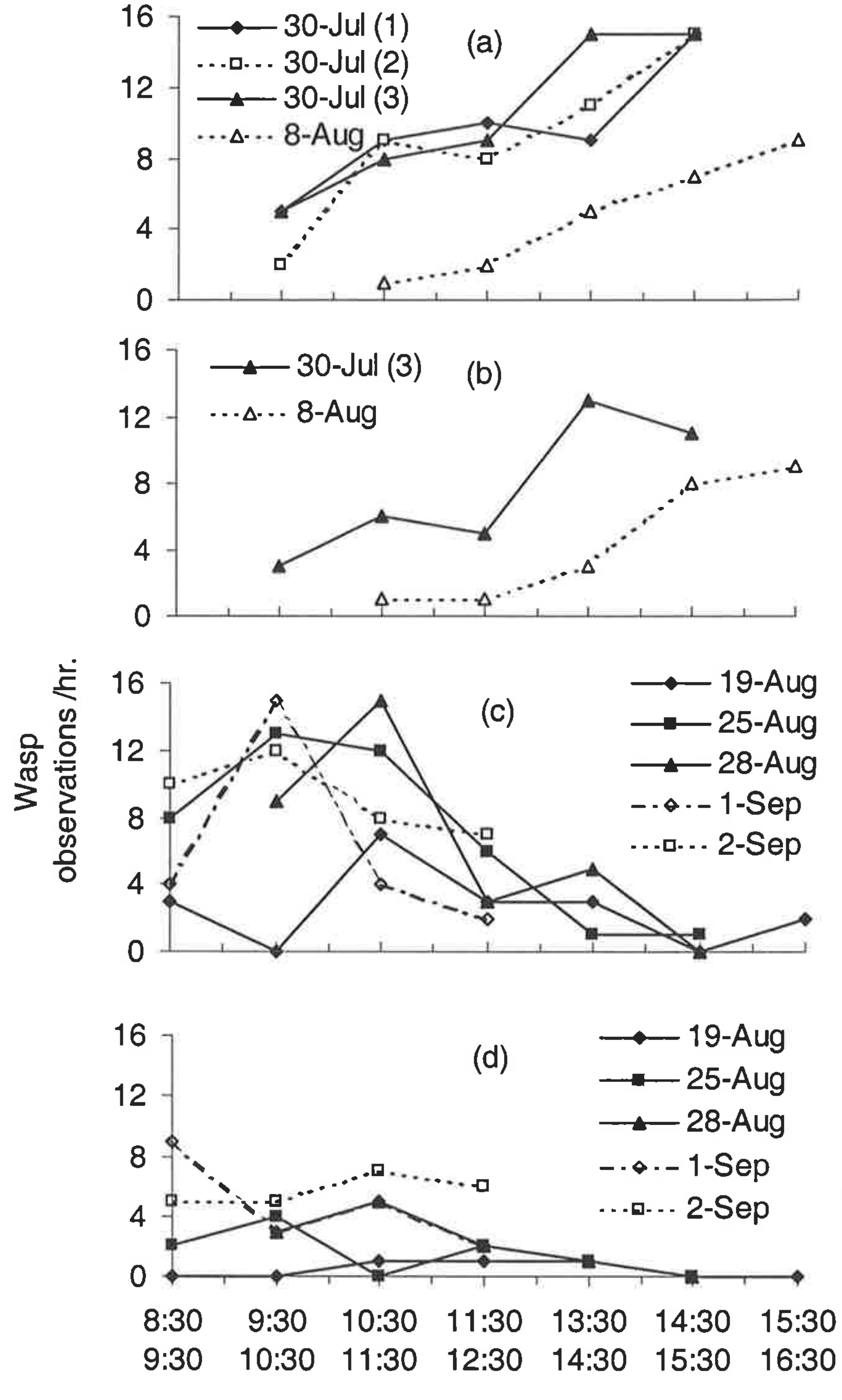


Figure 8.7 Cumulative number of *D. semiclausum* females observed per hour per plot during the experiments in the field. Field 1: (a) high host density plot; (b) low host density plot. Field 2: (c) high host density plot; (d) low host density plot.

8.3.2 *Field experiments*

During the experiments male wasps were rarely observed in the experimental plots (0-10 males per experimental observation), except on 8th August, when a high proportion of males (72%) was observed. Superparasitism was generally low (0-0.12%).

The cumulative number of wasp observations per hour per plot during the experiments reflected the daily rhythm of wasp activity and the relative aggregation response of the field population (Figure 8.7). The quality of the resource fields influenced the overall aggregative patterns. In Field 1 the number of wasps observed per hour per plot increased with time, while in Field 2 the number of wasps observed increased in the morning and then declined in the afternoon. Within the same resource field, the patterns of wasp aggregation and dispersal were consistent between the two plots over time.

The relative density of *D. semiclausum,* as estimated by the number of the wasps observed per unit time, varied during the experimental dates (Figure 8.8a). In Field 1, the population density decreased dramatically during the experimental dates, while in Field 2 the population density was low at the beginning of the experiment, and gradually increased.

Because the relative population density was highly variable over time and space, the 'aggregative index' and 'relative host density index' were used to compare the daily pattern of the aggregative response at the two spatial scales. At the plot level, the daily aggregative index I_{plot} (I_{plot} = total number of wasp observations on the high host density plot / total number of wasp observations on the two plots) was low in Field 1, compared with the relative density index D_{plot} (D_{plot} = total number of hosts present in the high host density plot / total number of hosts present in the two plots, which was 0.77) (Figure 8.8). In Field 2, I_{plot} increased with increasing parasitoid population density and then decreased when the parasitoid density reached a peak

(Figure 8.8). When I_{plot} was higher than D_{plot}, the patterns of parasitism in the high host density plot were also higher than the low host density plot (Figure 8.8).

At the plant level, the aggregative index I_{plant} (I_{plant} = total number of wasp observations on the high host density plants / total number of wasp observations on the whole plot) was lower than the relative density index D_{plant} (D_{plant} = total number of hosts present on the high host density plants / total number of hosts present in the whole plot, which was 0.67 and 0.80 for the low and high host density plot, respectively), and the resultant patterns of parasitism were density-independent over time (Figure 8-9; Figure 8-10). Overall, the percentage of wasp observations increased linearly with host density in the low host density plot, but negatively accelerated in the high host density plot (Figure 8.11).

8.4 Discussion

8.4.1 *Optimal patch exploitation*

The laboratory experiment showed the positive aggregation response of *D. semiclausum* to high host density patches (Figure 8.3a). However, in contrast to the predictions of the optimal foraging models, *D. semiclausum* neither exploited patches progressively based on their profitability (Cook & Hubbard, 1976; Comins & Hassell, 1979), nor selected patches at random with oviposition rate-dependent patch-leaving (Charnov, 1976b). Also, in contradiction with the optimal foraging model predictions, the degree of aggregation over patches did not change during the course of patch exploitation (Figure 8.3a). The parasitoids made more and longer visits to high-density patches (Figure 8.3; Figure 8.4). Obviously, aggregation arose from non-random patch selection and patch leaving rules.

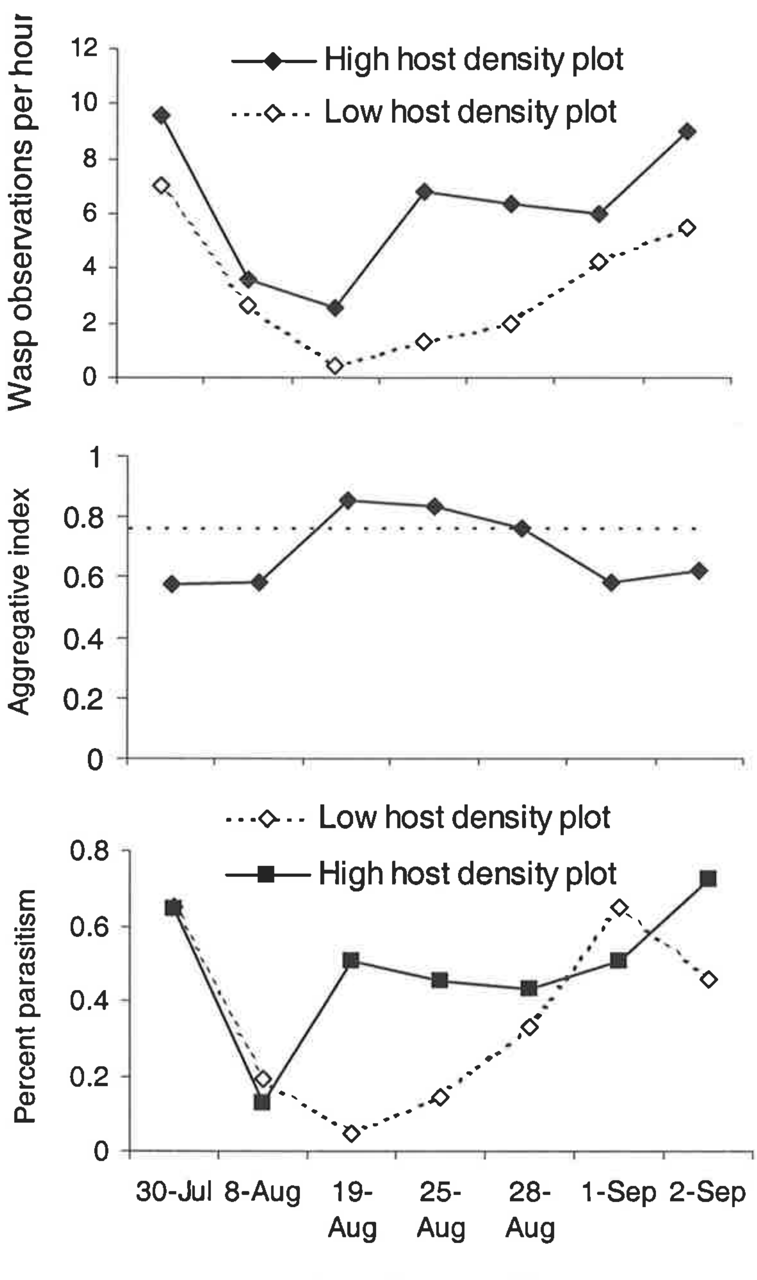


Figure 8.8 The relationship between the aggregative response of the field *D. semiclausum* population and percentage parasitism at the plot level. Top: the relative field population density of female *D. semiclausum*, estimated as total wasp observations per hour per plot. Middle: the aggregative index I_{plot} (I_{plot} = total number of wasp observations on the high host density plot / total number of wasp observations on the two plots). The dotted line represents the relative density index D_{plot} (D_{plot} = total number of hosts present in the high host density plot / total number of hosts present in the two plots, which is 0.77). Bottom: the pattern of percentage parasitism over time.

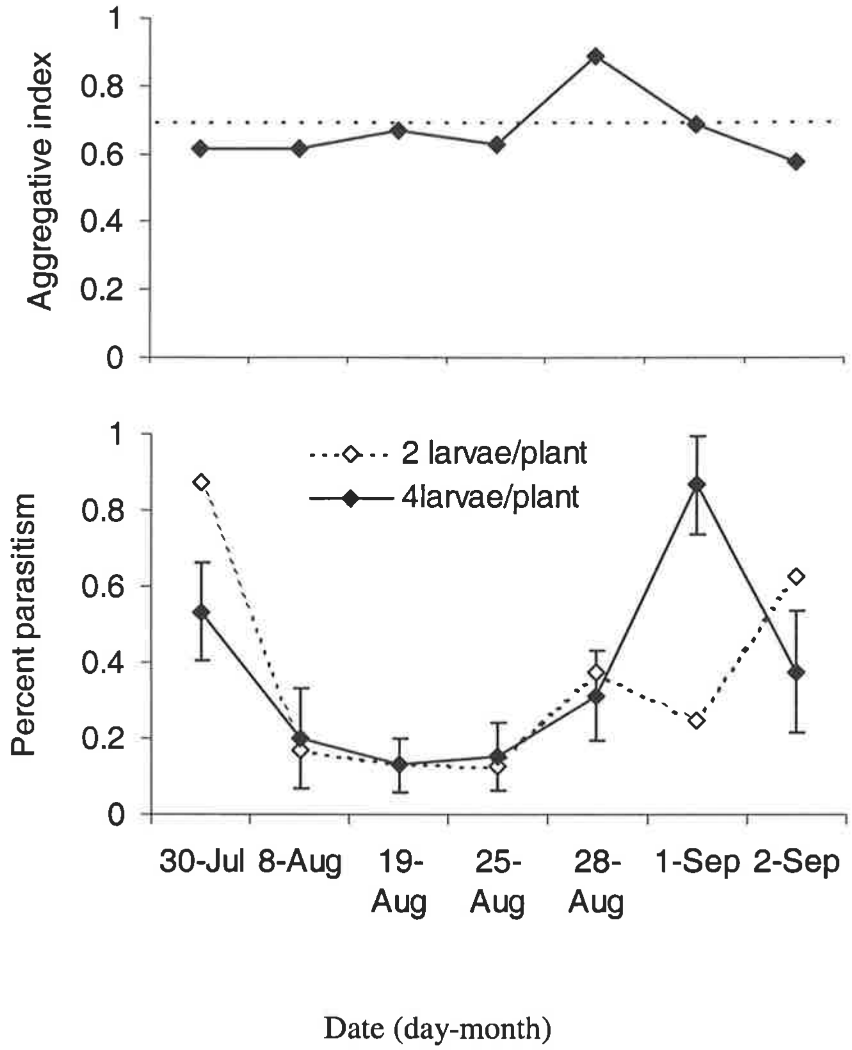


Figure 8.9 The relationship between aggregative response of the field *D. semiclausum* population and percentage parasitism per plant in the low host density plot. Top: the aggregative index I_{plant} (I_{plant} = total number of wasps observations on the higher host density plants / total number of wasp observations on the whole low host density plot). The dotted line represents the relative density index D_{plant} (D_{plant} = total number of hosts present on the higher host density plants / total number of hosts present in the whole low host density plot, which is 0.67). Bottom: the pattern of mean (± SE) percent parasitism (SE only present for the high host density plants). There was no significant difference in percentage parasitism between low and high host density plants (Student t-test, $p<0.05$, adjusted by the Sequential Bonferoni method (Rice, 1989))

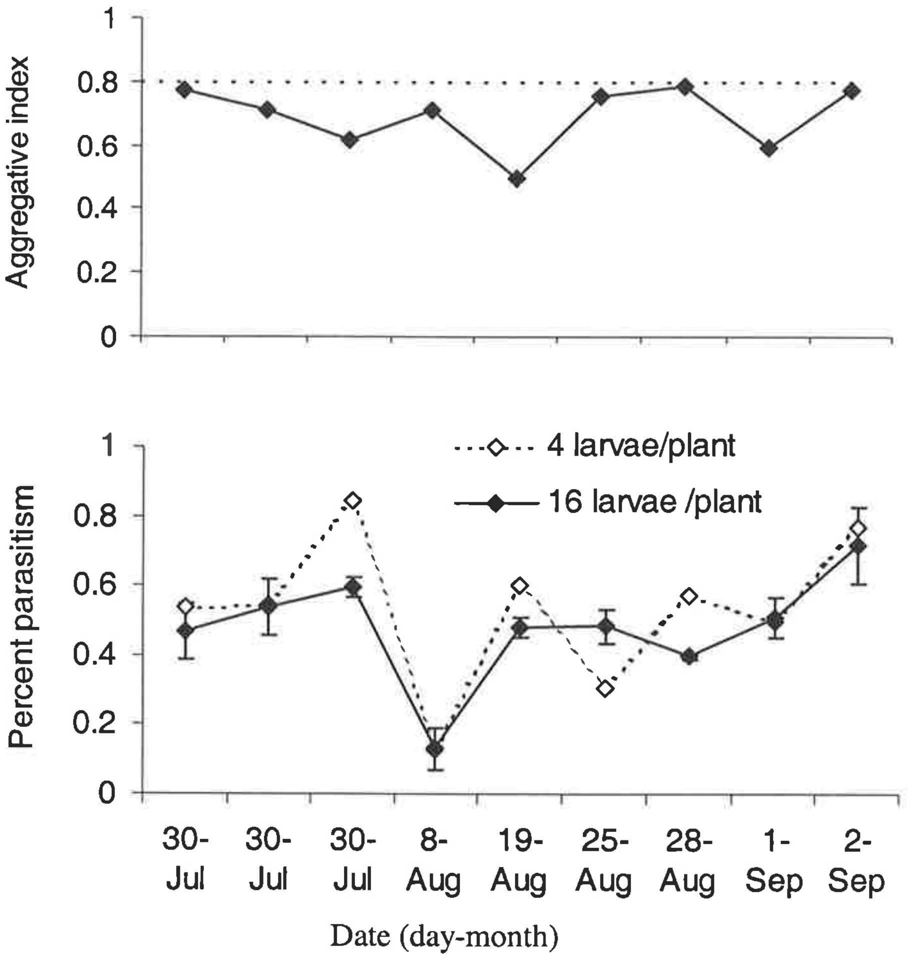
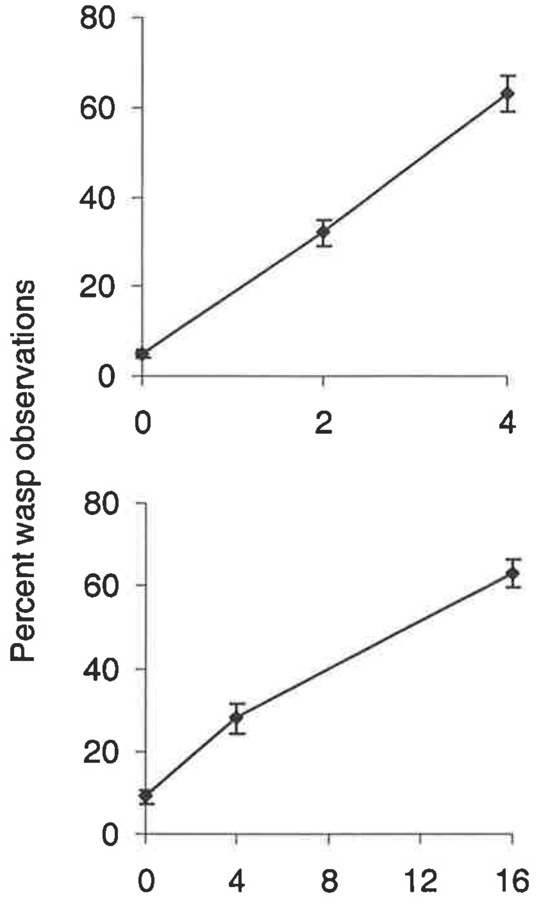


Figure 8.10 The relationship between aggregative response of the field *D. semiclausum* population and percent parasitism per plant at the high host density plot. Top: the aggregative index I_{plant} (I_{plant} = total number of wasps observations on the higher host density plants / total number of wasp observations on the whole low host density plot). The dotted line represents the relative density index D_{plant} (D_{plant} = total number of hosts present on the higher host density plants / total number of hosts present in the whole low host density plot, which is 0.80). Bottom: the pattern of mean (± SE) percentage parasitism (SE only present for the high host density plants). There was no significant difference in percent parasitism between low and high host density plants (Student t-test, $p<0.05$, adjusted by Sequential Bonferoni method (Rice, 1989)).



Host density per plant

Figure 8.11 Mean (± SE) percentage of *D. semiclausum* females observed on different host density plants. Top: low host density plot (n = 7); Bottom: high host density plot (n = 9).

Optimality models make the specific assumptions that the parasitoid is able to assess correctly the relative profitability of patches prior to or during patch exploitation (Charnov, 1976; Cook & Hubbard, 1976). Thus 'optimal' parasitoids should select patches based on their profitability, and leave patches based on the rate of oviposition (Charnov, 1976b; Cook & Hubbard, 1977; Comins & Hassell; 1979). Eventually all parasitoids should distribute themselves among patches such that each obtains the same encounter rate with hosts, i.e. the ideal free distribution (Sutherland, 1983). At equilibrium all patches are reduced to the same rate of oviposition, thus the proportion of parasitoids in the *i*th patch is equal to the proportion of available hosts in that patch. However, absolute assessment of patch profitability could be biologically unrealistic or unnecessary in this parasitoid. *D. semiclausum* do discriminate between the patches of different host densities as the frequencies of first visit increased nearly linearly with the host density per patch (Figure 8.4). However, with exploitation, the visits to patches of the highest host densities became less biased (Figure 8.3d), although this result could be affected by the non-random arrangement of experimental plants. *D. semiclausum* uses semiochemicals associated with the feeding activities of their hosts to locate host patches (Chapter 2). It is unknown how *D. semiclausum* assesses the initial patch quality based on odour cues (Chapter 6). *D. semiclausum* is a host specialist species. A specific parasitoid may utilise highly specific to semiochemicals while foraging for hosts (Geervliet *et al.*, 1998), but not be highly sensitive to them, as a high level of sensitivity might actually be disadvantageous. Under real conditions, an estimate of patch profitability based on kairomone concentration could be an unreliable measure of actual quality, because the effective host density depends on the number of available unparasitised hosts (Chapter 6). Hence, in this parasitoid patch selection could be less important than patch-leaving decisions.

In *D. semiclausum*, the patch residence time increased with host density, presence of host damage and unsuccessful encounters with hosts, and decreased with successful oviposition and cumulative visits to the same patch (Chapter 5-6). Thus, the parasitoids should make more and longer visits to high host density patches, and this has also resulted in an aggregation response of patch time allocation.

There was a large variation in searching activity among individual wasps (Table 8.2), but no relationship between searching activity and egg load was detected. This variation could be caused by individual genetic or phenotypic differences, or other factors beyond the behavioral control of the parasitoid such as host defense, which decreases the efficiency of parasitoid behaviour (Comins & Hassell, 1979). For a quantitative description, the non-optimal fixed aggregation response model (Hassell & May, 1973) might be used to describe statistically the observed patterns of patch time allocation by *D. semiclausum* in this study.

In the field the observed patterns of aggregation depended on the quality of the resource field, field parasitoid population density, and spatial scales. When the resource field was depleted, the parasitoid population dispersed to and aggregated in the artificial plots over time, and the relative degrees of aggregation to the high host density plot were low (Figure 8.7; Figure 8.8). When the habitat quality of the resource field seemed comparable to the adjacent experimental plots, the parasitoid population returned to the resource field as exploitation increased. The high level of dispersal in Field 1 could be a combination of active and passive aggregation responses.

At the plot level, when the field parasitoid population density was relatively low, the parasitoids aggregated more in the high host density plot (Figure 8.8). However, at the level of individual plants, the relative degree of aggregation decreased with increased host density (Figure 8.9; Figure 8.10). This suggested the importance of spatial heterogeneity or spatially hierarchical patch structure to which the parasitoid responds differentially (Chapter 7). Sheehan & Shelton (1989) found infestation level and patch size influenced patch leaving, but not patch finding, in a field population of the aphid parasitoid *Diaeretiella rapae*.

8.4.2 *Density-dependence of parasitism*

Although *D. semiclausum* was observed to have a positive aggregative response to high host density 'patches' over time and space, the laboratory test failed to detect density-dependent parasitism. Under field conditions, the daily pattern of parasitism varied over time and space.

The laboratory study identified the mechanisms that substantially govern the pattern of parasitism. Sufficient aggregative strength was necessary to outweigh the decreased foraging efficiency because of the interplay of host defense and adaptive superparasitism in *D. semiclausum*. Previous models explain diverse patterns of parasitism as a result of egg limitation and handling time (Hassell, 1982; Waage, 1983; Lessells, 1985; Smith & Maelzer, 1986), or interference (Sutherland, 1983), or stochastic variation in foraging behaviour (Morrison, 1986) or non density-dependent aggregation response (e.g. Reeve *et al.,* 1989). Optimality models are based on the premise that all patches should be reduced to the same oviposition rate (Cook & Hubbard, 1977; Comins & Hassell, 1979), while analytical models often assume the relative searching rate a is a fixed, species-specific constant (Hassell, 1982; Morrison, 1986). Thus these models are based on general predictions about the mechanisms deciding the patterns of parasitism. The laboratory study showed that the oviposition rate was always host density-dependent in *D. semiclausum* (Figure 8.5), and that the relative foraging efficiency varied with host density per patch. Direct interference rarely occurred and was independent of the host density per unit time. Egg limitation can not explain the observed pattern of wasp behaviour or parasitism (Table 8.2). In addition, avoidance of superparasitism was low in *D. semiclausum*. With single wasps in the wind tunnel, the parasitoid was found to readily superparasitise hosts (Chapter 3). In a multi-wasp experiment, the degree of superparasitism could be expected to be higher (Visser *et al.,* 1992).

Morrison (1986) explicitly predicted that stochastic variability in foraging time could result in different patterns of parasitism. But he did not address the mechanisms, which could have affected parasitism. There are indeed many sources of variability (Table 8.2). Some variability could be explained in this study: (1) density-dependent host defense should influence the encounter rate of a parasitoid (Chapter 2). In high host density plants, the hosts did not have enough space to move away to avoid the feeding site. In low host density plants, the host defensive behaviour would result in large variation in the oviposition rate. For example, the initial time to find a host is shorter when the host is on the feeding damage than when the host is resting away from it. Although the actual host density does not change, the effective host density depends on the feeding behaviour of the host. Furthermore, successful oviposition-motivated patch leaving decisions in *D. semiclausum* (Chapter 4-7) influence the variability. (2) On average, more time will be spent on waiting at high host density plants where the rate of unsuccessful encounters with hosts increased with host density (Figure 8.5). This balances out the increasing encounter rate on the high host density plants. (3) Due to adaptive superparasitism (Chapter 3), parasitism would be lower than the oviposition rate, particularly on high host density plants. However, as parasitised hosts often dropped off plants, re-encounter with the parasitised hosts could be low, particularly under field conditions.

The spatial scale or grain of environment can influence the density-dependence of host or prey mortality (Bernstein *et al.*, 1988; 1991; Walde & Murdoch, 1988). Bernstein et al. (1988; 1991) reported that at least three aspects of the interaction between predator or parasitoids and their resource population influence the spatial distribution of mortality in a host population: learning, distance between patches and structure of the environment. Their results suggest that at a small-scale if the parasitoids do not perceive variation in host density or at a very large scale if the parasitoids can not track the spatial changes in host availability, the resultant patter of parasitism should be spatially inverse density-dependent. While at the intermediate level, with small dispersal cost between patches learning parasitoids should be able to track spatial changes in the distribution of their hosts, and parasitism should be spatially directly density-dependent. However, in many previous studies the density-dependence in parasitism was found more often at larger spatial scales, possibility due either to the parasitoid's responses to these different scales or non-behavioral aggregation occurring at the larger scales (Walde & Murdoch, 1988). Thus the influence of spatial scale on density-dependence in parasitism depends on the parasitoid's ability to track the variation in host availability. It was found that *D. semiclausum* did respond to differences both in habitat quality and spatial scales in the field experiment. The resultant pattern of parasitism was related to the degree of aggregation relative to host density at the particular spatial level: (1) when the aggregative index was low, the resultant pattern of parasitism was approximately density-independent; (2) when the relative aggregation index was high, the resultant pattern of parasitism was directly density-dependent. Other factors such as variable handling times could also have influenced the pattern of parasitism as several field studies have suggested (Morrison & Strong, 1980, Waage, 1983; Smith & Maelzer (1986).

8.4.3 *Linking individual foraging behaviour and population dynamics*

Theoretical host-parasitoid interaction models have been based on inferred foraging behaviour to explain the population consequences of host-parasitoid interactions (Ives, 1995). Any parasitoid behaviour causing spatially heterogeneous parasitism contributes to the persistence of the interacting species (Beddington *et al.*, 1978; May, 1978; Chesson & Murdoch, 1986; Pacala & Hassell, 1991; Ives, 1995). However, the direct link between the aggregation response and density-dependence in parasitism appears to be weak. This was also demonstrated in the current study. Even though there was an obvious aggregation response, the resultant pattern of parasitism could be variable. Although population ecology studies are concerned with the pattern of parasitism, not foraging behaviour *per se*, conclusions drawn from these models concerning relationships between parasitoid searching behaviour and the spatial pattern of parasitism that occurs in field or laboratory systems should be viewed with caution until they are based a direct examination of parasitoid foraging rather than indirectly through an examination of spatial patterns of parasitism (Rosenheim *et al.*, 1989).

Although no single study of foraging in field parasitoid populations is going to lead to a general understanding of parasitoid behaviour, it is especially valuable to incorporate more biologically realistic behavioral attributes based on field-orientation studies into theory-driven host-parasitoid interaction models. Such an approach would bridge the gap between individual behavioral studies and ecological studies on host-parasitoid population dynamics.

Chapter 9 Experimental assessment of the impact of *D. semiclausum* on *P. xylostella* in the field

9.1 Introduction

The diamondback moth (DBM) *P. xylostella* is the most destructive insect pest of crucifer crops worldwide (Talekar & Shelton, 1993). It has developed resistance to many insecticides in many tropical and subtropical areas of the world (Cheng, 1988; Tabashnik *et al*, 1990; Talekar & Shelton, 1993), including Australia (Endersby & Ridland, 1997; Baker & Kovaliski, 1999). As a consequence, there is increasing interest in the development of biologically-based integrated management of DBM, in which the role of natural control is maximized to reduce the reliance on insecticides (Talekar & Shelton ,1993; Verkerk & Wright ,1996; Liu & Sun, 1998).

More than 10 parasitoids are associated with DBM in Australia (Yarrow, 1970; Goodwin, 1979). Of these, *D. semiclausum* is the most widely established and abundant species since its introduction into Australia in the early 1940's (Waterhouse & Norris, 1987). The parasitoid has been successfully introduced from Europe to many other countries (Waterhouse & Norris, 1987; Talekar & Shelton, 1993). In South Asia, *D. semiclausum* is well established in the cooler highlands of Malaysia, Philippines and Taiwan, and is providing substantial control of DBM in combination with *Bacillus thuringiensis* (*Bt*) in these areas (Ooi, 1992; Talekar & Shelton, 1993; Amend & Basedow, 1997). The successful establishment of *D. semiclausum* in these areas is most likely due to the parasitoid preferring a cooler temperatures in range of 15 to 25 °C (Talekar & Yang, 1991).

In Australia, the impact of natural enemies on DBM has not been experimentally evaluated in the field (Hatami, 1996). The main objective of this study was to evaluate the impact of natural enemies on DBM mortality using cage exclusion methods. The particular interest was the role of

D. semiclausum, a dominant larval parasitoid of DBM during the winter in Australia (Heisswolf *et al.*, unpublished data, 1996).

Cage exclusion is the most direct way to evaluate the impact of natural enemies on pest populations, and has been widely used in biological control programs (DeBach *et al.*, 1976; Luck *et al.*, 1988; Kidd & Jervis, 1996). The principle behind this method is that prey or host populations on sentinel plants from which natural enemies have been excluded, suffer lower predator-induced mortality or parasitism than populations on plants to which natural enemies are allowed access. This information can be used to show the effectiveness or shortcomings of existing natural enemies, the need for introducing new species, or the need to manipulate either the environment or the natural enemies to make biological control agents more effective (DeBach *et al.*, 1976). The exclusion cage method was employed to compare the survival of DBM cohorts when protected from natural enemy populations with that of cohorts exposed to natural enemies in the field.

9.2 Materials and methods

9.2.1 *Experimental design*

The experiment was conducted during winter at Gatton, Queensland 1999. A 50m x 20m field with transplanted broccoli seedlings in June 1999 was evenly divided into three plots subject to different management practices: integrated pest management (IPM), conventional insecticides spray schedule (CSS) and an unsprayed control (UNSP). During the experiments, the plots were monitored weekly for pest levels by sampling randomly 10 plants per plot. When a control threshold of 4-6 small DBM larvae per 10 plants was reached, the IPM plot was sprayed with *Bt* while the CSS plot was sprayed with either mevinphos or chlorfenapyr. No insecticides were applied to the unsprayed plot. Standard agronomic practices were used to grow the broccoli.

The experiment was repeated twice. The first trial started on 4th July, soon after transplanting, and the second trial started on 5th August and finished with the harvest of the broccoli on the 26th of August. Each trial consisted of four treatments: (a) fully caged; (b) open-caged; (c) open cage with a sticky barrier (selectively caged); and (d) no cage. The cage (40 cm high, 40 cm diameter) was constructed of a fine chicken wire net and covered by a fine Nylon mesh sleeve. The mesh size (25 cells per cm) was sufficiently small to exclude all natural enemies except tiny parasitoids such as *Trichogramma*. Each cage was held in place by two or three bamboo stakes. The bottom edge of each cage was buried approximately 10 cm in the soil. In the fully caged treatment, the sleeve was tied at the top to allow access for sampling. In either the open-caged or the selectively caged treatments, the cage was left open at the top to allow access of natural enemies, which still maintaining an environment similar to that in the fully closed cage. Additionally, with the selectively caged treatment, a sticky barrier (TAC-GEL, Rentokil Pty. Ltd., Australia) was set up on the outside of the cage to allow flying natural enemies to enter but selectively exclude entry of walking natural enemies. The uncaged plants were placed directly in the field.

The broccoli plants used as sentinel plants in the trials were grown in 14 cm diameter pots in a greenhouse until 6-8 fully extended leaves were present. The potted plants were then exposed to DBM adults from a laboratory culture in an insectary until each plant contained enough eggs for the trials. The initial density of 15-16 eggs (first trial) and 21-25 eggs (second trial) per plant was chosen in order to detect any treatment effects, yet low enough to ensure that no plants became severely defoliated during the experiment. All the experimental plants were carefully checked for eggs and any extra eggs were removed. Prior to the hatching of eggs, the plants were randomly placed into the three different field plots. In each of the three experimental plots, each cage treatment was replicated 4 to 5 times, and the experimental plants were placed 5-7 m apart.

The immigration of wild DBM into the experimental plants might be inevitable in the uncaged and open-caged treatments. Therefore, at the beginning of each trial, the plants in those treatments were checked at 3 day intervals to remove any newly oviposited DBM eggs. When most of the larvae on the trial plants had nearly completed their development, the trial was terminated, and the numbers of large DBM larvae (healthy, diseased, and parasitised) and pupae, and *D. semiclausum* cocoons were recorded. The DBM larvae and pupae were collected and maintained in a laboratory until DBM adults or parasitoids emerged.

In order to investigate the possible activities of egg parasitoids, 10 potted broccoli plants each containing about 20 freshly laid DBM eggs (collected within 12 hours) were placed 5-7 m apart into the unsprayed plot four times during the course of the trials. The eggs were collected from a laboratory culture of DBM and their positions on the leaves were marked. After a three-day exposure in the field, the plants were collected and the number of eggs remaining recorded, and reared in the laboratory to determine the levels of parasitism.

During the weekly monitoring of the DBM population in the field, the number of *D. semiclausum* cocoons was recorded. Although the comparison of parasitoid cocoons and DBM larval density could not give an accurate assessment of parasitism, such a measure indicates the population trends of both DBM and the parasitoid in the field. At the end of the second trial, a destructive sample of DBM larvae and pupae from different plots was collected to determine the overall levels of parasitism as a result of the different management practices.

9.2.2 *Data analysis*

The percentage loss and percentage parasitism was calculated to estimate the impact of natural enemies and other abiotic factors on DBM, and to compare the differences between different pest management practices. All proportional data were transformed by arcsin square root before an analysis of variance (LSD test, Statistix for Windows), and were back-transformed to proportions for presentation.

9.3 Results

The weekly monitoring only found a few other pests including aphids, thrips and *Pieris rapae* L. DBM was the dominant pest species, and its population density increased quickly at the beginning of the crop in the unsprayed plot (Figure 9.1). With the increasing *D. semiclausum* population, the DBM population decreased (Figure 9.2).

Within each pest management treatment, both trials showed that the DBM populations suffered the greatest loss in the uncaged treatment (Table 9.1). There was no significant difference in the mean percentage of DBM recovered, including those that were parasitised, among the fully caged, open-caged and selectively-caged treatments (Table 9.1).

Within each cage treatment, there was no significant difference in the mean percentage of DBM recovered between the different spray management treatments (Table 9.1).

Of the larvae that remained, 72 to 94% were parasitised by *D. semiclausum* in the uncaged treatments, much more than in the open or selectively-caged treatments (8-37% first trial, and 38-63 % second trial; Table 9.2). Within each cage treatment, this percentage parasitism did not differ significantly between the different pest management practice, except in the second experiment, where in the open-caged treatment, the percentage parasitism was significantly lower in the IPM plot than the other two management plots. The overall parasitism by *D. semiclausum* in both the open-caged and selectively-caged treatments was higher in the second trial than in the first trial (Table 9.2).

Table 9.1 Percentage (Mean ± SE) of recovered DBM at the end of the experiments from closed, open, selectively open and uncaged treatments in plots of different pest management practices.

		Initial egg	% Population recovered		
Treatment	Replicates	density	IPM	UNSP	CSS
First trial					
Closed cage	4	15-16	91.6 ± 4.2 Aa	91.7 ± 1.7 Aa	93.3 ± 7.3 Aa
Open cage	4	15-16	73.3 ± 8.6 Aa	70.9 ± 6.9 Aa	81.9 ± 4.2 Aa#
Selective cage	4	15-16	72.3 ± 5.3 Aa	72.3 ± 6.5 Aa	75.9 ± 9.8 Aa
No cage	8	15-16	36.7 ± 7.0 Ab	26.3 ± 8.6 Bb	45.7 ± 5.9 Ab
Second trial					
Closed cage	5	21-24	95.6 ± 3.2 Aa	93.2 ± 5.1 Aa	94.7 ± 3.0 Aa
Open cage	5	23-24	86.3 ± 3.2 Aa	86.2 ± 5.1 Aa	85.5 ± 7.3 Aa
Selective cage	5	22-25	89.5 ± 9.2 Aa	87.2 ± 9.3 Aa	83.4 ± 5.7 Aa
No cage	5	23-24	31.7 ± 15.8 Ab	20.2 ± 8.6 Ab	16.7 ± 5.4 Ab

* IPM – Integrated pest management; CSS – Conventional spray schedule; UNSP – Unsprayed. Percentage of initial DBM recovered was calculated as [(live DBM 4th instar larvae + live DBM pupae + diseased DBM 4th instar larvae + *D. semiclausum* cocoons) / initial egg density] x 100. Results of each experiment were compared between different treatment within same pest management practices and between different pest management practice with same treatment, respectively, using one-way ANOVA (STATISTIX for Windows). Within each plot, means in the same column followed by the same lower case letter are not significantly different (*P*>0.05, LSD). Within each treatment, means in the same row followed by the same capital letter are not significantly different (*P* > 0.05, LSD).

All larvae in one of the replicates were diseased, and the replicate was not included in the analysis.

Table 9.2 Percent parasitism (Mean ± SE) by *D. semiclausum* of the recovered DBM larvae at the end of each trial from closed, open, selectively open caged and uncaged treatments in plots of three different pest management practices.

Treatment	Replicates	% Parasitism		
		IPM	UNSP	CSS
First trial				
Closed cage	4	-	-	-
Open cage	4	8.5 ± 3.2 A a	36.7 ± 17.3 A a	25.5 ± 13.2 A a
Selective cage	4	8.3 ± 3.2 A a	8.6 ± 3.4 A a	4.8 ± 4.1 A a
No cage	8	93.6 ± 3.2 A b	84.6 ± 6.8 A b	92.6 ± 2.3 A b
Second trial				
Closed cage	5	-	-	-
Open cage	5	38.2 ± 6.2 A a	55.3 ± 5.6 AB a	63.2 ± 5.8 B a
Selective cage	5	49.8 ± 19.6 A ab	44.9 ± 3.8 A ab	43.9 ± 9.9 A ab
No cage	5	75.9 ± 21.8 A b	71.7 ± 9.0 A b	88.5 ± 15.7 A b

* IPM – Integrated pest management; CSS – Conventional spray schedule; UNSP – Unsprayed. Means in the same column followed by the same lowercase letter are not significantly different within each plot (*P*>0.05,Duncan's multiple range test). Results of each experiment were compared between different treatment within same pest management practices and between different pest management practice with same treatment, respectively, using one-way ANOVA (STATISTIX for Windows). Within each plot, means in the same column followed by the same lower case letter are not significantly different (*P*>0.05, LSD). Within each treatment, means in the same row followed by the same capital letter are not significantly different (*P* > 0.05, LSD).

On average, about 50% of the eggs were lost during the three-day exposures, and no egg parasitoids were discovered during the experiments (Table 9.3). The overall parasitism by *D. semiclausum* was not significantly different among the plots ($p = 0.2210$, Table 9.4).

9.4 Discussion

DBM is the dominant pest of brassica vegetables during winter in Australia. This study demonstrated that *D. semiclausum* is active during the winter, and plays an important role in suppressing larval DBM in the field. No other larval and egg parasitoids were discovered during the experiments (Table 9.3). Two DBM pupal parasitoids, *Diadromus collaris* (Gravenhorst) (Hymenoptera: Ichneumonidae) and *Brachymeria* spp, were recorded from the collections of DBM pupae on the experimental plants. Parasitism by the pupal parasitoids was low, except in the open-caged treatment in one trial where pupal parasitism by *D. collaris* was up to 22%. However, the pupal parasitism was probably underestimated because the experiments were terminated before all larvae had pupated. One fungal disease, possibly a *Zoöphthora* was found in a few of the cages. Up to 13% of the recovered DBM larvae were diseased by this fungal pathogen at the end of experiments. Predators observed on the experimental plants included spiders, Coccinellids and few Hemiptera. The loss of eggs and larvae was high in the open-caged treatment (Table 9.1). These losses are presumed to be abiotic induced mortality factors, such as rain and wind, as well as predator activity. The separate effects of biotic (parasitoids and predators) from the abiotic factors were not examined in this study.

Table 9.3 Percentage loss (Mean ± SE) of DBM eggs over the three-day exposure in the unsprayed plot.

Exposure date	Replicate	Initial egg density/ plant	% Loss*	% Parasitism
12-14 July	10	21	56.1 ± 8.4	No
18-20 July	10	19	41.4 ± 8.1	No
12-14 August	10	16	53.5 ± 9.7	No
25-27 August	10	23	51.6 ± 8.6	No

* Percentage loss between experimental dates was not significantly different ($P = 0.6501$, One-way ANOVA, STATISTIX for Windows).

Table 9.4 Field sample of DBM larvae and the parasitism (Mean ± SE) by *D. semiclausum* at the end of the second trial.

Plot	N	DBM density /plant*	% Parasitism
UNSP	20	1.6 ± 0.35 a	81.3 ± 7.3 a
IPM	10	11.6 ± 1.43 b	76.7 ± 4.1 a
CSS	20	1.6 ± 0.38 a	91.8 ± 4.8 a

*Numbers include the parasitised and unparasitised DBM individuals. All means in the same column were compared using one-way ANOVA (STATISTIX for Windows), and that followed by the same letter are not significantly different ($P>0.05$, LSD test).

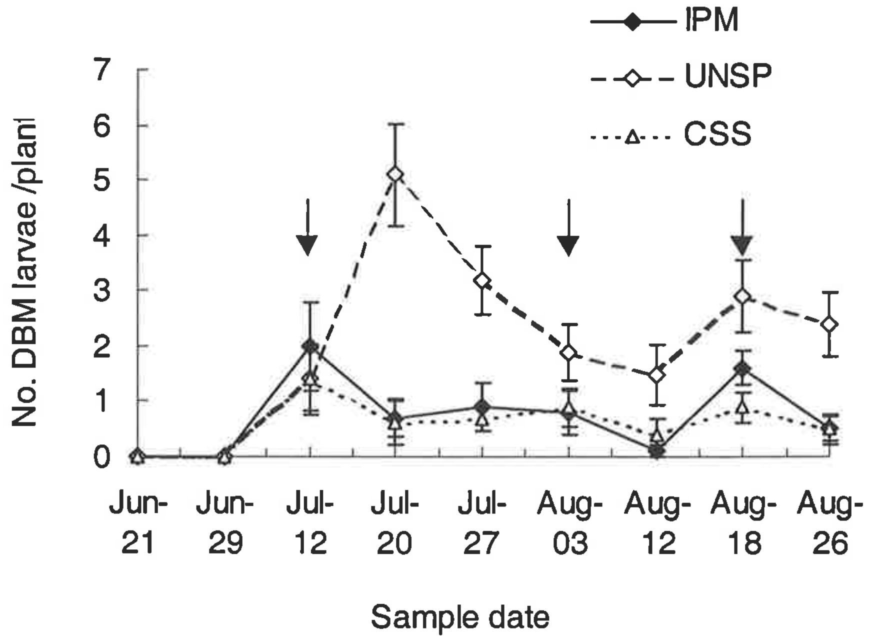


Figure. 9.1 Seasonal abundance (Mean ± SE) of *P. xylostella* larvae in plots with different pest management practices. IPM – Integrated pest management; CSS – Conventional spray schedule; UNSP – Unsprayed. Arrows indicate the spraying dates in both IPM and CSS plot.

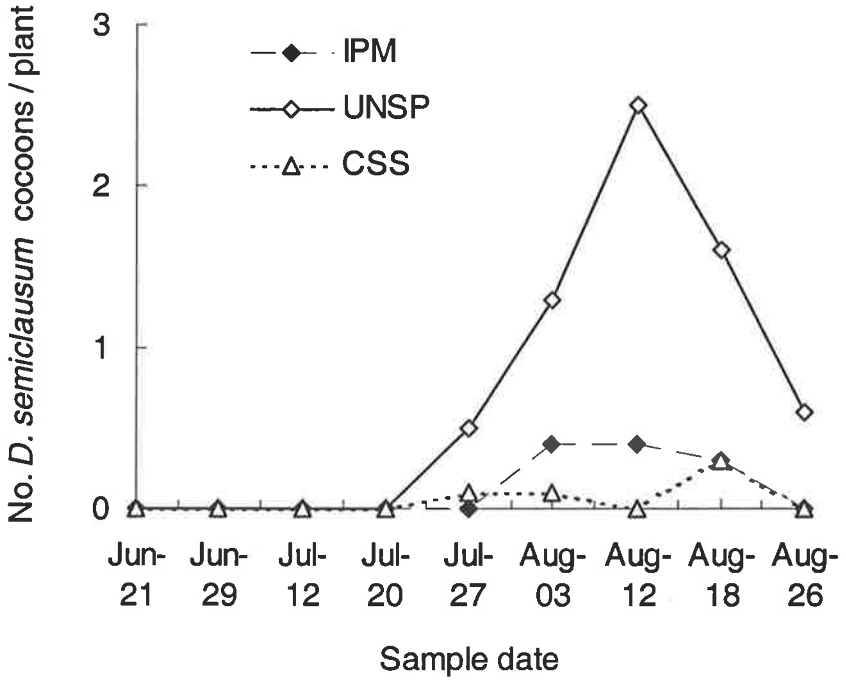


Figure 9.2 Seasonal abundance (Mean ± SE) of *D. semiclausum* cocoons in plots with different pest management practices. IPM – Integrated pest management; CSS – Conventional spray schedule; UNSP – Unsprayed.

The cages may influence the suitability or accessibility of DBM to predators and parasitoids. The major disadvantages of cage exclusion experiments are that cages might alter microclimate (temperature, humidity and light) and restrict the movement of pests and natural enemies (Luck *et al.*,1988; Kidd & Jervis, 1996). The effect of such microclimatic changes on the natural plant-host system can be important (Luck *et al.* 1988), depending on factors such as the plant species, the phytophage, cage design, and weather. Ambient temperature differences between the interior and exterior of the cage may influence the rate of development of the host, and consequently influence the rate at which the host is attacked. Humidity differences may influence the rate of disease development. Light intensity may affect the growth of the caged plants and thus influence the host insects and the behaviour of their natural enemies by changing host behaviour and physiology, as a result of microclimate-induced alterations in plant physiology. In this study, no obvious difference in the developmental rate of the DBM larvae in the caged, open-caged and uncaged treatments was observed. Also, there was no trend showing higher rates of disease development of the DBM larvae in the fully-caged (2-7%) and open-caged (1-6%) treatments. Although microclimatic variables such as light intensity, ambient temperature and humidity were not measured but presumed to be similar in the caged, open or uncaged treatments.

There was no significant difference in the mean percentage of DBM recovered and parasitism of the larvae remained between the open-caged and or selectively-caged treatments. Thus, the selectively-caged treatment appears to be not necessary for further experiments.

The levels of DBM parasitised by *D. semiclausum* were much higher in the uncaged treatment than the open-caged or selectively-caged treatments. There are two possible explanations. Firstly, the cages may create a physical barrier for the access of parasitoids to the host, and thus have directly affected the host-finding behaviour of the parasitoid. This suggests changes in cage design may be appropriate for further experiments, and the effect of the cage structure on the parasitoid's host-finding behaviour needs to be tested in separate experiments. Secondly, the inversely density-dependent parasitism could result in insufficient aggregation on high host densities in field. The foraging time allocation of a field population of *D. semiclausum* was observed during the same experimental dates and found a significant aggregation response of the parasitoid on high host density plants or plots (Chapter 8). However, the resulting pattern of parasitism largely depended on the relative degree of aggregation of the parasitoids to host density at different spatial scales. When the relative degree of aggregation was low, the resultant pattern of parasitism was generally density-independent (Chapter 8). In this study, low parasitoid density in the field probably could affect the relative degree of aggregation on high host density plants, and consequently result in the inversely density-dependent parasitism.

Although the CSS plot was sprayed using mevinphos and chlorfenapyr, and the IPM plot was sprayed using only *Bt* three times during the experiments (Figure 9.1). The overall parasitism by *D. semiclausum* was not significantly different among the plots (Table 9.4). The minimal influence of pest management practices on the percentage parasitism in this experiment could be due to the relatively small-area of the plots.

Based on the work presented here, the dominant role of *D. semiclausum* parasitising DBM has been noted during the winter growing season. It is likely that both the larval and pupal parasitoids can achieve a high level of natural biological control. Integrating parasitoids with *Bt* and other microbial agents should deliver effective pest management. This would minimize the problems of insecticide resistance and excessive insecticide use during the winter. However, further experiments with modified cage design are needed to test the effects of different pest management practices on natural control role.

Chapter 10 General discussion

From a practical perspective, this study has produced in a better understanding of the relatively higher foraging efficiency of *D. semiclausum* compared to *C. plutellae*. From a theoretical perspective, the detailed investigation into oviposition decisions and patch use rules of *D. semiclausum* has delivered new insight into the individual decision-making processes of parasitoids while foraging in patchy environment. Both contributed to the conceptual link between individual behaviour and population dynamics. This concluding chapter presents an overview of the major research findings of this thesis and their significance, along with a discussion of the possibilities for future work in these two complementary lines of behavioural ecology of insect parasitoids.

10.1 Project overview

10.1.1 *A practical perspective*

This study has elucidated the foraging behaviour of *C. plutellae* and *D. semiclausum,* the two major parasitoids of larval *P. xylostella* (Chapter 2). The specialist species *D. semiclausum* is more effective than the oligophagous species *C. plutellae* in parasitising and overcoming host defence. In host detection, *D. semiclausum* employs a strategy that includes visual contact and *wide-area* search while *C. plutellae* displays *area-restricted* search. In host location, *D. semiclausum* often *waits* for escaping hosts while *C. plutellae* usually *pursues* a host. As a consequence, *D. semiclausum* shows a more stereotyped behavioural pattern leading to oviposition, but *C. plutellae* exhibits a more plastic behavioural pattern. This shows that specialist parasitoids display specialised adaptations for host location, while generalist parasitoids display a relatively plastic foraging behaviour (Vet *et al.*, 1993; Godfray, 1994; Wiskerke & Vet, 1994; Geervliet *et al.,* 1996; Cortesero *et al.*, 1997).

The relationship found here between host defence and the parasitoids' counter ploys suggest several predictions for the outcomes of interactions between the two species and their host. Firstly, older larvae would be less susceptible to aggressive attack and thus should be less likely to be parasitised by *C. plutellae* than by *D. semiclausum.* Smaller larvae, however, should be easily located by both species, particularly *C. plutellae*. Previous studies on the pattern of host stage acceptance by both parasitoids assumed that both parasitoids preferred specific instars based on observed patterns of parasitism (Talekar & Yang, 1991). However, this apparent preference could have reflected the comprehensive outcome of behavioural interactions between the host and parasitoids. Secondly, the defensive behaviour elicited by the presence of parasitoids significantly increases larval movement and thus contact with infective units of the entomopathogenic fungus such as *Zoöphthora radicans* Brefeld (Furlong & Pell, 1996). There should be a greater increase in the probability of infection when *D. semiclausum* is present than *C. plutellae*, due to the response of host larvae to the wide-area searching behaviour of *D. semiclausum.* Thirdly, the difference in the parasitoid's ability to overcome the host defence between the two species should reduce their extrinsic competition for hosts.

In most Asian counties, where *C. plutellae* and *Oomyzus sokolowskii* (Kudj.) usually occur (Wang *et al.,* 1999), the desired level of DBM control is not achieved by the native parasitoids alone. Thus the introduction of *D. semiclausum* into such areas may result increasing exploitation of the host population, particularly when host-densities are low (see Chapter 9).

10.1.2 *A theoretical perspective*

A major theme of this thesis is the central issue of foraging theory in parasitoids, i.e. patch time allocation, which includes (1) patch selection; (2) oviposition decisions; and (3) patch-leaving decisions. The key to understanding these individual decision-making processes in parasitoids is to determine how they gather information about resource abundance and distribution over patches. The proposed foraging theory models are based on *a priori* modelling (Charnov, 1976b; Waage, 1979; Stephens & Krebs, 1986; Driessen *et al.*, 1995), and obviously do not capture the dynamic nature of parasitoid foraging behaviour. The derivation of behavioural rules directly from the observed data using statistical modelling, as employed in this study, permits incorporation of the effects of many factors on behavioural activity.

The oviposition decisions of *D. semiclausum* were investigated in detail (Chapter 3). The parasitoid randomly encounters a host and the decision to oviposit requires ovipositor probing, which appears to be an adaptation to the host's defence. The parasitoid's decisions to accept or reject a host are dependent on host quality, and are dynamically adjusted by the patch residence time and egg depletion. No other experience had a detectable significant effect on oviposition decisions. Further statistical modelling showed that the parasitoid was able to distinguish between hosts: unparasitised, parasitised by a conspecific female (overall), parasitised by herself (past), and parasitised by herself (present). However, contrary to the theoretical predictions of a static optimality model (Hughes, 1979; Iwasa *et al.* 1984; Harvey *et al.*, 1987; Janssen, 1989; van Alphen & Visser, 1990) and an evolutionarily stable strategy (ESS) model (Visser *et al.*, 1992) of oviposition decisions in solitary parasitoids, both self- or conspecific- superparasitism were very common in *D. semiclausum*. The common occurrence of superparasitism in *D. semiclausum* may confer an adaptive advantage in terms of the relatively high cost involved in locating hosts and overcoming host defence. Alternatively, it may reflect the parasitoid's inability to reliably assess host quality, which would constrain its host discrimination ability. The dynamic oviposition decisions in *D. semiclausum* highlighted the importance of the debate of time- vs. egg-limitation for pre-ovigenic parasitoids (Roitberg, 1989; Rosenheim, 1996; Heimpel *et al.*, 1998; Mangel & Heimpel, 1998; Sevenster *et al.*, 1998; Rosenheim, 1999; Casas *et al.*, 2000; Papaj, 2000).

D. semiclausum has limited information prior to a patch visit. This has been supported by the analysis of first landings and the frequency of visits to patches of different host density (Chapter 2 & 8). The first landing occurs mostly on infested plants, indicating host-associated cues such as kairomones are used in patch location or selection. Two factors may influence the patch selection decisions. Firstly, the parasitoid may not be able to assess correctly the exact concentration of kairomone in a given area, particularly at higher spatial scales of reference. Secondly, there is no close relationship between kairomone concentration and the number of available hosts. Because the value of the pre-patch information about the its environment is very limited, this may be the reason that the parasitoid's patch selection might not be so closely correlated with host density, while patch-leaving based on the within-patch experience has a more significant role in determining the patch time allocation.

The patch-leaving rules of *D. semiclausum* were analysed by means of Cox's proportional hazards model (Chapter 4-6). The patch-leaving tendency decreased with increased host density or presence of host damage, clumped host distribution, unsuccessful encounters with hosts, and increasing inter-patch distances. Successful oviposition, self-superparasitism, rejection of parasitised hosts, and unsuccessful search time since last oviposition increased the patch-leaving tendency. None of the simple rules of thumb (Stephens & Krebs, 1986) such as fixed searching time, fixed oviposition number, or fixed giving-up time was likely to have been employed by *D. semiclausum*. The results agreed with the general predictions of the Marginal Value Theorem (Charnov, 1976b) that both patch residence time and number of ovipositions of *D. semiclausum* increased with increasing inter-patch distance and host density. The incremental influence of oviposition on the patch-leaving tendency regardless to host density and distribution, indicated that a count-down mechanism gave the best predictions of the patch-leaving decisions of *D. semiclausum* (Driessen *et al.*, 1995). Overall, the patch-leaving tendency is adjustable based on the experience in a particular environment. The patch residence time of parasitoids is determined in a more complex way than described by simple models. The parasitoid is considered as continually adjusting its probability per unit time to leave, according to 'rewarding' and 'unrewarding' experience. A conceptual model of the patch exploitation for *D. semiclausum* is proposed based on this work and the proportional hazard analysis of the patch-leaving tendency (Figure 10.1). However, it is expected that additional factors could also influence the patch-leaving tendency of *D. semiclausum*. The true hazard functions for a specific individual and a specific event could largely depend on the conditions that the individual experienced.

It should be noted that the covariates used in the analysis of patch leaving tendency of parasitoids using proportional hazards model are set only at the start of the foraging period when the wasp enters the patch or when the wasp self-censors the observation by ovipositing or performing some other act (Haccou *et al.*, 1991). The covariates that are found to influence the leaving tendency are those which exist at the start of each measured period of searching. Some of these may change during the course of a patch visit, but these changes in covariates are not included in the analysis. Thus, it is impossible to determine factors that finally trigger patch leaving (Keller & Tenhumberg, 2000). The statistical modeling of behavioral rules should be considered as an extension to the usual *a priori* modelling. On the one hand, *a priori* consideration is used to determine which covariates are potentially relevant. On the other hand, the results of such statistically derived behavioral rules should provide important information for refinement of evolutionary and mechanistic behavioral models and directions for further testing or the development of more realistic behavioural models. The statistical method can only provide a test of the relative importance of factors influencing the patch-leaving tendency under particular conditions, which usually have been considered separately in optimality models. While the importance of some covariates and their effects may be consistent under different conditions, some factors could cease to be important when the environment has changed due to the interactions among variables. Thus, the general context of this work is the understanding of decision-making by a foraging parasitoid from both the functional and mechanism point of view, rather than absolute predictions of parasitoid behaviour. The interplay between experimental and theoretical studies is important for furthering the understanding of foraging behaviour in parasitoids.

The patch leaving tendency of *D. semiclausum* changes as its gains experience and moves within and among different spatial levels in a multi-plant environment (Chapter 7; Figure

10.1). The parasitoid seemed to use different patch-leaving rules at different spatial levels. At the largest of the spatial levels, the parasitoid appeared to gather less information or cues to decide when to leave a given area. This suggested that the parasitoid may perceive a multi-plant environment as a hierarchy of patches, and its patch-leaving decisions might be spatially hierarchical. However, it is possible that the different results at the three spatial scales of reference were artefacts of the statistical method used. The effects of some covariates many be masked by others at different spatial scales due to their concurrent occurrence or lower statistical power at large spatial scales.

The optimal foraging predictions and the population consequences of the differential patch exploitation by *D. semiclausum* were tested under laboratory and field conditions (Chapter 8). In the laboratory, the parasitoid displayed an unchanging pattern of aggregation in patches of varying host densities during patch exploitation. In contrast to the optimal foraging predictions (Cook & Hubbard, 1977; Comins & Hassell; 1979; Sutherland, 1983), patches were neither exploited in order of profitability nor exploited differentially until the same oviposition rate was achieved in each patch. The resulting pattern of parasitism was density-independent, which mainly depended on the interaction between the tendency to aggregate and the conflicting mechanisms that determine the relative foraging efficiency such as density dependent host defence and handling time. In addition, superparasitism in this parasitoid also countered the density dependence of parasitism. In the field, the parasitoid population also showed a positive aggregation response to plants with a high host density over time at two spatial scales: single plants and groups of plants. However, the resultant pattern of parasitism depended on the relative degree of aggregation to host density at a given spatial scale. The results suggest that the direct link between the optimal foraging predictions and aggregation responses appears to be weak in *D. semiclausum*, and the spatial density dependence in parasitism could depend on the relative degree of aggregation in the field.

10.2 Future research

10.2.1 *Evaluating parasitoids for biological control: behavioural approaches*

The host-parasitoid system of *P. xylostella* and the two major larval parasitoids is a well-documented case of classical biological control. In spite of the world-wide importance of biological control of *P. xylostella*, previous studies have mostly focused on the comparison of the basic characteristics of the biology and ecology of these two parasitoids (Talekar & Shelton, 1993), and very little was known about their relative foraging efficiency until now (Verkerk & Wright, 1996). In order to predict levels of parasitism by these parasitoids, a thorough understanding of the behavioural ecology of the tritrophic interactions involved is needed (Verkerk & Wright, 1996). The behavioral approach, when combined with other aspects of the host-parasitoid interaction, including chemical ecology and analysis of population dynamics, will give insight into the evolutionary dynamics of the host selection strategies in parasitoids (Vet & van Alphen, 1985; van Alphen & Vet, 1986; Vet & Dicke, 1992). Thus future research on these topics will substantially improved our understanding of this particular host-parasitoid system, and will help in development of more useful criteria for predicting the impacts of effective natural enemies.

10.2.2 *Link between individual behaviour and population dynamics*

While practical biological control is concerned with the control efficiency of a biological control agent, the ecological theory that supports biological control is concerned with the persistent host population regulation of a pest by its parasitoids (host-parasitoid stability) and attempts to predict the host-parasitoid population dynamics based on demographic and behavioral processes. In particular, the relevant theory of population dynamics is concerned with the behaviour of parasitoids in an environment in which hosts have a patchy distribution.

In order to better understand the link between individual behaviour and population dynamics, two types of research are needed. Firstly, laboratory and simple patch-level experiments are needed to obtain a thorough understanding of the individual decision-making of parasitoids on patch exploitation. An important issue is to understand how parasitoids behave to maximize their lifetime rate of host encounter and reproduction. Thus, testing of evolutionary and mechanistic behavioral models can elucidate the link between individual behaviour and host-parasitoid population dynamics, such as how a behavioral trait affects the fitness of individual parasitoids and parasitism, and thus parasitoid-host population dynamics. Substantial progress in understanding the foraging behaviour of *D. semiclausum* has been achieved. Though *a priori* models have been taken to propose useful hypotheses for experimentation in the past, statistical analysis of behavioral rules should play a greater role in testing and refining those models. As demonstrated in this thesis, the combined use of descriptive statistical models and general predictions from theoretical optimality models makes it possible to analyze empirical data in a functional context. Furthermore, this approach can indicate the relative importance of different factors for modeling optimization problems. It can expected that there will be a much greater use of statistical modeling in parameterising host-parasitoids models. The model parameters can be used in simulations aimed at comparing the performance of complex and simple decision rules in different environments, taking into account the constraints on the animal's informational state.

Secondly, it is necessary to create stronger links between experiments, both in the laboratory and in the field, and theoretical population models. Host-parasitoid population dynamic models have usually been derived from theoretically optimal behavioral processes. Empirical results from experimental studies on the behaviour of natural enemies in a multi-patch environment, preferably in natural environment, can provide the information needed both to test and refine theoretical models. It would be especially valuable to incorporate more biologically realistic behavioural attributes based on field-oriented studies into theory-driven host-parasitoid interaction models in the future. The approach would bridge the gap between individual behavioural studies and ecological studies on host-parasitoid population dynamics. Otherwise, the theoretical models could miss important biological phenomena and thus could be misleading. For example, theretical studies have shown that aggregation can be an important factor stabilizing host-parasitoid interactions as a result of density-dependence in parasitism. However, the exact role of aggregation on the density dependence in parasitism is not always certain, as this study has shown. Both theoretical and experimental studies are required to resolve these problems. There will be a move from the classical simple models of host-parasitoid population dynamics to more realistic mechanisms models designed to understand specific interactions. More types of behavioral ecological studies of parasitoids in the fields are urgently needed.

Thus, in behavioral ecology of insect parasitoids, there will surely be continuing interest in applying optimality theory to parasitoid behaviour. Understanding how parasitoids exploit patchy resources will not only provide better predictions of parasitoid foraging efficiency from a practical perspective, but will also provide a link between individual behaviour and population ecology.

Appendix Insect culture

A laboratory population of the diamondback moth (DBM) *Plutella xylostella* was established from field collected larvae from the suburb of Adelaide, South Australia in 1994, and was maintained at a constant temperature of 24-25 °C, and a photoperiod of 14L:10D in the insectary of the Department of Applied and Molecular Ecology, Adelaide University. Common cabbage (*Brassica oleracea* var. *capitata*, cv. Green Coronet) was grown in pots (12.5 x 12 x 8 cm) in potting soils in a glasshouse, and were used as food plants of *P. xylostella*.

Separate cages (50 x 30 x 30 cm) were provided for moth oviposition, rearing of larvae, and pupae and adult emergence. Each cage consisted of four sides confined by fine mesh nylon and the bottom was a metal plate. One vertical side that could be closed and opened served as a door.

A potted-young plant was placed into the oviposition cage containing hundreds of adult DBM. 20% honey solution in a container with wick on it was provided in the cage. After one or two days, the plant with newly deposited eggs was removed from the oviposition cage and put into a rearing cage. Dead moths in the oviposition cage were removed with a vacuum. The wick and honey solution was changed regularly.

The rearing cages containing plants and larvae were set-up in a sequence based on the larvae developmental stages. The plants were watered regularly and when the leaves were almost consumed by the larvae, fresh plants were supplied for them in close contact to ensure that plenty of food plants were always available. The larvae moved onto these new plants and the following day the remnants of the old plants were discarded.

Newly formed pupae were collected in a plastic cup and transferred into the oviposition cage.

The used and dirty cages were washed with detergent, and some bleach (sodium hypochlorite) was added as a disinfectant.

Cotesia plutellae was imported from the Asian Vegetable Research Development Center, Twain in 1998 (Dr. N. S. Talekar). The laboratory population was reared on larval DBM from the culture under the same condition as the DBM culture. It required four cages (50 x 30 x 30 cm) for oviposition and development of the parasitoids, and one small cage (16 x 16.5 x 21 cm) as a holding cage that only kept adult wasps. A new rearing cage was set up each week as an oviposition cage. A young potted cabbage plant bearing about 200 larval DBM in the second and third instars was placed into the cage. Another fresh plant was added to give plenty of food for the hosts. After placing water in a container with a wick and some drops of honey were smeared on the top of the cage, about 20 mated female parasitoids were taken from the holding cage and released into the oviposition cage. They were left to oviposit for about 3-4 days, then the wasps were removed from the oviposition cage with a vacuum.

Fresh plants were added to cages as necessary to ensure that the food for hosts was always sufficient. Any moths that had emerged were removed from the cage using a vacuum. Wasp cocoons were collected (usually 3 weeks after a cage was set up) and were placed into the holding cage if not for experiments. The water and the wick were replaced regularly and honey was added to the holding cage if necessary. Dead wasps were removed from the holding cage using an aspirator.

A culture of *Diadegma semiclausum* was established from parasitised larval DBM collected from Adelaide suburb in October 1997. Each year new field collected individuals were added to the laboratory population to maintain genetic diversity. All the wasps used in the experiments were the offspring of the individuals that have been reared for 1-2 generations in the laboratory. The *D. semiclausum* culture was maintained in the same way as *C. plutellae*.

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