

Parasitoid Learning and Intraguild Predation and their Effects on the Biological Control of Aphids



Von der Naturwissenschaftlichen Fakultät
der Gottfried Wilhelm Leibniz Universität Hannover
zur Erlangung des akademischen Grades eines

Doktorin der Gartenbauwissenschaften

- Dr. rer. hort. -

genehmigte

Dissertation

von

Chantal Jazzar (MSc)

geboren am 01.01.1972 in Tripoli, Libanon

2007

Referent: Prof. Dr. Hans-Michael Poehling

Korreferent: Prof. Dr. Felix Wäckers

Tag der Promotion: 29.1.2007

To my everything and my everybody,

to my Jesus



Abstract**Optimising Parasitoid Learning as a Strategy to Enhance the Biological Control of Aphids in Protected Environment****Chantal Jazzar**

Myzus persicae (Sulzer) and *Macrosiphum euphorbiae* (Thomas) (Homoptera: Aphididae) are important cosmopolitan pests of vegetables and ornamentals in greenhouses. They are responsible for direct and indirect plant damage. Through extensive feeding, *M. persicae* and *M. euphorbiae* can interfere in quite dramatic ways with the growth processes of the plant. Indirect damage is linked to their proficiency in viral transmission. Because of their hidden feeding habit on the underside of the leaf, insecticidal control of those aphids with conventional sprayers is not warranted. *M. persicae* and *M. euphorbiae* resistance to several groups of insecticides has been observed. Therefore, the avenues of research are now directed towards green and sustainable control strategies. Most popular is the use of natural enemies such as parasitoids and predators.

In a permanently changing environment, it is by no means an easy task to distinguish potentially important events from negligible ones. Yet, to survive, every animal has to continuously face that challenge. The capacity to learn through experience and then modify her responses to prevailing environmental cues equips a foraging parasitoid with behavioral plasticity. *Aphelinus abdominalis* (Dalman) (Hymenoptera: Aphelinidae) is a generalist parasitoid of aphids using associative learning to locate hosts from distance. Aphidophagous systems are subject to diversified guilds of natural enemies. Intraguild predation (IGP) occurs when one species in a predatory guild feeds on another predatory species within the guild. IGP is ubiquitous among aphid antagonists.

The general objective of this study is to enhance the control of *M. persicae* and *M. euphorbiae* through the manipulation of the learning ability of the *A. abdominalis* wasp. Specific objectives are (i) to study if learning is implicated in the host recognition and handling of the wasp through aphid species switching, (ii) to examine if *Aphelinus* female learns predation risks and the resulting antipredator behaviors, (iii) to investigate the direct (IGP) and indirect (behaviorally mediated) effects of pairing *Aphelinus* wasp with *Chrysopa carnea* (Stephens) (Neuroptera: Chrysopidae) predator.

Sweet pepper *Capsicum annuum* (L.) leaf discs sustaining one of the aphid species were used. The behavioral decisions of the foraging females were traced with a multiple video camera set up for an ensuing analysis with the “Observer Video Pro” software system. The combination of the two protagonists’ trial was conducted in microcosms each housing a single pepper plant in a growth chamber.

When switching the aphids between patches, *M. persicae* and *M. euphorbiae* experienced *Aphelinus* showed similar reproductive success on *M. persicae* or *M. euphorbiae* patches. Handling of successive hosts improved the host handling skills of the females after alternating or conserving the aphid species between patches.

When assessing the learned recognition of predation threat, predator naïve and predator experienced *A. abdominalis* displayed similar behavioral decisions and oviposition success in patches with or without the L2 *C. carnea*. Antipredator behaviors (e.g. leaving the patch or depressed oviposition) were trivial. It seems more adaptive for the *Aphelinus* wasp to respond to predation risk through associative learning rather than through sensitization. The aphid species was found to promote the mutual interactions between the two antagonists.

The effect of combining *A. abdominalis* and *C. carnea* on *M. persicae* or *M. euphorbiae* population reduction was dependent on predator induced behavioral changes of the aphids. This induced antipredator response is species specific. Indirect fitness costs (lower reproductive success) were more important than direct IGP (mummy destruction or adult killing) in shaping the outcome of *A. abdominalis*-*C. carnea* interactions.

Results of this study clearly indicate that *A. abdominalis* averted any suboptimal behavior mostly due to learning about the foraging environment. Thus *Aphelinus* made the optimal decision by exploiting patches maintaining the switched aphid host, handling both hosts more efficiently due to experience and foraging in the presence of a benign L2 *C. carnea*. IGP interactions have direct and indirect effects on the parasitoid, the indirect effects appear to be more detrimental than the direct ones under certain conditions. Exploiting learning in integrated pest management programs is a credit but it should be considered within the food web context.

Keywords: learning, *Myzus persicae*, *Macrosiphum euphorbiae*, *Aphelinus abdominalis*, *Chrysopa carnea*, antipredator behavior, intraguild predation.

Zusammenfassung

Die Optimierung des Lernverhaltens von Parasitoiden als Strategie zur Verbesserung der Biologischen Blattlausbekämpfung unter Glas

Chantal Jazzar

Myzus persicae (Sulzer) und *Macrosiphum euphorbiae* (Thomas) (Homoptera: Aphididae) sind wichtige kosmopolitische Schädlinge an Gemüse- und Zierpflanzen in Gewächshäusern. Sie sind für direkten und indirekten Schaden an den Pflanzen verantwortlich. Auf Grund ihrer beträchtlichen Nahrungsaufnahme können *M. persicae* und *M. euphorbiae* die Wachstumsprozesse der Pflanzen deutlich beeinflussen. Indirekter Schaden kann dabei durch die Übertragung von Viren entstehen. Wegen ihrer versteckten Lebensweise auf der Unterseite des Blattes ist die Bekämpfung mit Insektiziden dieser Blattläuse mit konventionellen Applikationsgeräten nicht immer gewährleistet. Außerdem sind Resistenzen von *M. persicae* und *M. euphorbiae* gegenüber mehreren Gruppen von Insektiziden beobachtet worden. Aus diesem Grund richtet sich die breite Forschung besonders auf umweltfreundliche und nachhaltige Bekämpfungsstrategien. Dabei ist die populärste Strategie die Verwendung von natürlichen Feinden wie beispielsweise Parasitoiden und Prädatoren.

In einer sich permanent verändernden Umgebung oder Umwelt ist es auf keinen Fall eine leichte Aufgabe, potentiell wichtige Ereignisse von unwesentlichen zu unterscheiden. Doch um zu überleben, muß sich jedes Tier ununterbrochen dieser Herausforderung stellen. Die Fähigkeit durch Erfahrung zu lernen und entsprechend Antworten zu aktuellen Umweltreizen zu modifizieren, stattet einen nach Futter suchenden Parasitoiden mit der Verhaltensweise der Formbarkeit aus. *Aphelinus abdominalis* (Dalman) (Hymenoptera: Aphelinidae) ist als Generalist ein Parasitoid von Blattläusen, der durch assoziatives Lernen seinen Wirt aus Entfernung zu finden vermag. Aphidophage Systeme unterliegen unterschiedlichen Gilden natürlicher Feinde. Intraguild predation (IGP) kommt vor, wenn eine räuberische Art auf einen weiteren Räuber trifft und sich beide von der gleichen Beute ernähren. IGP ist unter Antagonisten von Blattläusen weit verbreitet.

Die allgemeinen Ziele dieser Forschungsarbeit sind, die Bekämpfung von *M. persicae* und *M. euphorbiae* mit Hilfe der Schlupfwespe *A. abdominalis* durch die Manipulation ihrer Lernfähigkeit zu verbessern. Im Speziellen wurde hierbei untersucht, (i) ob Lernen an der

Wirtserkennung und - handhabung beteiligt ist, (ii) ob *Aphelinus abdominalis* - Weibchen Prädationsrisiken und die daraus resultierenden Anti-Prädatoren-Verhaltensweisen erlernen und (iii) ob direkte und indirekte (verhaltensvermittelte) Effekte bei der Paarung der Schlupfwespe *Aphelinus* mit dem Prädatör *Chrysopa carnea* (Stephens) (Neuroptera: Chrysopidae) entstehen.

In Mikrokosmosversuchen in Klimakammern, jeweils bestückt mit einer Pflanze des Paprika, *Capsicum annuum* L., wurden die Kombinationen der Versuchsprotagonisten durchgeführt. Blattscheiben des Paprika, auf denen sich jeweils eine der Blattlausarten befanden, wurden hierbei benutzt. Die Verhaltensentscheidungen der nach Futter suchenden Schlupfwespenweibchen wurden mit einem multiplen Videokamerasystem verfolgt, das nachfolgend für die Analyse mit der "Observer Video Pro" Software diente.

Beim Austausch zwischen den Blattläusen zeigten *Aphelinus*-Weibchen gleiche Vermehrungserfolge in *M. persicae* oder *M. euphorbiae* Versucheinheiten, wenn die Schlupfwespe zuvor in *M. persicae* oder *M. euphorbiae*-Einheiten Erfahrungen machen konnten. Die Fähigkeiten im Umgang der Weibchen mit dem Wirt verbesserten sich mit der Handhabung aufeinanderfolgender Wirte, nachdem die Weibchen zwischen den Versucheinheiten ausgetauscht oder darin gehalten wurden.

Des Weiteren wurde die Prädatorenbedrohung auf *A. abdominalis* analysiert. Hierbei zeigten Prädatoren-erfahrene und Prädatoren-unerfahrene *A. abdominalis* ähnliche Verhaltensentscheidungen und Ovipositionserfolge in Versuchseinheiten mit und ohne den L2-Larven von *C. carnea*. Das Anti-Prädatoren-Verhalten (z.B. das Verlassen der Einheit) war unbedeutend. Es scheint für die *Aphelinus* Schlupfwespe angepasster zu sein, auf das Prädationsrisiko durch assoziatives Lernen zu reagieren, als durch Sensibilisierung. Hierbei fördern die Blattlausarten die gegenseitigen Wechselwirkungen zwischen den zwei Antagonisten.

Die Auswirkungen der Kombinationen aus *A. abdominalis* und *C. carnea* auf die Reduktion der Populationen von *M. persicae* oder *M. euphorbiae* war abhängig von der durch Prädatoren ausgelösten Verhaltensänderungen der Blattläuse. Dies bedeutet, dass die Anti-Prädatoren-Verhaltensweisen durch die Art bestimmt ist. In der Darstellung des Ergebnisses der Wechselwirkungen zwischen *A. abdominalis* und *C. carnea* waren die indirekten Kosten der Fitness (z.B. niedriger Fortpflanzungserfolg) bedeutsamer als die der direkte IGP (Mumiezerstörung oder Erwachsenenentötung).

Die Ergebnisse dieser Arbeit zeigen eindeutig, daß *A. abdominalis* unspezifische, suboptimale Verhaltensweisen in der Regel wegen des Lernens unterbindet, die Umgebung nach Nahrung abzusuchen. So traf *Aphelinus* Schlupfwespen beim Ausbeuten der Versuchseinheiten die optimalen Entscheidungen, den gewechselten Blattlauswirt beizubehalten, auf Grund der gemachten Erfahrungen beide Wirte effizienter zu handhaben und in der Gegenwart eines gütigen L2 *C. carnea* weiter nach Futter zuzusuchen. IGP-Wechselwirkungen haben direkte und indirekte Wirkungen auf den Parasitoiden, wobei unter bestimmten Voraussetzungen die indirekten schädlicher, als die direkten Wirkungen zu sein scheinen. Das Erforschen von Lernvermögen ist eine wertvolle Aufgabe in Integrierten Pflanzenschutzprogrammen, die auch innerhalb des Kontextes der Nahrungsnetzwerke betrachtet werden sollte.

Stichwörter: Lernen, *Myzus persicae*, *Macrosiphum euphorbiae*, *Aphelinus abdominalis*, *Chrysopa carnea*, Anti-Prädatoren-Verhaltensweisen, Intraguild Predation.

Table of Contents

ABSTRACT.....	I
ZUSAMMENFASSUNG.....	III
TABLE OF CONTENTS.....	VI
ABBREVIATIONS	VIII
1. GENERAL INTRODUCTION	1
2. SWITCHING APHID SPECIES: GENERAL CHEMICALS BETRAY THE PREY IDENTITY TO A GENERALIST PARASITOID.....	7
2.1. ABSTRACT.....	7
2.2. INTRODUCTION	8
2.3. MATERIALS AND METHODS	10
<i>Rearing</i>	10
<i>Aphid preparation</i>	10
<i>Parasitoid treatments</i>	11
<i>Behavioral decisions of naïve females: expression of innate responses</i>	12
<i>Behavioral decisions of experienced females</i>	12
<i>Data compilation</i>	13
<i>Statistical analysis</i>	13
2.4. RESULTS	14
<i>Behavioral repertoire of female <i>Aphelinus abdominalis</i> on the sweet pepper patch</i>	14
<i>Behavioral decisions of naïve females: expression of innate responses</i>	15
<i>Behavioral decisions of experienced females</i>	18
2.5. DISCUSSION.....	28
3. TWO PROTAGONISTS ON APHIDOPHAGOUS PATCHES: EFFECTS OF LEARNING AND INTRAGUILD PREDATION.....	33
3.1. ABSTRACT	33
3.2. INTRODUCTION	33
3.3. MATERIALS AND METHODS	35
<i>Rearing</i>	35
<i>Aphid preparation</i>	36
<i>Handling of the predator and the parasitoid</i>	36
<i>Recording and quantification of the two protagonists' interactions</i>	38
<i>Data compilation</i>	38
<i>Measurement of the protagonists' behavioral observations</i>	39
<i>Statistical analysis</i>	39
3.4. RESULTS	40
<i>Parasitoid predator interactive behaviors</i>	41
<i>Parasitoid prey behavioral repertoire</i>	45
<i>Effect of extraguild prey species on intraguild interactions</i>	53
3.5. DISCUSSION.....	54

4. DIRECT AND INDIRECT INTERACTIONS BETWEEN APHELINUS ABDOMINALIS AND CHRYSOPA CARNEA: EFFECTS OF INTRAGUILD PREDATION AND PREY ANTIPREDATOR BEHAVIORS.....	58
4.1. ABSTRACT	58
4.2. INTRODUCTION	59
4.3. MATERIALS AND METHODS	61
<i>Rearing</i>	61
<i>Experimental plants</i>	61
<i>Protagonists' treatments</i>	62
<i>Statistical analysis</i>	63
4.4. RESULTS	64
<i>Time related combined and independent impacts of <i>Aphelinus abdominalis</i> and <i>Chrysopa carnea</i> on aphid population growth</i>	64
<i>Effect of the extraguild prey species on the performance of the antagonists</i>	68
<i>Intraguild predation effects on the parasitoid</i>	71
<i>Predation efficiency of <i>Chrysopa carnea</i></i>	75
4.5. DISCUSSION.....	76
5. GENERAL DISCUSSION	80
REFERENCES.....	84
ACKNOWLEDGEMENTS.....	95

Abbreviations

ANOVA	Analysis of variance
ca.	Circa
cm	Centimetres
cv.	Cultivar
df	Degree freedom
F	Statistical <i>F</i> -value
GLM	General linear model
Km	Kilometers
h	Hours
L:D	Relation of light to darkness
L1	First larval instar
L2	Second instar larvae
L3	Third instar larvae
min	Minutes
mm	Millimeters
mg	Milligram
ns	Non-significant
<i>P</i>	<i>P</i> -value (statistical significance level)
rh	Relative humidity
s	Seconds
SAS	Statistical analyses system
SE	Standard error of the mean
μm	Micrometers
√	Square root transformation

1 General Introduction

Aphids are an extremely successful group, which occurs throughout the world, with the greatest number of species in temperate regions. They are small and inconspicuous. However, they frequently become so numerous that the number of individuals feeding on the leaves and shoots per acre of ground is 2000 million (Dixon, 1973). Many species are agricultural pests. Several generations are born a year. They have a high reproductive rate. Their complex life cycles and polymorphism enable them to optimally exploit their host plants and respond adaptively to every contingency of their environment. They can migrate great distances up to 1300 Km and in the temperate regions few plants species are without a specific aphid (Dixon, 1973).

Aphids originated from the Archescytinidae in the Carboniferous era, or early Permian, 280 million years ago (Heie, 1967). A conspicuous evolution of aphids was later associated with the appearance of flowering plants, the Angiosperms. Those constitute the host plants of most currently present aphids, although some aphids live on Gymnosperms and a few species attack ferns and mosses (Dixon, 1973).

Aphids belong to the superfamily Aphidoidea, within the order Homoptera, the plant-sucking bugs. The Aphidoidea are all soft-bodied insects, whose wings if present are membranous. They are sap feeders. Aphids are characteristic by the viviparous parthenogenetic mode of reproduction of the females i.e. they give birth to live offspring without fertilization. The asexual females can be apterous or alate. The initial advantage of apterousness is an increase in fecundity because the development and maintenance of wing musculature possibly competes with the development of embryos for the limited amount of nitrogen available to the aphid (Dixon, 1973).

Host alternation is common amongst aphids. It is an adaptive strategy to benefit from an incessant supply of nutritionally favorable foliage, which is either growing or senescent (Dixon, 1973). This host alternation tactic sustains the continuity of the aphid colony. When a dense aphid population develops on the plant, some of the alates escape from the large accumulation of predators and parasitoids. Those winged females move to an enemy free plant to establish a new colony.

The green peach aphid *Myzus persicae* (Sulzer) is a highly polyphagous aphid. Its winter host is peach, *Prunus persica*. Although some eggs overwinter on peach, overwintering is usually in the mobile stages on herbaceous plants, weeds and brassicas. The summer hosts are very numerous and spread over 40 plant families. *M. persicae* numbers reach a peak in July. This aphid does not form massive colonies, but tends to move when crowded by walking to infest other parts of the same or neighboring plants. Redistribution in late summer to other crops or wild herbaceous plants is followed by a return migration to winter hosts in late September and early October.

M. persicae is considered a noxious virus vector. Its wide host range grants this aphid a proficiency in transmitting more than 120 plant viruses. Some of the more important viruses transmitted include Potato leaf roll virus, Beet western yellows virus, Beet mild yellowing virus, Pea enation virus and Lettuce mosaic virus.

The potato aphid *Macrosiphum euphorbiae* (Thomas) is a key pest of the Solanaceae plants, especially potato. Firstly introduced into Europe in 1917, this species is very polyphagous and cosmopolitan. It rarely overwinters as eggs on *Rosa* spp., but predominantly spends winter in the mobile stages on weeds, potato sprouts in store houses, and on lettuce under glass. In early May/June alate morphs are produced and migrate to potato and other crops. In the summer, *M. euphorbiae* alternates among over 200 plant species in more than 20 plant families. *M. euphorbiae* is of little importance in the field as a vector of potato viruses. Conversely, in hot dry weather, *M. euphorbiae* population rapid build up can reduce plant growth, thereby lowering yields. Leaves become twisted and cupped as a result of feeding by clusters of aphids on the underside of the foliage (Howard et al., 1994). The excreted honeydew supports the growth of sooty mold fungi and thus affects the marketability of the fruit. *M. euphorbiae* can transmit over 50 plant viruses, mainly of the non-persistent variety, but with less efficiency than *M. persicae*. In particular it is known to transmit Potato leaf roll virus, Beet mild yellowing virus, Beet yellows virus and Lettuce mosaic virus.

Chemical control of aphids is not warranted and is difficult to achieve because of poor under foliage coverage when insecticides are applied with conventional sprayers (Howard et al., 1994). *M. persicae* has documented resistance to 71 synthetic chemical insecticides (Georghiou and Lagunes-Tejada, 1991). *M. euphorbiae* insecticide resistant phenotypes were recently observed (Foster et al., 2002). Although a number of tomato, eggplant and

pepper cultivars express a level of resistance to some aphid-transmitted viruses, there is no apparent resistance to aphid attacks.

Biological control offers an environmentally benign and sustainable strategy to suppress or mitigate aphid effects through the use of natural enemies. Aphidophagous systems constitute attractive preys for diversified guilds of antagonists. Those include the larvae of lacewings and most ladybeetles, the larvae of some hoverflies and cecidomyid larvae. Certain birds also eat aphids, especially when these are abundant or when other food is scarce (Dixon, 1973). Aphids are also the hosts of hymenopterous parasitoids and pathogenic species (Minks and Harrewijn, 1988). The hymenopterous parasitoids insert their eggs into the body of the aphids and the parasitoid larva develops within the hosts finally killing it. The parasitoid larva glues the skin of the dead aphid to the surface of the leaf and then spins a cocoon within it. This cocoon, together with the skin of a dead aphid is called a “mummy”. Only one parasitoid reaches maturity in each parasitized aphid.

Parasitoids are broadly considered as pest specialists whereas predators are classified as generalists. Specialists attack one or few prey species and their dynamics are thus tightly linked to those of their hosts. This close relationship with a host species may allow a specialist to mount a strong numerical response and thereby control the host population (e.g. Murdoch, 1994). In contrast, generalists feed on a cocktail of species and thus respond less strongly to density fluctuations of any single prey species. Recently, two avenues of research focus on the enhancement of pest control using natural enemies. The first deals with parasitoid behavioral improvement through learning. The second tackles the implementation of multiple natural enemy species to combat a specific pest.

In the past decade, awareness has grown the importance of learning in the life history of insects in several taxa. However tiny an insect is and however small its brain, insects are able to learn (Bleeker, 2005). Learning is a change in the behavior as a result of experience. Associative learning is one of the ways through which parasitoids can optimize their host search (Vet et al., 1991). Most parasitoids species employ classical Pavlovian conditioning. In this conditioning, the relationship between two stimuli, the unconditioned stimulus (US) and the conditioned stimulus (CS), is learned (Kupfermann, 1991). The US is a biologically meaningful stimulus to which the parasitoid exhibits innate response. The CS is a neutral stimulus to which the parasitoid has limited or no responsiveness. A

rewarding US like food results in appetitive conditioning. A noxious stimulus can also be used as US, resulting in defensive conditioning (Kupfermann, 1991). Therefore associative learning is not only limited to food or host finding but possibly to risk assessment and predator avoidance. In parallel, there are energetic costs related to formation and maintenance of memory (Dukas, 1999; Mery and Kawecki, 2005) and ecological costs to learning (Bleeker, 2005). Learning takes time and is vulnerable to mistakes. Hence it is likely that when innate behavior can suffice learning is not favored. According to Roitberg et al. (1993), learning is adaptive when a large number of decisions have to be made.

A major constraint on the evolution of efficient host searching mechanism in parasitoids is the continuous selection on hosts for inconspicuousness to avoid being detected by predators or parasitoids. Although direct cues from hosts are highly reliable, they are difficult to detect (Vet and Dicke, 1992). In contrast stimuli from plant are more detectable but less reliable indicators of hosts' presence (Vet and Dicke, 1992). To solve the reliability-detectability problem, many parasitoids recruit the odors that the plants emit in response to herbivory to locate their host from distance. Depending on the diet breadth of the host, the parasitoids respond to the plant odors innately or learn them. It is likely that parasitoids using hosts that are restricted to one or several similar plant species are innately attracted by the induced plant odor (Vet and Dicke, 1992). However, parasitoids attacking hosts foraging on several unrelated plants differing in odor composition and are variably available in space and in time are assumed to learn the induced plant odors (Vet and Dicke, 1992). Steidle and van Loon (2003) modifying the concept of dietary specialization and infochemical use in carnivores according to the present literature found that the innate use of infochemicals occurs in all carnivores regardless of dietary specialization.

A body of studies have shown improved parasitoid performance due to learning (e.g. Lewis and Tumlinson, 1988; Lewis and Martin, 1990; Mölck et al., 2000; Papaj and Vet, 1990; Turlings et al., 1993; Wajnberg, 1989). Parasitoids learn to respond to kairomones or visual cues associated with the substrate of their prey (Dukas and Duan, 2000; Gandolfi et al., 2003; Kerguelen and Cardé, 1998; Steidle, 1998; Vet and Groenewold, 1990) Associative learning is also implicated in the host-evaluation process, as parasitoids reject fewer potential hosts after experiencing a poor environment, and in some cases learn to discriminate hosts that are already parasitized (Roitberg et al., 1992; van Baaren and Boivin, 1998). A reward such as an oviposition in a suitable host

constitutes a reinforcement that is strong enough to influence the persistence of the learned experience. But the often erratic performance of parasitoids is limiting their use as pest control agents. The ability of females to locate and attack hosts is a key component of how well a given parasitoid population performs. Thus the variation in this host location ability even of females having a complete pre-release experience with the plant host complex could be a major source of inconsistent results in biological control with parasitoids (Lewis et al., 1990). Therefore a possible candidate to compensate for irregular parasitoid performance is the use of multiple antagonists such as a predator and a parasitoid.

In agricultural system the deployment of natural enemy complexes as opposed to a single enemy strategy has been a controversial issue in the management and biological control of insect pests. In arthropod food webs, many predators are generalists and they may not restrict their diets to herbivore species but feed also on other antagonists. Generalist predators are therefore expected to engage in intraguild predation (IGP). This is defined as the killing and eating of species that otherwise use similar resource and are thus potential competitors (Polis et al., 1989). IGP is a ubiquitous phenomenon among aphidophagous predators. Temporal and spatial distributions of aphids promote IGP interactions (Lucas, 2005). Between parasitoids and predators, IGP interaction is asymmetrical in favor of the predator. The predator is always the IG-predator, the parasitoid the IG-prey and the common resource, the aphid, is the extraguild prey. This raises the possibility of relatively ineffective generalists disrupting efficient pest control by specialists (e.g. Snyder and Yves, 2001). Predation affects prey behavior (Lima and Dill, 1990; Lima, 1998). Because of the adaptive flexibility in the prey behavior in response to a changing risk of predation (that is antipredator decision making), the predator may have large impacts on the ecological system independent from actual predation (Lima, 1998). This impact is termed behaviorally mediated non-lethal predator-prey interactions (Lima, 1998). For example, to reduce the risk of predation, prey individuals may alter activity time or location and degree of mobility (Abramsky et al., 1996; Coll and Izraylevich, 1998; Kotler et al., 1991). Thus antipredator decision making has costs. The immediate cost of antipredator behavior is lowered energy intake, which translates into non-optimal foraging and reduced resource utilization (Lima and Dill, 1990; Lima, 1998). Therefore, the ability to accurately assess the risk of predation should be beneficial and the costs associated with antipredator behaviors should act as a driving force to develop efficient risk assessment systems (Helfman, 1989). Since predation fluctuates in space and in time and not all predators are

equally dangerous, it seems more adaptive to the prey to respond to the risk of predation through associative learning rather than through sensitization. Predators and prey can use odors associated with the presence of other con or heterospecifics on the patches for avoiding competition or IGP (Lima and Dill, 1990). Additionally, chemically labelled diet of the predator (e.g. concentration of conspecific alarm cues) endows the prey the capacity to distinguish dangerous from harmless predators (e.g. Dicke and Grostal, 2001; Kats and Dill, 1998; Mirza et al., 2006).

Aphelinus abdominalis (Dalman) (Hymenoptera: Aphelinidae) is a solitary polyphagous endoparasitoid of aphids. The parasitoid is generalist at the plant and the herbivore level. The wasp attacks *M. persicae* and *M. euphorbiae* in greenhouses and *Sitobion avenae* (F.) and *Metopolophium dirhodum* (Walker) on cereals. The female associatively learns to locate hosts from distance (Mölck et al., 2000). *Aphelinus* wasps are synovigenic i.e. continue to mature eggs throughout part or all of their adult life. The females practice destructive host feeding to meet their protein, amino acids and vitamin needs necessary to sustain egg maturation. Like other aphelinids, aphid excreted honeydew or nectar offers a source of sugar to the foraging females (Vigianni, 1984).

The general objective of this study is to exploit the learning feature of *A. abdominalis* parasitoid to enhance *M. euphorbiae* and *M. persicae* control.

First, we inspected if the infochemicals used by *Aphelinus* females to recognize and accept the two hosts *M. euphorbiae* and *M. persicae* offered in alternation are innate and share common general components. In addition, we traced the behavioral responses they elicit. Also, we investigated if learning to handle one aphid species improves the handling skills of the other species.

Second we investigated the learned recognition of *Aphelinus* wasps of predation risk. Therefore, the females were paired with *Chrysopa carnea* (Stephens) (Neuroptera: Chrysopidae) predator in a patch or third in a microcosm setup sustaining either of the aphid hosts. We examined the learning of *Aphelinus* parasitoids to adjust their behavioral responses to predation cues through constant informational update. We studied whether the type of interaction between the two protagonists is direct (IGP) or indirect (behaviorally mediated).

2 Switching Aphid Species: General Chemicals Betray the Prey Identity to a Generalist Parasitoid

2.1. Abstract

Learning has been shown to play a vital role in the parasitoids' host finding, recognition and handling, thus in their reproductive success. The generalist *Aphelinus abdominalis* (Dalman) (Hymenoptera: Aphelinidae) associatively learns to recruit plant synomones for host habitat location. The overall goal of this study is to test whether *A. abdominalis* wasp innately uses general components (chemical similarities) to recognize new hosts by the naïve parasitoids or by parasitoids that have already a foraging experience but on a different host. We also aim to demonstrate that *A. abdominalis* alternatively trained on two of her hosts, *Myzus persicae* (Sulzer) or *Macrosiphum euphorbiae* (Thomas), eventually learns to handle both of them efficiently.

We tested first naïve wasps on patches harboring either *M. persicae* or *M. euphorbiae* aphids. Then the experienced females collected from each patch were offered on a second patch the same or the switched aphid host. The behavioral decisions of the parasitoids were traced with a multiple video camera set up for an ensuing analysis with the "Observer Video Pro" software system. We have categorized the females' behavioral states into rewarding and non-rewarding. Rewarding behaviors are those linked to physiological needs of the wasps. They include oviposition, host feeding and honeydew feeding. Non-rewarding behaviors are related to the handling of the host. They comprise searching, contact, oviposition attempts, standing and patch leaving.

Results reveal that *M. euphorbiae* and *M. persicae* experienced females achieved similar reproductive success (percent mummy formed) on *M. euphorbiae* and *M. persicae* patches. Those wasps showed no behavioral discrimination when performing host and honeydew feeding behaviors irrespective of the host species on the second patch.

Within a patch sustaining one of the aphid hosts, we found an overall similarity in the frequency and time spent with the rewarding behaviors between the naïve *Aphelinus* and the *Aphelinus* having a foraging experience with the switched aphid species. This result highlights that *A. abdominalis* may rely on cues shared by both aphid species triggering their innate recognition and thus acceptance.

In a patch hosting a specific aphid species, we recorded little improvement in the rewarding behaviors between naïve *Aphelinus* and the *Aphelinus* experienced with the

same aphid species. Consequently, our results agree with the concept that rewarding behaviors are influenced by the parasitoid's physiological state rather than by learning.

Comparison of non-rewarding behaviors between naïve and experienced wasps demonstrated that experienced *Aphelinus* exhibited an improvement in host handling after alternating or conserving the aphid species between the patches. Experienced *A. abdominalis* learned how to save time in contact, oviposition attempts and pausing behaviors contrasted with the naïve wasps. The biological significance of those results is discussed.

2.2. Introduction

Foraging for hosts in insect parasitoids is divided into three steps: host habitat location, host location and host recognition and acceptance resulting in oviposition (e.g. Vinson, 1976; Vinson, 1998). Parasitoids enjoy behavioral plasticity allowing them to make optimum use of the prevailing foraging opportunities (Powell et al., 1998) through learning. Learning is the process of acquiring knowledge about the world (Kupfermann, 1991). Associative learning is a form of classical conditioning through which the wasps innately recognize host derived stimuli (kairomones) upon contact and they associate these stimuli with surrounding stimuli (plant synomones) to which originally they show no or limited responsiveness (Turlings et al., 1993). Consequently, the more detectable plant synomones are used for host habitat location, and the more reliable stimuli such as host kairomones, not easily modified by adult experience (Vet and Dicke, 1992), are used for host location and acceptance (Vinson, 1976; Vinson, 1998). After a female has encountered a potential host, she evaluates its suitability and nutritive quality by antennation and ovipositor probing (Mackauer et al., 1996). If the host is perceived to exceed the female's response threshold and is deemed suitable for larval development it is accepted and an egg is deposited (Henry et al., 2005).

In addition to classical conditioning, insects learn how to handle their prey more efficiently as they gain experience (Chittka and Thomson, 1997). This learning to handle the prey is referred to as operant learning and is extensively studied in the flower handling skills of honeybees. It has been suggested that bees are limited in their ability to learn the handling of more than one flower type quickly and accurately. In other words, the "know-how" of handling one flower morphology may interfere with the ability to handle a second one (Darwin, 1876). But studies have found that bees that are trained on two motor tasks in

alternation eventually learn to execute both of them efficiently (Chittka et al., 1997; Dukas, 1995).

A body of literature has recently explored the role of infochemicals as foraging cues deployed by generalist natural enemies (e.g. Steidle et al., 2001a; Steidle et al., 2001b; Steidle et al., 2003; Steidle and van Loon, 2003). It is assumed for generalist natural enemies that parasitoids innately use general components that are common for all hosts or host plants (Vet and Dicke, 1992) to find and recognize a host not encountered before. In line with this hypothesis, Godfray (1994) assumed that chemical similarities between hosts determine the host range of parasitoids.

Aphelinus abdominalis (Dalman) (Hymenoptera: Aphelinidae) is a solitary endoparasitoid reported to accept a number of cereal aphid species as hosts, e.g. *Sitobion avenae* (F.) and *Metopolophium dirhodum* (Walker) (Kalina and Stary, 1976), and *Myzus persicae* (Sulzer) and *Macrosiphum euphorbiae* (Thomas) on sweet pepper, aubergine, tomato etc. in greenhouses (e.g. Colombo and Fasce, 1994). Thus, *A. abdominalis* can be considered generalist on both the plant and the herbivore level. Furthermore, the parasitoid through associative learning exploits infochemicals for host habitat location (Mölck et al., 1999; Mölck et al., 2000).

The general objective of this study is to test the hypothesis that generalist parasitoids deploy innate cues to recognize new hosts by the naïve parasitoids or by parasitoids that have already a foraging experience but on a different host (Steidle et al., 2001b). We also aim to prove that a generalist parasitoid trained on two of her hosts alternatively ultimately learns to handle both of them efficiently.

Our model system consists of the parasitoid *Aphelinus abdominalis* foraging in sweet pepper leaf discs sustaining either *Myzus persicae* or *Macrosiphum euphorbiae* as aphid hosts.

The specific objectives are first, to investigate the innate response of *Aphelinus* female to both aphid hosts. The second objective is to examine the effect of experience on potential behavioral improvement of the parasitoid. Therefore, we can explore 1) if *A. abdominalis* may rely on cues shared by both aphid species triggering their innate recognition and thus acceptance and if so 2) is the female able to show improved handling skills when compared to the naïves? The third objective is to detect if switching host species between patches affects the behavioral states of the experienced *A. abdominalis* wasps. We try to tackle two questions: will a previous foraging experience on a different host impose on the parasitoid fitness costs? One way to answer this question is to inspect if the oviposition success of

experienced females expressed as percent mummy formed statistically diverges when switching the aphid species. The next question is: will a previous foraging experience on a host different from the one currently offered on the patch develop the handling skills of the wasp due to the parasitoid's handling of successive hosts?

2.3. Materials and Methods

Rearing

Sweet pepper (*Capsicum annuum* L., cv. "Mazurka") plants and eggplants (*Solanum melongena* L., cv. "Ecavi") (Solanaceae) were grown in the nursery of the Institute of Plant Diseases and Plant Protection (Leibniz University of Hannover, Germany) at a temperature of 20°C, 60-70 % rh and 16:8 L:D regime. Aphid cultures were kept in climatic chambers in gauze cages at a temperature of 20 ± 1°C, 16:8 L:D photophase and 60 % rh. *M. persicae* was exclusively reared on sweet pepper plants whereas a mixture of sweet pepper and eggplants was offered to *M. euphorbiae* to enhance colony proliferation and alate production. A stock culture of the grain aphid *S. avenae* feeding on wheat (*Triticum aestivum* L.) (Triticae) was supplied to generate *A. abdominalis* mummies. The rearing on the wheat system for the parasitoid cultures was chosen to prevent any interference of pre-adult or emergence related experience with the oviposition experience treatment following Mölck et al. (2000). After pupation, mummies were harvested and transferred into fine gauze-covered acrylic cylinders that were placed on plastered pots. Emerged wasps were supplied with 15 % sucrose solution at 16:8 L:D photoperiod, 22 ± 1°C and 90 % rh until their experimental deployment.

Aphid preparation

Ten to twelve alate *M. persicae* or *M. euphorbiae* collected from the stock culture were clip caged overnight on a sweet pepper plant to generate synchronized progeny. The subsequent day, eight L1 of each aphid species were transferred using a fine Kolinsky hairbrush to a patch made of a 2 cm diameter sweet pepper leaf disc which was laid over a similar diameter piece of cotton imbibed with water. The wet cotton carrying the leaf disc was immersed in a thick film of water in a 3 cm diameter Petri-dish. The water film was intended to obstruct the free movement of the antagonist or the aphids from the leaf disc. The patches were placed in 13×15×5 cm plastic containers whose cover and sides were perforated and replaced with fine mesh to facilitate ventilation. The containers were kept

for three days in climatic chambers at a temperature of $20 \pm 1^\circ\text{C}$, 16:8 L:D photoperiod and 60 % rh. At the fourth day, the aphids reaching the L3 nymphal stage were used in the experiment.

Parasitoid treatments

Parasitoids with different types of experiences (Figure 2.1) were tested when they were three to seven day old. Naïve parasitoids were obtained by collecting the wasps from the stock culture. Throughout the experiment, the behavioral decisions of the parasitoids were recorded with a multiple video camera set up described by Meyhöfer (2001) for an ensuing analysis with the Observer Video-Pro software system (Noldus Technology, 1997). Sixteen cameras were used simultaneously, allowing the recording of eight replications per treatment for naïve females and four replications per treatment for experienced ones.

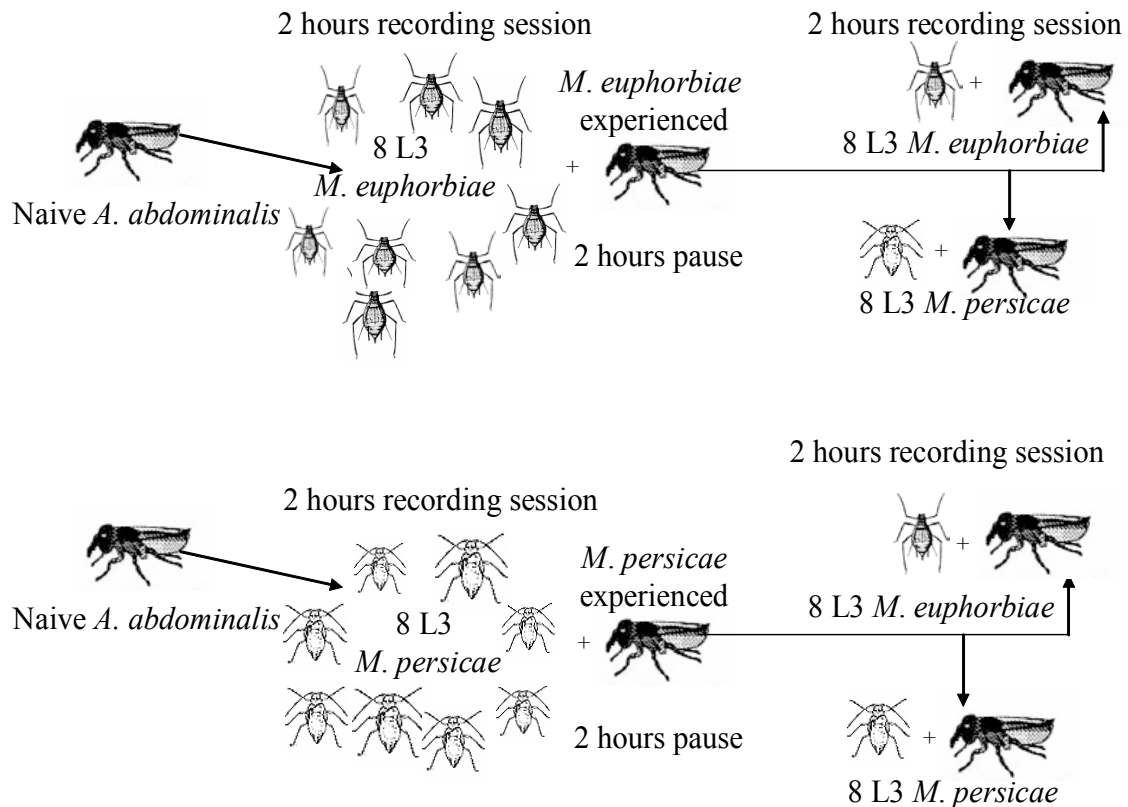


Figure 2.1. Types of experiences of *Aphelinus abdominalis* females and leaf-disc host complexes used in the different treatments.

Behavioral decisions of naïve females: expression of innate responses

To study the innate behavioral responses to the aphid prey, naïve wasps obtained from the stock culture were transferred to a sweet pepper leaf disc sustaining either eight *M. persicae* or eight *M. euphorbiae*. A two-hour video recording session permitted to trace the behavioral states of those females. Then each wasp was collected and given a two-hour resting period isolated from any infochemical source under ambient laboratory conditions. This pause was necessary to manipulate the mechanism of sensitization and desensitization to chemicals associated with hosts and patches so that the experienced females respond adaptively on the second patch (Thiel and Hoffmeister, 2004) (Figure 2.1).

Behavioral decisions of experienced females

We have undertaken two types of behavioral comparisons for experienced wasps. The first aims to study the effects of host learning. Hence we made a within patch behavioral comparison between naïve and experienced *Aphelinus*. The second is to examine the effects of switching the aphid species on the behavioral decisions of the parasitoid. Therefore, we made a within and between patches behavioral comparisons of experienced females.

Effects of host learning

Within an aphid patch, we have contrasted the behaviors of 1) naïve *Aphelinus* versus wasps experienced with the same aphid species, and 2) naïve *Aphelinus* relative to wasps experienced with the switched aphid species (Figure 2.1). The *Aphelinus* experienced with the alternate aphid species is naïve with respect to the offered host. The behavioral decisions of this experienced wasp will be the result of the information gained on the previous patch interacting with the information acquired on the second patch. If *Aphelinus* recognizes and accepts the switched aphid as the naïve, this represents a first indicator of common cues between the hosts eliciting innate responses in the female.

Effects of switching the aphid species

M. persicae or *M. euphorbiae* experienced *Aphelinus* were released on *M. persicae* and *M. euphorbiae* patches for another two hour recording session (Figure 2.1) and the behaviors of the experienced females within and between patches were compared. The purpose of this behavioral comparison is to provide a second indicator supporting the hypothesis that *Aphelinus* accepts the two aphid hosts based on chemical similarities between them. For

example, if a *M. persicae* experienced female foraging in a *M. euphorbiae* patch exhibits similar behavioral states as a *M. persicae* experienced female foraging in a *M. persicae* patch, it is highly probable that the female wasp chemically recognizes the two species as a “single host”.

In the first and second types of comparisons, we test whether handling of successive hosts improves the host handling skills of the wasps even when the hosts are switched.

Data compilation

While foraging in the patch, *A. abdominalis* displayed two categories of behavioral states: rewarding and non-rewarding.

Rewarding behaviors are mostly induced by the physiological needs of the wasp and are hardly modified by learning. Those include, 1) oviposition, 2) host feeding referring to the consumption of the host hemolymph exuding from a wound made by the female ovipositor and 3) honeydew feeding defined as the wasp’s intake of the aphid honeydew droplets deposited on the leaf surface. At the end of each recording session, the leaf discs were stored in a climatic chamber at a temperature of 20°C, 90 % rh for eight days until mummification. The number of black aphid mummies, when observed, was noted.

Non-rewarding behaviors are those behaviors related to the operant learning capabilities of the wasp, namely learning to perform a behavioral sequence quickly and accurately to obtain a reward. Non-rewarding behaviors are as follows, 1) searching, included all the displacements on the leaf disc from one location to another, 2) contact, the parasitoid approaches to the vicinity of the aphid and antennation, 3) oviposition attempts, the parasitoid turns around and attacks the aphid with her ovipositor, 4) standing, the female pauses motionless, and 5) patch leaving.

Statistical analysis

Behavioral observations yielded three basic types of measures. The dependent variables are latency (measured in seconds), which is the onset of the first occurrence of the behaviour, frequency (measured / 2h) and percent total duration allocated to a specific behavior.

For data gathered from the frequency and percent total duration variables, the number of replications of naïve females performing a certain behavior ranged between eighteen and twenty-one (each female being a replicate) and for experienced ones between eight and eleven. For data collected from the variable latency, the number of replications for naïve wasps executing a certain behavior ranged between two and twenty-one and for

experienced ones between one and eleven. When sample size was insufficient to carry out an ANOVA, the percent of the individuals responding to the behavior was calculated. When the sample size was large enough and the factors' analysis by ANOVA could be conducted, the data for the parameters frequency and latency were $\sqrt{(x+0.5)}$ transformed whereas percent total duration data were arcsine transformed to normalize the data. The data were analyzed using the PROC GLM procedure in SAS to determine single or interaction effects of factors (SAS Institute, 1999).

To investigate the innate responses of the wasps to aphid derived cues, interspecific prey comparison of the behaviors of naïve *A. abdominalis* females was conducted using Bonferroni T test in SAS version 8 (SAS Institute, 1999).

To study the effects of host learning on the foraging behaviors of the female *A. abdominalis*, comparison of the behavioral decisions between naïve and experienced wasps was conducted on *M. persicae* and *M. euphorbiae* patches. Whenever significant interactions were observed between factors, the level of one factor was compared at each level of the other factor with either *M. persicae* or *M. euphorbiae* as herbivore victim using Dunnett's two-sided test in SAS version 8 (SAS Institute, 1999).

To examine whether switching preys alters the behavioral decisions of *A. abdominalis*, the behavioral states of the experienced females within and between prey species were compared. Whenever significant interactions were observed between factors, means at different levels of the respective factor were compared using Tukey's multiple means comparison procedure in SAS version 8 (SAS Institute, 1999).

Within an aphid species, the number of mummies generated from the different treatments was collected and the percent mummy formation calculated and arcsine transformed before being subjected to statistical analyses. Analysis was carried out using Tukey's multiple means comparison procedure in SAS version 8 (SAS Institute, 1999).

A significance level of $\alpha = 0.05$ was used in all analyses. Data are presented as means \pm SE.

2.4. Results

Behavioral repertoire of female *Aphelinus abdominalis* on the sweet pepper patch

Visual observations agreeing with the video recordings showed that the parasitoid starts immediately after release in the patch with searching and drumming until encounter with the host. Thereafter, four behaviors are displayed: the female inspects the host with her

antennae or contact behavior, instantly followed by turning around and attacking the aphid with her ovipositor, a behavior termed oviposition attempts. The host could be either accepted for oviposition with a mean duration ranging from 5.05 min (± 0.69 SE) to 10.16 min (± 3.44 SE), or used as a food source with an average time spent in host feeding ranging from 14.7 min to 50.11 min (± 22.30 SE). Some females were also observed to feed on the aphid honeydew as a nutrient rich alternative with a mean duration ranging from 4.11 min (± 0.59) to 10.20 min (± 1.72 SE). The female parasitism also exhibited a lengthened period (≥ 1 min) of immobility, or a shorter phase (< 1 min) of preening after a host or honeydew meal, a behavior described as standing, and finally, patch leaving behavior.

Behavioral decisions of naïve females: expression of innate responses

Results elucidate that naïve parasitoids oviposited 46 minutes earlier in *M. euphorbiae* than in *M. persicae* (ANOVA $df = 1$, $F = 15.07$, $P = 0.0008$). The naïve *A. abdominalis* oviposited three times more frequently (Figure 2.2) and invested 3.5 fold higher proportion of time to oviposit in *M. euphorbiae* than in *M. persicae* (Figure 2.3).

Moreover, naïve wasps performed host and honeydew feeding behaviors at similar frequencies between the two aphid species (Figure 2.2). Naïve parasitoids allocated equal proportion of time to host feeding behavior on *M. euphorbiae* or *M. persicae*, but spent 9 fold higher proportion of time with feeding on *M. persicae* produced honeydew (Figure 2.3) despite that the latency of honeydew feeding was similar between *M. euphorbiae* and *M. persicae* patches (ANOVA $df = 1$, $F = 0.77$, $P = 0.410$).

Considering the non-rewarding behaviors, no difference in the behavioral activities between the two aphid species was detected (Table 2.1) except that naïve parasitoids in *M. euphorbiae* patches 29.33 min (± 6.60 SE) displayed oviposition attempts 18 minutes earlier than naïve females in *M. persicae* patches 47.63 min (± 7.96 SE).

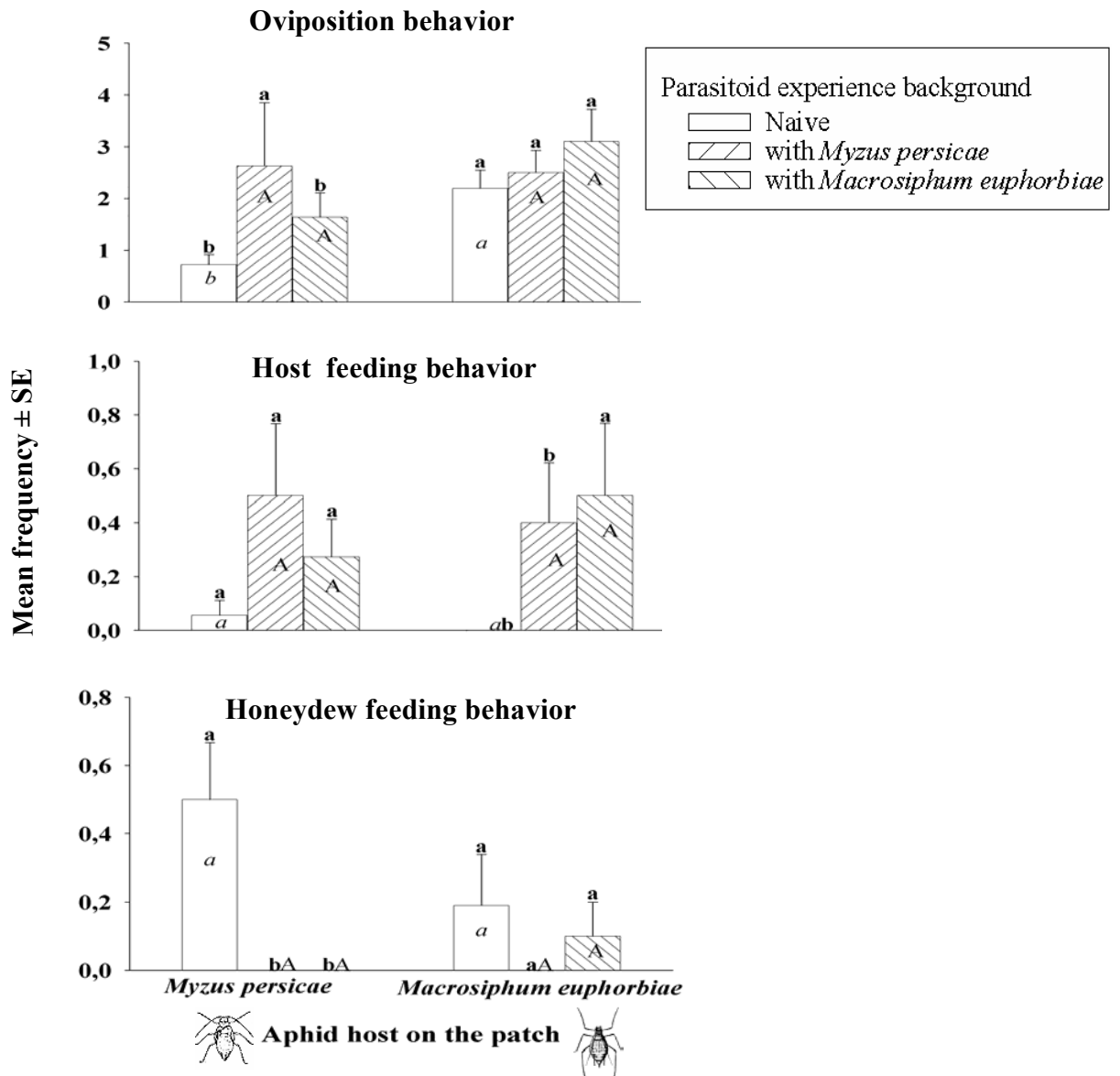


Figure 2.2. Mean frequency (\pm SE) of rewarding behaviors of female *Aphelinus abdominalis* in *Myzus persicae* or *Macrosiphum euphorbiae* patches.

The experienced females forage in patches harboring the same or the alternate aphid species.

Bars followed by the italic lower case represent between patch comparison of naïve females.

Bars followed by the bold lower case represent within patch comparison of naïve vs. experienced wasps.

Bars followed by upper cases represent within and between patches comparison of experienced females.

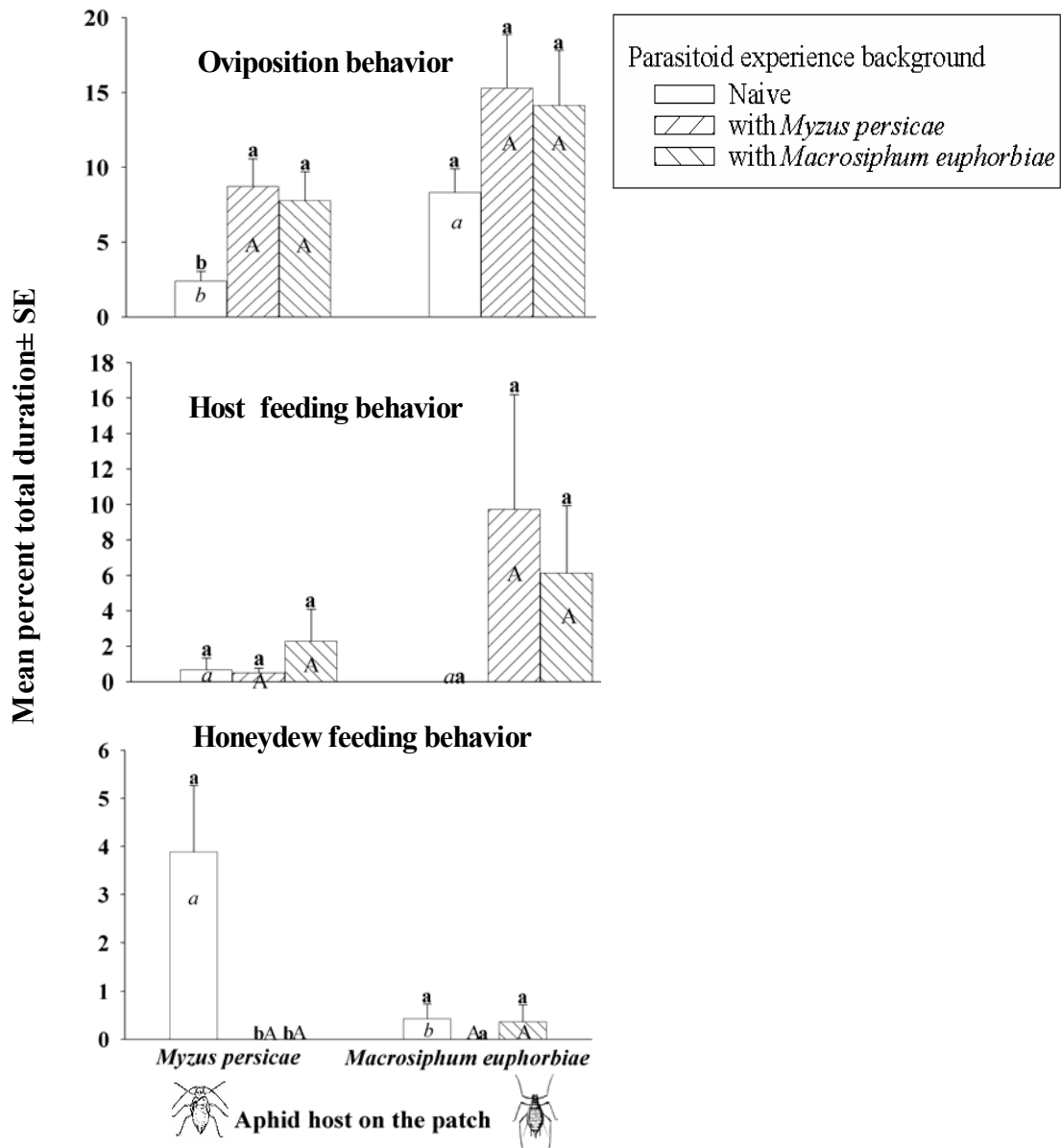


Figure 2.3. Total foraging time (\pm SE) spent with rewarding behaviors of female *Aphelinus abdominalis* in *Myzus persicae* or *Macrosiphum euphorbiae* patches.

The experienced females forage in patches harboring the same or the alternate aphid species.



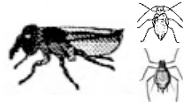

Bars followed by the italic lower case represent between patch comparison of naïve females.

Bars followed by the bold lower case represent within patch comparison of naïve vs. experienced wasps.

Bars followed by upper cases represent within and between patches comparison of experienced females.

Table 2.1. Summary of ANOVA results showing the non-rewarding behavioral states of naïve *Aphelinus abdominalis* females foraging in patches harboring eight late L2 early L3 nymphs of *Macrosiphum euphorbiae* or *Myzus persicae* prey.



Behavior	Variable	df	<i>F</i>	<i>P</i>
	Frequency	1	1.57	0.219
Searching	Latency	-	-	-
	Percent total duration	1	0.14	0.711
	Frequency	1	0.56	0.458
Contact	Latency	1	1.01	0.323
	Percent total duration	1	2.58	0.117
	Frequency	1	0.02	0.876
Oviposition attempts	Latency	1	4.19	0.048
	Percent total duration	1	2.53	0.120
	Frequency	1	2.94	0.095
Standing	Latency	1	4.09	0.051
	Percent total duration	1	0.71	0.406

Behavioral decisions of experienced females

Effect of host learning

To study if host learning is implicated in the foraging decisions of the parasitoids, comparison of naïve versus *M. persicae* and *M. euphorbiae* experienced females in *M. persicae* or *M. euphorbiae* patches was undertaken.

Rewarding behaviors

We have categorized rewarding behaviors as those behaviors that support life history traits of the parasitoid and thus realize fitness. Those behaviors are: oviposition, host feeding and honeydew feeding.

Oviposition behavior

In *Macrosiphum euphorbiae* patches

Experience with the host has not affected the initiation of laying an egg i.e. latency of oviposition between naïve and experienced wasps when *M. euphorbiae* (ANOVA $df = 2$, $F = 0.54$, $P = 0.590$) was the herbivore victim on the patch. Furthermore, no significant difference in the mean frequency of oviposition (ANOVA $df = 2$, $F = 0.99$, $P = 0.382$) (Figure 2.2) and in the time spent with oviposition behavior (ANOVA $df = 2$, $F = 2.68$, $P = 0.082$) (Figure 2.3) was found between naïve and experienced parasitoids.

In *Myzus persicae* patches

Naïve and *M. persicae* or *M. euphorbiae* experienced females displayed a similar latency of oviposition (ANOVA $df = 2$, $F = 2.78$, $P = 0.083$) in *M. persicae* patches.

On the other hand, a higher frequency of oviposition behavior (ANOVA $df = 2$, $F = 3.62$, $P = 0.038$) (Figure 2.2) and longer time spent with oviposition activity (ANOVA $df = 2$, $F = 6.70$, $P = 0.0035$) (Figure 2.3) were detected. Particularly, *M. persicae* experienced females displayed a three fold more frequent oviposition behavior $2.63 (\pm 1.22 \text{ SE})$ in *M. persicae* host compared to the naïves $0.72 (\pm 0.19 \text{ SE})$ ($P = 0.027$) (Figure 2.2). Furthermore, those females spent about 3.5-fold higher mean proportion of time in oviposition activity $8.71 \% (\pm 1.84 \text{ SE})$ in *M. persicae* as opposed to the naïve wasps ($P = 0.0043$) (Figure 2.3). *M. euphorbiae* experienced females $1.64 (\pm 0.47 \text{ SE})$ oviposited in *M. persicae* host as frequently as the naïve wasps $0.72 (\pm 0.19 \text{ SE})$ ($P = 0.222$) (Figure 2.2) but allocated three fold the proportion of the foraging time in oviposition behavior $7.78 \% (\pm 1.93 \text{ SE})$ compared to naïve *Aphelinus* $2.38 \% (\pm 0.67 \text{ SE})$ ($P = 0.027$) (Figure 2.3).

Host feeding behavior

In *Macrosiphum euphorbiae* patches

No naïve females host fed when *M. euphorbiae* was the herbivore offered. In parallel, the frequency of host feeding had a significant impact on the experienced females in *M.*

euphorbiae patches (ANOVA $df = 2$, $F = 3.88$, $P = 0.029$). In particular, the mean frequency of host feeding of *M. euphorbiae* experienced females $0.50 (\pm 0.27 \text{ SE})$ is significantly higher than that of the naives overlooking this behavior ($P = 0.037$) whereas the mean frequency of host feeding of *M. persicae* experienced females $0.40 (\pm 0.27 \text{ SE})$ is comparable to that of the naives ($P = 0.088$) (Figure 2.2). Naive and experienced wasps were found to allocate a similar duration of time to host feeding behavior in the presence of *M. euphorbiae* (ANOVA $df = 2$, $F = 3.01$, $P = 0.061$) (Figure 2.3).

In *Myzus persicae* patches

The latency of host feeding was similar between naïve and *M. persicae* or *M. euphorbiae* experienced females (ANOVA $df = 2$, $F = 6.83$, $P = 0.051$) with *M. persicae* as prey.

Naïve and experienced parasitoids host fed at equivalent frequencies (ANOVA $df = 2$, $F = 2.65$, $P = 0.086$) (Figure 2.2) and spent a comparable duration of time with host feeding behavior (ANOVA $df = 2$, $F = 2.46$, $P = 0.101$) (Figure 2.3) in *M. persicae* patches.

Honeydew feeding behavior

In *Macrosiphum euphorbiae* patches

A comparison between naïve and experienced wasps revealed that no *M. persicae* experienced females consumed *M. euphorbiae* produced honeydew. In parallel, 9.52 % naïve parasitoids fed on this sugar resource significantly earlier than 10 % *M. euphorbiae* experienced ones 8.36 min ($\pm 0.26 \text{ SE}$) vs. 119.68 min, respectively, (ANOVA $df = 1$, $F = 10353.0$, $P = 0.0063$) (Figure 2.2). Moreover, naïve and *M. persicae* or *M. euphorbiae* experienced wasps displayed similar frequency (ANOVA $df = 2$, $F = 0.48$, $P = 0.623$) (Figure 2.2) and percent duration of the foraging time (ANOVA $df = 2$, $F = 0.48$, $P = 0.622$) (Figure 2.3) to feeding on *M. euphorbiae* produced honeydew.

In *Myzus persicae* patches

In *M. persicae* patches, naïve wasps performed honeydew feeding behavior significantly more frequently than *M. persicae* experienced females ($P = 0.038$) (Figure 2.2).

Consequently, naïve wasps spent a significantly higher proportion of the foraging time to feeding on *M. persicae* produced honeydew than *M. persicae* experienced females ($P = 0.037$) (Figure 2.3). In addition, naïve wasps collected *M. persicae* ejected honeydew more frequently than *M. euphorbiae* experienced wasps ($P = 0.020$) (Figure 2.2). Hence, those naïves devoted a significantly higher proportion of their foraging time to feeding on honeydew relative to *M. euphorbiae* experienced wasps ($P = 0.020$).

Non-rewarding behaviors

We have defined four behavioral states as non-rewarding behaviors. Those behaviors are related to handling the host to obtain a reward. They include the search for a host followed by antennation or contact behavior, aphid attack with the ovipositor or oviposition attempts, standing and patch leaving. To study the effect of operant learning on host handling skill improvement, we compared the naïve to each experienced wasps (*M. persicae* or *M. euphorbiae* experienced) within a *M. euphorbiae* or a *M. persicae* patch (Figure 2.1).

In Macrosiphum euphorbiae patches

Results reveal that the onset of the parasitoid searching on the patch, the frequency of searching and the time spent in searching activity were comparable between naïve and *M. persicae* or *M. euphorbiae* experienced females (Table 2.2).

The parasitoid experience with a specific aphid species (*M. persicae* or *M. euphorbiae*) significantly influenced the latency and time allocated in contacting the *M. euphorbiae* aphid, but not the frequency (Table 2.2). We found that *M. euphorbiae* experienced females had their first contact with the *M. euphorbiae* aphids around 5 minutes earlier than the naïves, 3.05 min (± 1.58 SE) vs. 7.77 min (± 1.88 SE) respectively, ($P = 0.027$), and the *M. persicae* experienced parasitoids around 7 minutes earlier than the naïves, 7.77 min (± 1.88 SE) vs. 0.87 min (± 0.18 SE), respectively, ($P = 0.002$). The naïves spent about 1.5 fold higher proportion of the foraging time contacting *M. euphorbiae* as compared to the *M. persicae* experienced females, 54.89 % (± 3.47 SE) vs. 37.80 % (± 4.68 SE), respectively, ($P = 0.016$). The 1.3 fold higher proportion of time in contacting *M. euphorbiae* host between naïves and *M. euphorbiae* experienced parasitoids was close to significance 54.89 % (± 3.47 SE) vs. 40.95 % (± 5.63 SE), respectively, ($P = 0.058$).

Naïve and *M. euphorbiae* or *M. persicae* experienced females exhibited similar frequency and time spent in oviposition attempts in *M. euphorbiae* but displayed a significantly different latency of this behavior (Table 2.2). *M. euphorbiae* experienced females attacked *M. euphorbiae* prey with their ovipositor approximately 18 minutes earlier than the naïves 11.48 min (± 5.98 SE) vs. 29.33 min (± 6.60 SE), respectively, ($P = 0.010$), whereas the *M. persicae* experienced parasitoids performed their first oviposition attempt in the *M. euphorbiae* aphid 27 minutes earlier than the naïves 2.26 min (± 0.44 SE) vs. 29.33 min (± 6.60 SE), respectively, ($P = 0.0002$).

When evaluating the pausing behavior of the parasitoid, we found that being a naïve or an experienced *A. abdominalis* resulted in a significant impact on the latency, frequency, and proportion of time spent motionless (Table 2.2).

The parasitoids revealed a trend in the latency to stand still: experienced wasps tend to stand later than the naïves. The naïves stood almost 20 minutes before the *M. euphorbiae* experienced females, 14.08 min (\pm 3.21 SE) vs. 34.89 min (\pm 12.40 SE), respectively, ($P = 0.114$), and about 17 minutes earlier than the *M. persicae* experienced parasitoids, 14.08 min (\pm 3.21 SE) vs. 34.14 min (\pm 8.89 SE), respectively, ($P = 0.059$).

Furthermore, naïve wasps paused 3 times more frequently than *M. euphorbiae* experienced females, 5.19 (\pm 0.62 SE) vs. 1.60 (\pm 0.60 SE), respectively, ($P = 0.001$). Those naïves allocated a 3 fold higher proportion of the foraging time to standing as compared to *M. euphorbiae* experienced females, 9.53 % (\pm 1.69 SE) vs. 3.09 % (\pm 1.59 SE), respectively, ($P = 0.006$). In addition, the naïve *Aphelinus* paused 2.7 times more frequently than *M. persicae* experienced parasitoids, 5.19 (\pm 0.62 SE) vs. 1.90 (\pm 0.78 SE), respectively, ($P = 0.002$). Those naïve *Aphelinus* devoted 3 fold higher proportion of the foraging time to standing behavior in comparison to *M. persicae* experienced parasitoids, 9.53 % (\pm 1.69 SE) vs. 3.06 % (\pm 1.29 SE), respectively, ($P = 0.008$) in a patch sustaining *M. euphorbiae* host.

We found that 9.09 % (1/11) *M. persicae* experienced *A. abdominalis* departed from the experimental patch at a latency of 9.70 seconds.

In Myzus persicae patches

The latency, frequency and percent duration of the searching activity were statistically alike between naïve and *M. persicae* or *M. euphorbiae* experienced wasps (Table 2.3).





Being a naïve foraging *Aphelinus* or an experienced one only significantly influenced the onset of *M. persicae* contact but had no effect on the frequency or total time allocated in the contact behavior (Table 2.3). In a *M. persicae* patch, *M. euphorbiae* experienced females contacted *M. persicae* host 9 minutes earlier than the naïve females, 1.32 min (\pm 0.36 SE) vs. 10.62 min (\pm 2.49 SE), ($P = 0.0002$), respectively, and *M. persicae* experienced wasps about 9.9 minutes ahead of the naïve parasitoids, 0.70 min (\pm 0.37 SE) vs. 10.62 min (\pm 2.49 SE), respectively, ($P = 0.0001$).

The latency and the time spent in oviposition attempts behavior but not the frequency of the behavioral state “*M. persicae* attack” were significantly different between naïve and experienced *A. abdominalis* (Table 2.3). *M. euphorbiae* experienced females launched their first oviposition attempt around 40 minutes earlier than the naïve *Aphelinus*, 8.37 min

(± 2.85 SE) vs. 47.63 min (± 7.96 SE), respectively, ($P = 0.0001$), and *M. persicae* experienced parasitoids about 45 minutes prior to the naïve wasps, 1.95 min (± 0.72 SE) vs. 47.63 min (± 7.96 SE), ($P = 0.0001$), respectively.

Table 2.2. Summary of ANOVA results showing within patch comparisons of non-rewarding behavioral states between naïve and *Myzus persicae* or *Macrosiphum euphorbiae* experienced *Aphelinus abdominalis* females when offered patches sustaining eight late L2 early L3 nymphs of *Macrosiphum euphorbiae* prey.



Behavior	Variable	df	<i>F</i>	<i>P</i>
	Frequency	2	0.08	0.928
Searching	Latency	2	-	-
	Percent total duration	2	0.49	0.617
	Frequency	2	0.27	0.763
Contact	Latency	2	7.62	0.0017
	Percent total duration	2	4.92	0.013
	Frequency	2	0.45	0.641
Oviposition attempts	Latency	2	10.92	0.0002
	Percent total duration	2	2.88	0.068
	Frequency	2	10.23	0.0003
Standing	Latency	2	3.78	0.036
	Percent total duration	2	7.36	0.002

Contrasted with naïve *Aphelinus*, *M. euphorbiae* experienced females allocated twice the proportion of time to attack *M. persicae* prey, 13.52 % (± 3.24 SE) vs. 5.93 % (± 1.45 SE), respectively, ($P = 0.025$), whereas naïve and *M. persicae* experienced females invested identical proportion of the foraging time in performing this behavioral state ($P = 0.083$).





The pausing frequency in a *M. persicae* patch was the only variable carrying statistical significance between naïve and experienced wasps (Table 2.3). The naïves stood still 3

times more frequently than the *M. persicae* experienced females, $1.26 (\pm 0.56 \text{ SE})$ vs. $3.67 (\pm 0.65 \text{ SE})$, ($P = 0.016$), respectively, but displayed a similar frequency of immobility as *M. euphorbiae* experienced wasps foraging in *M. persicae* patches ($P = 0.109$).

12.5 % (1/8) *M. persicae* experienced *A. abdominalis* departed from *M. persicae* patches at a latency of 40.35 minutes.

Table 2.3. Summary of ANOVA results showing comparisons of non-rewarding behavioral states between naïve and *Myzus persicae* or *Macrosiphum euphorbiae* experienced *Aphelinus abdominalis* females when offered patches sustaining eight late L2 early L3 nymphs of *Myzus persicae* prey.



Behavior	Variable	df	<i>F</i>	<i>P</i>
	Frequency	2	0.51	0.603
Searching	Latency	2	2.52	0.095
	Percent total duration	2	2.30	0.116
	Frequency	2	0.63	0.541
Contact	Latency	2	15.55	<.0001
	Percent total duration	2	2.58	0.091
	Frequency	2	0.13	0.877
Oviposition attempts	Latency	2	24.61	< 0001
	Percent total duration	2	4.24	0.023
	Frequency	2	4.53	0.018
Standing	Latency	2	1.19	0.318
	Percent total duration	2	1.28	0.290

Effects of switching the aphid species

We compared *M. persicae* and *M. euphorbiae* experienced females within and between *M. persicae* and *M. euphorbiae* patches. The purpose is to investigate whether switching aphid species between patches affects the behavioral decisions of the *A. abdominalis* female. If no significant behavioral modifications occur, it is possible to postulate that *A. abdominalis* innately uses general chemical components common for all hosts to recognize and accept the hosts. Furthermore, we test whether the “know-how” of host handling is improved as the parasitoid handles successive hosts.

Rewarding behaviors

Oviposition behavior

M. euphorbiae experienced females displayed a similar latency (ANOVA $df = 3$, $F = 0.33$, $P = 0.803$), frequency (ANOVA $df = 3$, $F = 1.23$, $P = 0.315$) (Figure 2.2) and time spent with oviposition activities (ANOVA $df = 3$, $F = 1.72$, $P = 0.181$) (Figure 2.3) as *M. persicae* experienced *A. abdominalis* whether foraging in a *M. euphorbiae* or a *M. persicae* patch.

Oviposition success is evaluated as the percent of mummy formed. Since the effect of the treatment and the interaction terms between the host and the treatment were not statistically significant (Table 2.4), the analysis was conducted based on the factor host. We found that the oviposition success of *M. euphorbiae* and *M. persicae* experienced females was not significantly different when the prey offered was either *M. euphorbiae* (ANOVA $df = 2$, $F = 0.09$, $P = 0.917$) or *M. persicae* aphid (ANOVA $df = 2$, $F = 1.67$, $P = 0.202$) (Figure 2.4).

Host feeding

No statistical difference between *M. euphorbiae* and *M. persicae* experienced females was found when comparing the onset of host feeding (ANOVA $df = 3$, $F = 1.41$, $P = 0.309$), the mean frequency (ANOVA $df = 3$, $F = 0.18$, $P = 0.907$) (Figure 2.2) and the time spent (ANOVA $df = 3$, $F = 0.31$, $P = 0.814$) (Figure 2.3) with this behavioral state when the herbivore victim was *M. euphorbiae* or *M. persicae*.

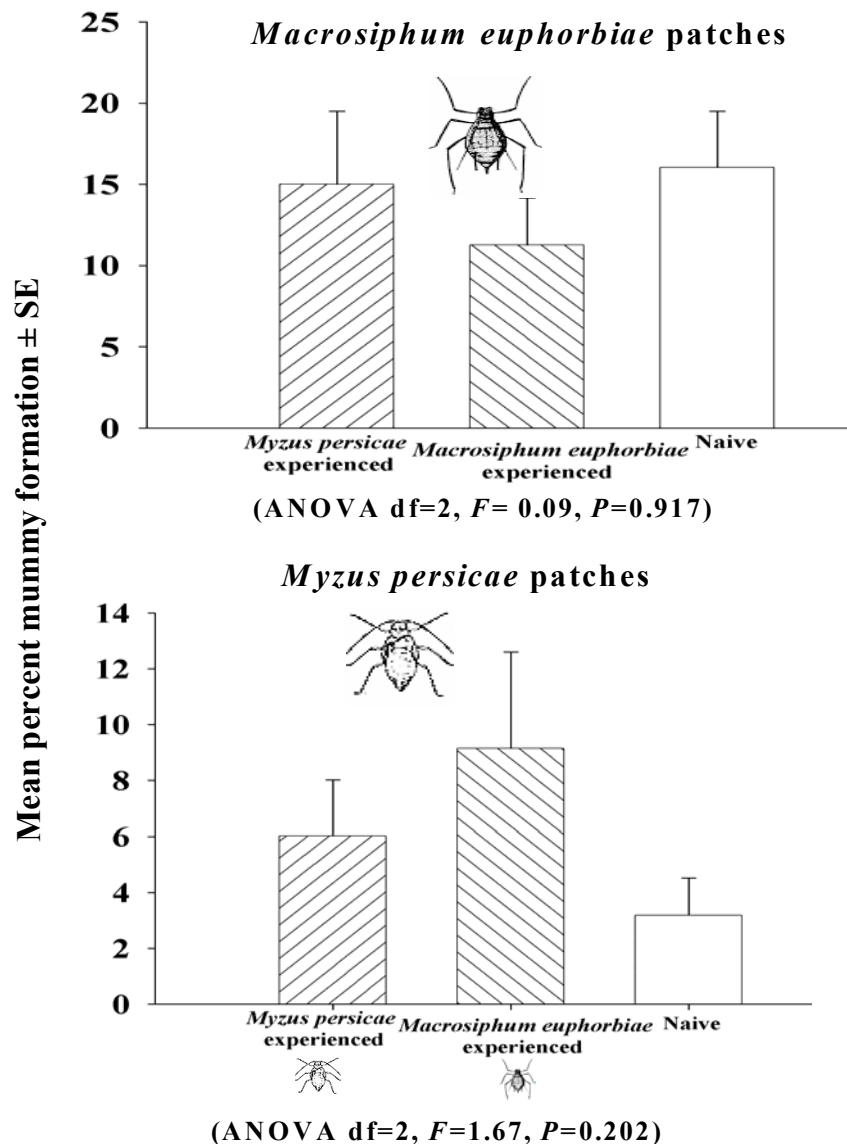



Figure 2.4. Mean percent mummy formed by *Aphelinus abdominalis* females with different host experience background and released in patches sustaining the same or the switched aphid species.

Honeydew feeding

Insufficient number of replication narrowed the analysis of the behavioral state honeydew feeding to the variables frequency and percent total duration. A comparison among experienced wasps foraging in *M. euphorbiae* or *M. persicae* patches divulged no significant difference in the mean frequency (ANOVA df = 3, $F=0.96$, $P=0.421$) (Figure 2.2) and time spent with honeydew feeding behavior (ANOVA df = 3, $F=0.96$, $P=0.421$) (Figure 2.3).


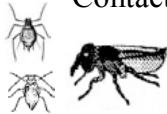


Table 2.4. Summary of ANOVA results showing comparisons of percent mummy formed by *Myzus persicae* and *Macrosiphum euphorbiae* experienced *Aphelinus abdominalis* when offered patches hosting eight late L2 early L3 nymphs of *Myzus persicae* or *Macrosiphum euphorbiae* prey. 

Source of variations	df	<i>F</i>	<i>P</i>
Host	1	7.00	0.010
Treatment	2	0.29	0.746
Host*Treatment	2	0.97	0.382

Non-rewarding behaviors

No behavioral discrimination was found between *M. euphorbiae* and *M. persicae* experienced females foraging in *M. euphorbiae* or *M. persicae* patches (Table 2.5).

Table 2.5 Summary of ANOVA results showing within and between patch comparisons of non-rewarding behaviors displayed by *Myzus persicae* and *Macrosiphum euphorbiae* experienced *Aphelinus abdominalis* when offered patches hosting eight late L2 early L3 nymphs of *Myzus persicae* or *Macrosiphum euphorbiae* prey.

Behavior	Variable	df	<i>F</i>	<i>P</i>
	Frequency	3	0.56	0.645
Searching	Latency	3	1.75	0.174
	Percent total duration	3	0.78	0.512
	Frequency	3	0.59	0.624
Contact	Latency	3	1.42	0.253
	Percent total duration	3	2.00	0.133
	Frequency	3	0.13	0.940
Oviposition attempts	Latency	3	1.88	0.151
	Percent total duration	3	1.15	0.342
	Frequency	3	0.28	0.839
Standing	Latency	3	0.17	0.915
	Percent total duration	3	1.62	0.203

2.5. Discussion

In the present study, we investigated if the generalist aphelinid wasp *A. abdominalis* uses innate cues to recognize new hosts by the naïve parasitoids or by parasitoids that have already a foraging experience but on a different host. Moreover, we studied whether the handling of one aphid species improves the handling skills of the other species.

Freshly emerged *A. abdominalis* without any experience have to rely exclusively on innate cues to find a host. In our experiment, naïve females in *M. euphorbiae* patches exhibited similar frequency and percent duration of the foraging time with the behaviors leading to a

reward acquisition (namely search, contact and oviposition attempts) as naives in *M. persicae* patches. This result shows that the wasps not only innately respond to the cues emitted from both hosts in a similar manner, but also exhibit similar types of behaviors elicited by those cues. Once the host is recognized and accepted, we found that naive *A. abdominalis* oviposited earlier, with a higher frequency and time spent in oviposition behavior in *M. euphorbiae* prey, possibly because *A. abdominalis* has an inherent preference for this aphid species. Honěk et al. (1998) found that the lowest *A. abdominalis* mortality and the heaviest mummy weight were recorded in *M. euphorbiae* (3.9 %, SE 2.9-4.9) aphids as compared to *Metopolophium dirhodum* (15.0 %, SE 12.0-18.7) and *Rhopalosiphum padi* (L.) (45.5 %, SE 41.8-49.2).

The parasitoids reveal another scenario when comparing the naïve to the experienced individuals. In *M. persicae* patches, the frequency and time spent in oviposition activities were higher with *M. persicae* experienced wasps, thus demonstrating that host experience reinforces operant learning i.e. the patterns of prey handling (e.g. Nurindah et al., 1999a) and results in a stronger response to the target stimuli (Bjorksten and Hoffman, 1995; Papaj and Vet, 1990). For example, we obtained that the time required recognizing the host decreased as the parasitoid handled successive hosts. Experienced females were more active foragers than naïve *Aphelinus* because they stood later than the naives, had a lower frequency and spent less time in the standing behavior relative to the naïve wasps. Moreover, experienced *Aphelinus* started the external host examination process through antennation (Nurindah et al., 1999b) or contact behavior earlier than the naive *A. abdominalis*. Subsequently the females launched earlier the process of internal host recognition (Nurindah et al., 1999b) through the oviposition attempt behavior to assess the suitability of the host (Henry et al., 2005). Host acceptance is ultimately contingent upon chemical cues that are examined during ovipositor probing (Michaud and Mackauer, 1994; Nurindah et al., 1999a). Thus, experienced parasitoid contrasted with the naïve ones learned how to save in the time required completing the handling sequence prior to obtaining an oviposition reward i.e. in contact, oviposition attempts and pausing behaviors. In *M. persicae* and *M. euphorbiae* patches, frequency and time allocated to oviposition were alike between the naive *Aphelinus* and the *Aphelinus* experienced with the switched aphid species (Figures 2.2 & 2.3). The response of the experienced wasp similar to the innate response of the naïve female may be due to priming (Turlings et al., 1993). *M. persicae* and *M. euphorbiae* aphids have different defensive strategies upon parasitoid attack: *M. euphorbiae* retreats its stylets and leaves the feeding location whereas *M.*

persicae remains at its feeding site and kicks. Nevertheless, we found that when switching the aphid species on the second patch, the experienced forager has shown a similar capacity to handle both aphid species. The latency, frequency and percent total duration of the host handling sequence were alike for both hosts. Thus, *Aphelinus* trained on each aphid species learned to handle both of them efficiently.

Furthermore, *M. euphorbiae* and *M. persicae* experienced females displayed similar frequency and time spent with oviposition activities in *M. euphorbiae* or *M. persicae* patches (Figures 2.2 & 2.3). Besides, the oviposition success of those wasps on each patch was not significantly different when switching the aphid species (Figure 2.4). Therefore, experienced wasps foraging in the presence of the alternate aphid species have acquired an experience type which is positive. Agreeing with the concept that the innate use of infochemicals occurs in all carnivores regardless of dietary specialization (Steidle and van Loon, 2003), it is possible that *A. abdominalis* uses general chemical cues present in all her host species to recognize an aphid as host. Le Ralec et al. (2005) offering *A. abdominalis* the two hosts *S. avenae* and *M. persicae* and the non-host *Aphis gossypii* Glover found that the reactive distance or the distance at which a host could be perceived did not appear to differ between host and non-host aphids. Those authors suggested that host species recognition seems not to be achieved from a distance. Hence, it is argued that for host recognition, *A. abdominalis* mostly relies on non-volatile cuticular kairomones derived from the aphid body and perceived at a distance of 3-4 mm (Le Ralec et al., 2005).

Hymenopterous parasitoids are equipped with mechanisms that enable them to deal with the dichotomy between searching for hosts and food foraging (Wäckers et al., 2002). Those mechanisms are expressed relative to their physiological needs (Wäckers, 1994). Overall, our results show that the foraging *Aphelinus* invested more in current reproduction i.e. oviposition than in future reproduction i.e. host feeding. For example, the frequency and time spent with the host feeding behavior were statistically alike between naïve and experienced *Aphelinus* particularly in *M. persicae* patches (Figures 2.2 & 2.3). In addition, within and between *M. persicae* and *M. euphorbiae* patches, the experienced *Aphelinus* host fed at a similar frequency and allocated similar time performing this behavior (Figures 2.2 & 2.3). *A. abdominalis* are experimentally used when having a full complement of mature eggs (3-7 days old) so the motivation to lay an egg is higher than to feed on a host (Heimpel and Rosenheim, 1995). We found that in *M. persicae* patches, 55.56 % naïve *Aphelinus* oviposited relative to 5.56 % performed host feeding. One hundred percent *M. persicae* experienced *Aphelinus* oviposited in *M. persicae* whereas

37.5 % host fed on this aphid. One hundred percent *M. euphorbiae* experienced wasps oviposited in *M. persicae* compared to 30 % exploiting this prey as a food source. In *M. euphorbiae* patches, 71.43 % of the naïve *Aphelinus* oviposited while none host fed. 90 % *M. euphorbiae* experienced females laid an egg in *M. euphorbiae* host when 30 % host fed. 73.73 % *M. persicae* experienced *Aphelinus* oviposited in *M. euphorbiae* in parallel to 27.27 % feeding on this prey species. Consequently, the tendency to oviposit that we observed might have been triggered by the high initial female egg load interacting with the parasitoid's assessing the L3 nymphal stage of the aphid as a high quality host. Within the four hour recording session, females having oviposited might have not experienced increased egg limitation triggered by a decreasing egg load. Therefore, under our experimental conditions, host feeding was not a requirement for egg replenishment.

We obtained that the sucrose fed females might have mostly exploited the kairomonal effect of honeydew (e.g. Grasswitz and Paine, 1993; Green and Ayal, 1998; Shaltiel and Ayal, 1998) possibly to heighten their innate searching behavior (Bouchard and Cloutier 1984; Budenberg, 1990; Budenberg et al., 1992) and as a directive (Green and Ayal, 1998) and arresting (Grasswitz and Paine, 1993) cue to the aphid aggregation but rarely as rich source of energy because the experimental females were satiated. But when fed upon, honeydew had a noteworthy impact. This relevance of honeydew was shown through the significant frequency and time spent with feeding on *M. persicae* produced honeydew between naïve and experienced wasps overlooking this behavior (Figures 2.2 & 2.3). We have not found such an importance of honeydew feeding in *M. euphorbiae* patches. This result can be attributed either to the physiological needs of the females or to the lower nutritious quality of *M. euphorbiae* produced honeydew.

Within the four-hour experimental recording, switching the aphid species between patches caused minimal departure from the patch. Since the assessment of a host's value by a parasitoid is governed by her recent foraging experience (Mackauer et al., 1996), under our trial conditions, the data show first, that both species are given the same value, and second, that *A. abdominalis* has not modified her original patch quality estimate and categorizes the first patch of equal value to the second one. This finding is to be verified by giving the female the opportunity for patch leaving by choice.

To conclude, switching *M. persicae* and *M. euphorbiae* between patches has not affected the foraging responses of *Aphelinus* females. Though the females have not learned to improve the rewarding behaviors dictated by the physiological state of the animal, they have learned how to efficiently handle the host when compared to the naïve wasps.

Furthermore, experienced wasps displayed similar successful parasitism when the aphid hosts were alternated. Therefore, we admit the hypothesis that the generalist *A. abdominalis* relies on the common occurrence of chemical cues used for host recognition and acceptance.



3 Two Protagonists on Aphidophagous Patches: Effects of Learning and Intraguild Predation

3.1. Abstract

In aphidophagous systems, parasitoid and predator guilds frequently display an asymmetric form of intraguild predation (IGP). Not all predators are equally dangerous. Therefore, defensive responses to non-threatening predators may result in lost foraging opportunities. We used sweet pepper patches sustaining *Macrosiphum euphorbiae* (Thomas) or *Myzus persicae* (Sulzer) aphids as a model system to trace the foraging behaviors of the aphelinid parasitoid *Aphelinus abdominalis* (Dalman) when confronted with L2 *Chrysopa carnea* (Stephens). We standardized the females by comparing behavioral reactions of host-experienced wasps. We split the IGP interactions into two categories. First, we presented a scheme delineating the bidirectional protagonists' interactions. Second, we investigated the effect of learned recognition of the predator on the rewarding and non-rewarding behaviors of the parasitoids. Finally, we explored whether the extraguild prey species promotes IGP in the antagonists' mutual interactions and the rewarding behaviors. The parasitoids' conditioning treatments are as follows: predator naive or predator experienced wasps foraging in a patch with or without *C. carnea*. Results reveal that presence of, or experience with the predator has not affected *Aphelinus* foraging activities. We propose three hypotheses to discuss the behavioral and ecological implications of our findings. Predation fluctuates in space and in time. Accordingly, the wasps exhibited a constant update of information regarding the relative predation risk. The predator induced mobility of *M. euphorbiae* yielded higher frequency of reciprocal antagonists' contacts. Besides, it has motivated the female for a higher frequency and allocation time for oviposition in *M. euphorbiae* host.

3.2. Introduction

The foraging behavior of animals is shaped by energetic costs and by the risk from natural enemies (Krebs and Davies, 1987). Intraguild predation (IGP) defined as a predation event where a member of the guild preys upon another member of the same guild is a ubiquitous phenomenon among aphidophagous predators (Lucas, 2005). When predators and parasitoids are implicated in intraguild interactions, the IGP is unidirectional that is the parasitoid is always the intraguild prey (IG prey) and the predator is the intraguild predator

(IG predator) (e.g. Lucas, 2005; Meyhöfer and Klug, 2002). Therefore, the failure to respond to an IG predator can cost the IG prey its life or that of her progeny, but responding to non threatening predators often comes at a relatively high cost to the prey through loss of feeding sites, decreased reproduction and/or high energy investment (Dicke and Grostal, 2001; Lima and Dill, 1990). Thus, the ability to accurately assess the risk of IGP should be beneficial, and the costs associated with antipredator behaviors should act as a driving force for the IG prey species to develop efficient risk assessment systems (Helfman, 1989). The prey should also develop the capacity to avoid a potentially lethal confrontation (Grostal and Dicke, 1999). There is a condition dependent plasticity of prey responses to predators (Mathis et al., 2003). Plasticity might be beneficial if the risk of predation is not always constant that is when the vulnerability of the prey to the predator may not be the same at all life stages (Mathis et al., 2003). For example, prey might outgrow the handling limits of some predators (e.g. Mathis et al., 2003) or may develop defenses such as toxins that provide protection (Pettersson et al., 2005).

The optimization of prey response suggests that recognition of predation risk through chemical information is crucial for the fitness of many animals (e.g. Dicke and Grostal 2001; Ferrari and Chivers, 2006; Grostal and Dicke, 1999; Kusch et al., 2004; Mathis et al., 2003; Persons and Rypstra, 2001) and facilitates learned recognition of novel predators (Ferrari and Chivers, 2006). The chemical signature of a predator may be direct such as kairomones (e.g. Dicke and Grostal, 2001) or indirect for instance information from disturbed /injured (alarm pheromones) or dead conspecifics (e.g. Dicke and Grostal, 2001; Ferrari and Chivers, 2006; Grostal and Dicke, 1999). In addition, a body of literature shows that the predator's diet serves as an indirect cue uncovering the identity of the predator to the prey (e.g. Chivers et al., 1996; Dicke and Grostal, 2001; Kortet and Hedrick, 2004; Meng et al., 2006) because it provides information about the proclivity of a predator to feed on a particular prey type (Persons and Rypstra, 2001).

The prey can assess the level of immediate threat (Ferrari and Chivers, 2006, Schmitz et al., 2004). A single prey species responds differently to different predator species or predator behavior (Schmitz et al., 2004). Different predator avoidance behavior may then represent different degrees of risk aversion resulting from the amount of information prey have about predators (Sih, 1992).

Predator size can be an important indicator of threat for some species (Kusch et al., 2004). For example, the two co-occurring wolf spiders *Pardosa milvina* (Hentz) and *Hogna helluo* (Walckenaer) engage in size structured IGP. Adult female *Pardosa* varies

antipredator responses towards kairomones produced by *Hogna* that varies in size. This was manifested by a decrease in the activity in the presence of kairomones from *Hogna* of equal or larger size but showed no change in the presence of a blank control or from single *Hogna* smaller than itself (Persons and Rypstra, 2001).

Though the parasitoid-predator interaction is asymmetrical (e.g. Lucas, 2005; Meyhöfer and Klug, 2002), the presence of an extraguild prey mitigates the intensity of the IGP (e.g. Hindayana et al., 2001; Lucas, 2005).

Our present research is aimed at investigating the effect of on patch learning of the predator's presence on the foraging behavior of the parasitoid. Our general hypothesis addresses the following question: can the IG prey learn the level of risk associated with a particular predation threat? Our tritrophic system is the aphelinid wasp *Aphelinus abdominalis* (Dalman) (Hymenoptera: Aphelinidae) as IG prey, the second larval stage (L2) *Chrysopa carnea* (Stephens) (Neuroptera: Chrysopidae) as IG predator, the two aphid species *Myzus persicae* (Sulzer) and *Macrosiphum euphorbiae* (Thomas) (Homoptera: Aphididae) as extraguild preys, and a sweet pepper leaf disc sustaining one of the two herbivores.

Three specific objectives were the focus of this study. First, to examine the “predator inspection” phenomenon, which may serve the parasitoid to gain information about the type of predator encountered (Magurran and Girling, 1986) or the predator's readiness to attack (Licht, 1989). Therefore, we have presented an outline of the mutual behavioral interactions between the parasitoid and the predator. Second, to investigate how the female *Aphelinus* processes the circuit of information gained on patches of different qualities. Thus, we have compared behavioral reactions of predator naïve or predator experienced *Aphelinus* in patches bearing or free from *C. carnea*. Third, we have highlighted the effect of the extraguild prey species on the IGP interactions when presenting the rewarding behaviors and the parasitoid-predator interactive behaviors.

3.3. Materials and Methods

Rearing

Sweet pepper (*Capsicum annuum* L., cv. “Mazurka”) plants and eggplants (*Solanum melongena* L., cv. “Ecavi”) (Solanaceae) were grown in the nursery of the Institute of Plant Diseases and Plant Protection (Leibniz University of Hannover, Germany), at a temperature of ca. 20°C, 60-70 % rh and 16:8 L:D photoperiod. Aphid cultures were kept

in climatic chambers in gauze cages at a temperature of $20 \pm 1^\circ\text{C}$, 16:8 L:D photophase, and 60 % rh. *M. persicae* was exclusively reared on sweet pepper plants whereas a mixture of sweet pepper and eggplants was offered to *M. euphorbiae* to enhance colony proliferation and alate production. A stock culture of the grain aphid *Sitobion avenae* (F.) feeding on wheat (*Triticum aestivum* L.) (Triticae) was sustained to generate *A. abdominalis* mummies. After pupation, mummies were harvested and transferred into fine gauze-covered acrylic cylinders that were placed on plastered pots. Emerged wasps were supplied with 15 % sucrose solution at 16:8 L:D photoperiod, $22 \pm 1^\circ\text{C}$ and 90 % rh until their experimental deployment. The lacewing predator (*C. carnea*) was supplied by Katz Biotech Company (Germany).

Aphid preparation

Ten to twelve alate *M. persicae* or *M. euphorbiae* collected from the stock culture were caged overnight on a sweet pepper plant to generate synchronized progeny. The subsequent day, twelve L1 of each aphid species were transferred using a fine Kolinsky hairbrush to a patch made of a 2 cm diameter sweet pepper leaf disc, which was laid over a similar diameter piece of cotton imbibed with water. The wet cotton carrying the leaf disc was immersed in a thick film of water in a 3 cm diameter Petri-dish. The water film was intended to obstruct the free movement of the antagonists or the aphids from the leaf disc. The patches were placed in 13×15×5 cm plastic containers whose cover and sides were perforated and replaced with mesh to facilitate ventilation. The containers were kept for three days in climatic chambers at a temperature of $20 \pm 1^\circ\text{C}$, 16:8 L:D regime and 60 % rh. At the fourth day, the aphids reaching the L3 nymphal stage were used in the experiment.

Handling of the predator and the parasitoid

Naïve 3-7 day old *A. abdominalis* were collected from the stock culture and transferred to the sweet pepper leaf discs sustaining *M. persicae* or *M. euphorbiae* as herbivore victim. The treatments are presented in Figure 3.1. To guarantee *Aphelinus* females' motivation for egg laying, they were allowed to forage for 15 minutes before L2 *C. carnea* were introduced. In a preliminary trial, we examined the suitability of L3 *C. carnea* to be used in the main experiment. Video assessment of the results revealed that in *M. euphorbiae* patches with both antagonists, 75 % of the female parasitoid left the patch by jumping

and/or flying and 25 % were exterminated by the L3 *C. carnea*. Since we needed the biological material in subsequent treatments, we opted for the L2 stage.

To standardize the *Aphelinus*' experience with respect to the extraguild prey, we have released naïve female *Aphelinus* on aphid patches with or without the L2 *C. carnea*. On this first patch, all the females gained experience with the extraguild prey but some of them were predator naïve while others were predator experienced. Then we have collected those wasps and subjected them to a second patch bearing or lacking the L2 *C. carnea*. The parasitoids' behavioral reactions in this second patch were the focal point of our research.

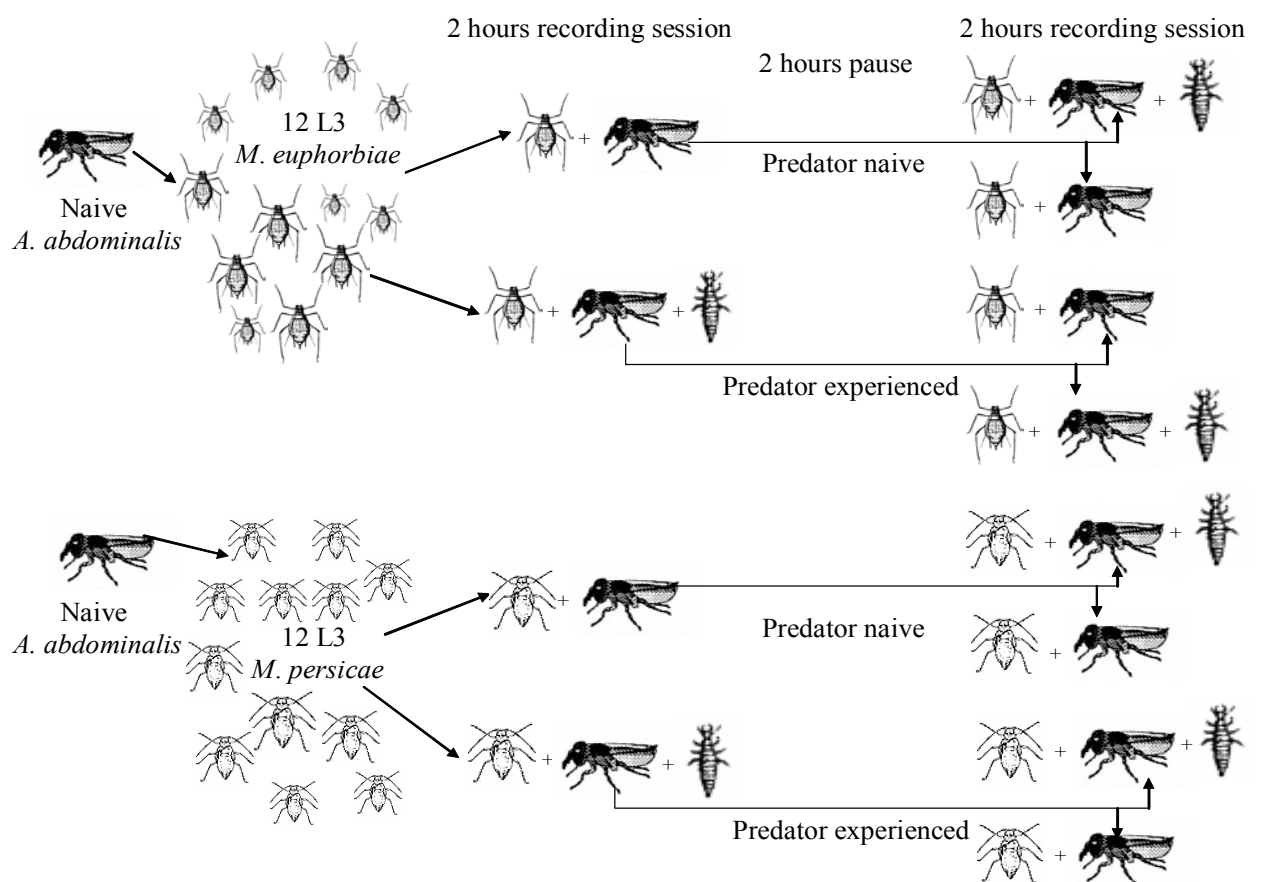


Figure 3.1. Schematic representation of the experimental treatments.

Naïve *Aphelinus abdominalis* were released in *Macrosiphum euphorbiae* or *Myzus persicae* patches with or without the second larval stage (L2) of the predator *Chrysopa carnea*. The parasitoids were allowed to forage for two hours. The collected *Aphelinus* were all host experienced but some were predator naïve while others were predator experienced. Those *Aphelinus* females were given a two-hour resting period. Afterwards, they were offered for two hours a second foraging opportunity in the presence or absence of L2 *C. carnea*.

Recording and quantification of the two protagonists' interactions

The behavioral reactions of *A. abdominalis* rivalling with L2 *C. carnea* in *M. persicae* or *M. euphorbiae* patches were recorded with a multiple video camera set up described by Meyhöfer (2001) and later analyzed with the Observer Video-Pro system (Noldus Technology, 1997). Sixteen cameras were used simultaneously and two recording sessions were held. In the first session, the behavioral decisions of naïve *Aphelinus* were traced. For each aphid species, four replications per treatment were jointly recorded. All of the *Aphelinus* wasps gained experience with the aphid host but some were predator naïve while others predator experienced (Figure 3.1). The *Aphelinus* females were collected and allowed to rest for two hours isolated from any infochemical source under ambient laboratory conditions. This pause was necessary to manipulate the mechanism of sensitization and desensitization to chemicals associated with hosts (Thiel and Hoffmeister, 2004) and opponents (Vet, 1999). Consequently, the *Aphelinus* wasps were able to respond adaptively on the second patch. The next recording session was done to depict the behaviors of the *Aphelinus* parasitoids in patches harboring or free from L2 *C. carnea*. Thus, predator naïve and predator experienced *Aphelinus* were released in patches with or without *C. carnea* (Figure 3.1). For each extraguild prey species (*M. persicae* or *M. euphorbiae*), two replications per treatment per recording session could be concurrently recorded. Six consecutive recording sessions were conducted at different dates. The number of replications ranged from 19-22 for the recording sessions prior to the pause of the *Aphelinus* and from 9-11 for the ones ensuing the *Aphelinus* resting period (Figure 3.1). At the end of each recording session, the patches sustaining the aphids were kept inside a climatic chamber at a temperature of $20 \pm 1^\circ\text{C}$ until mummification. After seven days, black mummies appear. The number of mummies, when observed, was noted.

Data compilation

We split the behavioral observations of the foraging *Aphelinus* females into two chief categories: parasitoid predator interactive behaviors and parasitoid prey behavioral repertoire.

Parasitoid predator interactive behaviors

The *Aphelinus* parasitoid and L2 *C. carnea* sharing a common aphid resource exhibited bidirectional interactions. We defined two different behavioral states for the parasitoid and two events for the predator.

The behavioral states of the parasitoid are 1) contact predator (physical contact and antennation) and 2) oviposition attempts in the predator (the female attacks the predator with ovipositor insertion). The predatory events are 1) predatory attack (predator directly attacks the parasitoid with its mandibles) and 2) predatory contact (implies nearby physical contact with the parasitoid).

Parasitoid prey behavioral repertoire

While foraging in the patch, *A. abdominalis* displayed two categories of behavioral states: rewarding and non-rewarding. Rewarding behaviors are mostly induced by the physiological needs of the wasps. They have a direct impact on the parasitoid fitness. Rewarding behaviors include oviposition, host feeding referring to the consumption of the host hemolymph by the adult wasp, and honeydew feeding defined as the female's intake of the aphid honeydew droplets deposited on the leaf surface.

Non-rewarding behaviors are related to the operant learning capabilities of the wasp, namely learning to perform a behavioral sequence quickly and accurately to obtain a reward. Non-rewarding behaviors are as follows, 1) searching (the parasitoid moves), 2) contact aphids (starts with the parasitoid approaching to the vicinity of the aphid and antennation), 3) oviposition attempts (the parasitoid turns around and attacks the aphid with her ovipositor), 4) standing (the parasitoid is motionless) and 5) patch leaving.

Measurement of the protagonists' behavioral observations

Behavioral observations yielded two basic types of measurements. The dependent variables are frequency (measured /2h) and percent duration spent with the behavioral states. To study the influence of the learning recognition of the predator on the parasitoid's foraging activities, we have standardized the *Aphelinus* females with respect to aphid host learning. Hence, we have compared behavioral reactions of host experienced *Aphelinus* in patches sustaining or devoid of L2 *C. carnea*. Those *Aphelinus* females have two experience levels with the predator: they are either predator naïve or predator experienced wasps (Figure 3.1).

Statistical analysis

To normalize the data distribution, frequency and percent total duration variables were $\sqrt{(x+0.5)}$ and arcsine transformed, respectively, before any statistical analyses. To determine the significance of single and interaction effects of three factors (i.e., two levels of predator, two host species, two parasitoid experience levels with the predator) on the

frequency and percent duration of a given behavior, a three-way ANOVA was conducted using the PROC GLM procedure in SAS (SAS Institute, 1999). To study if learning recognition of the predator affects *Aphelinus* performance when exposed to L2 *C. carnea*, factorial combinations of the parasitoid experience with the predator and the predator (presence or absence) were expressed as none (i.e., no encounter, predator naïve *Aphelinus* foraging in a patch without L2 *C. carnea*), encounter as experienced (predator naïve *Aphelinus* foraging in a patch with L2 *C. carnea*), encounter as naïve (predator experienced *Aphelinus* foraging in a patch without L2 *C. carnea*), and encounter as naive and experienced (predator experienced *Aphelinus* foraging in a patch with L2 *C. carnea*) (Figure 3.1). Whenever deficient number of observations was obtained for a particular behavior for a given factor, ANOVA analysis was reduced to a two-way analysis by dropping the factor with insufficient number of observations. In case of a significant interaction between factors, different levels of a factor were compared at a given level of the second factor; otherwise, data were pooled. When a significant effect of a factor is detected by means of ANOVA, the dependent variable means at different levels of the respective factor were compared using Tukey's multiple means comparison procedure.

The number of mummified aphids in the presence or absence of *C. carnea* was recorded and percent mummy formation was calculated. The percentage values were arcsine transformed before being subjected to statistical analyses.

In all analyses, a 0.05 alpha level was used and data are presented as means \pm SE.

3.4. Results

At first, we have hypothesized that direct confrontations of the two protagonists may confer the *Aphelinus* parasitoid with learned recognition of *C. carnea* predator. Therefore, we have presented a scheme delineating the bidirectional parasitoid-predator interactive behaviors.

Because predation level is not fixed and fluctuates in space and time (Lima and Dill, 1990), a continuous learning process could keep up to date the actual threat of a given predator (Ferrari and Chivers, 2006). Thus, we have evaluated the development of such responses to the predator through the comparison of behavioral decisions of predator naïve and predator experienced *Aphelinus* in patches with or without *C. carnea*.

IGP between natural enemies of aphids cannot be fully evaluated without referring to the aspect of aphid behavior (Brodeur and Rosenheim, 2000). Therefore, we have inspected

whether the extraguild prey species promotes IGP interactions when presenting the rewarding behaviors and the parasitoid predator interactive behaviors. We presume that those two categories of behavioral reactions are relevant in showing the learned recognition of the predator.

Parasitoid predator interactive behaviors

This comparison aims to present a detailed scheme of the mutual interactions between *A. abdominalis* and L2 *C. carnea*. We assume that the physical presence of the predator in the patch is an important mediator of “menace” learning process. In the presence of *C. carnea*, we compare the behavioral reactions of predator naïve and predator experienced *Aphelinus* wasps (Figure 3.1). Therefore, per aphid species, we contrast the frequency and percent total duration spent in contact predator, oviposition attempts in the predator, predatory contacts and predatory attack behaviors. Consequently, we can assess whether the predator naïve parasitoids learn to recognize the predator and if so, whether the intensity of their antipredator responses match those of the predator experienced *Aphelinus*.

One hundred percent predator naïve (average duration $11.4 \text{ s} \pm 1.15 \text{ SE}$) and 100 % predator experienced *Aphelinus* (average duration $13.5 \text{ s} \pm 2.77 \text{ SE}$) contacted the L2 *C. carnea* when the extraguild prey was *M. euphorbiae*. In parallel, 77.8 % predator experienced (average duration $22.4 \text{ s} \pm 6.99 \text{ SE}$) and 90.9 % predator naïve *Aphelinus* (average duration $16.5 \text{ s} \pm 6.26 \text{ SE}$) contacted L2 *C. carnea* in a *M. persicae* patch. A comparison between predator naïve and predator experienced *Aphelinus* revealed no significant difference in the mean frequency (Table 3.1) (Figure 3.2) and the percent duration of time spent in contact predator behavior when the host is *M. euphorbiae* (ANOVA $df = 1, F = 0.38, P = 0.547$) or *M. persicae* (ANOVA $df = 1, F = 0.48, P = 0.500$) (Figure 3.3).

Oviposition attempts in the predator behavior was restricted to 9.09 % predator naïve *Aphelinus* (mean duration 32.7 s) and 11.11 % predator experienced *Aphelinus* (mean duration 6.5 s) in *M. persicae* patches. Predator naïve *Aphelinus* attacked *C. carnea* as frequently as predator experienced *Aphelinus* (ANOVA $df = 1, F = 0.02, P = 0.889$) (Figure 3.2). Predator naïve *Aphelinus* spent 0.035 % ($\pm 0.035 \text{ SE}$) of the foraging time attacking *C. carnea* in *M. persicae* patches whereas predator experienced *Aphelinus* invested 0.010 % ($\pm 0.010 \text{ SE}$) time displaying this behavioral state in the presence *M. persicae* prey (Figure 3.3).

C. carnea contacted 81.82 % predator experienced and 90.91 % predator naïve *Aphelinus* when the herbivore victim was *M. euphorbiae*, and 77.78 % predator experienced and 63.64 % predator naïve *Aphelinus* with *M. persicae* as the offered host. The results divulge no significant difference in the mean frequency of predatory contact between predator naïve and predator experienced *Aphelinus* (Table 3.2) (Figure 3.2).

In *M. euphorbiae* patches, *C. carnea* attacked 36.36 % predator experienced and 45.45 % predator naïve *Aphelinus*. When the extraguild prey was *M. persicae*, *C. carnea* attacked 22.22 % predator experienced and 45.45 % predator naïve *Aphelinus*.

No statistical difference in the mean frequency of predatory attack was found between predator naïve and predator experienced *Aphelinus* in *M. euphorbiae* (ANOVA $df = 1$, $F = 0.63$, $P = 0.436$) or in *M. persicae* (ANOVA $df = 1$, $F = 1.40$, $P = 0.251$) patches (Figure 3.2).

Table 3.1. Summary of ANOVA results of mean frequency of *Aphelinus abdominalis* contact predator in a patch sustaining twelve L3 aphids as affected by the species of the aphid host and the parasitoid experience with the predator.



Source of variation	df	<i>F</i>	<i>P</i>
Host	1	4.50	0.041
Parasitoid experience	1	0.97	0.330
Host * Parasitoid experience	1	0.00	0.952

Contact predator behavior: parasitoid approaches the predator and antennation.

The parasitoids were offered either *Myzus persicae* or *Macrosiphum euphorbiae* hosts.

Parasitoid experience with predator implies predator naïve or predator experienced (Figure 3.1).

Table 3.2. Summary of ANOVA results of mean frequency of predatory contact behavior in a patch sustaining twelve L3 aphids as affected by the species of the aphid host and the parasitoid experience with the predator.



Source of variations	df	<i>F</i>	<i>P</i>
Host	1	9.27	0.004
Parasitoid experience	1	0.18	0.678
Host * Parasitoid experience	1	0.00	0.962

Predatory contact behavior: predator is physically in the vicinity of the parasitoid.

The parasitoids were offered either *Myzus persicae* or *Macrosiphum euphorbiae* hosts.

Parasitoid experience with predator implies predator naïve or predator experienced (Figure 3.1).

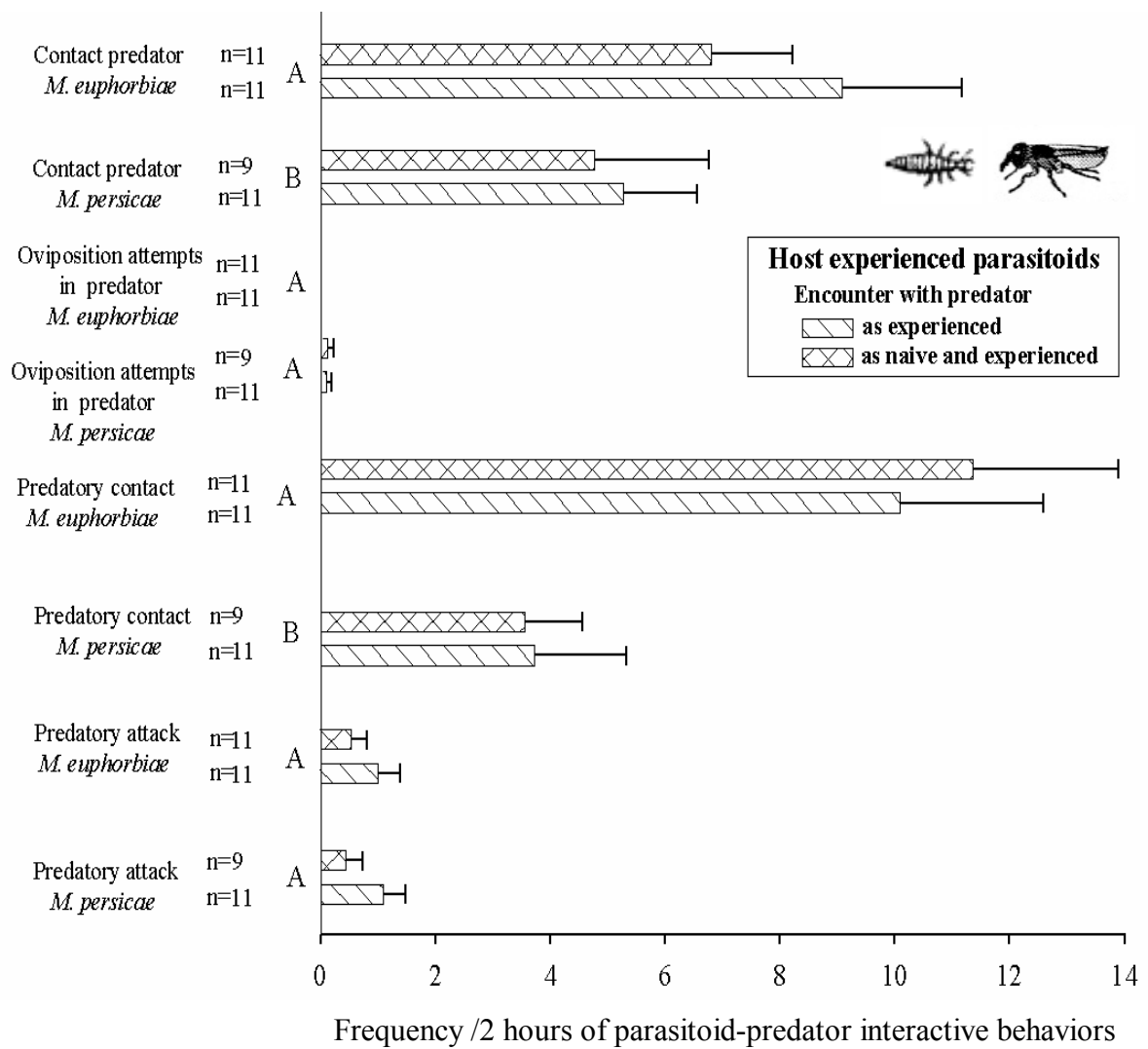


Figure 3.2. Mean (\pm SE) frequency / 2 hours of mutual interactive behaviors of predator naïve or predator experienced *Aphelinus abdominalis* and L2 *Chrysopa carnea* foraging in patches sustaining twelve L3 *Macrosiphum euphorbiae* or *Myzus persicae*.

Predator naïve implies encounter with the predator as host experienced (Figure 3.1).

Predator experienced implies encounter with predator as naïve and host experienced (Figure 3.1).

Parasitoid experience level with the predator had no effect on any behavior at a given aphid species.

Capital letters indicate the effect of the aphid species on the behavior of the *Aphelinus abdominalis* wasp or on the *Chrysopa carnea* predator.

(n) indicates the number of replications per treatment.

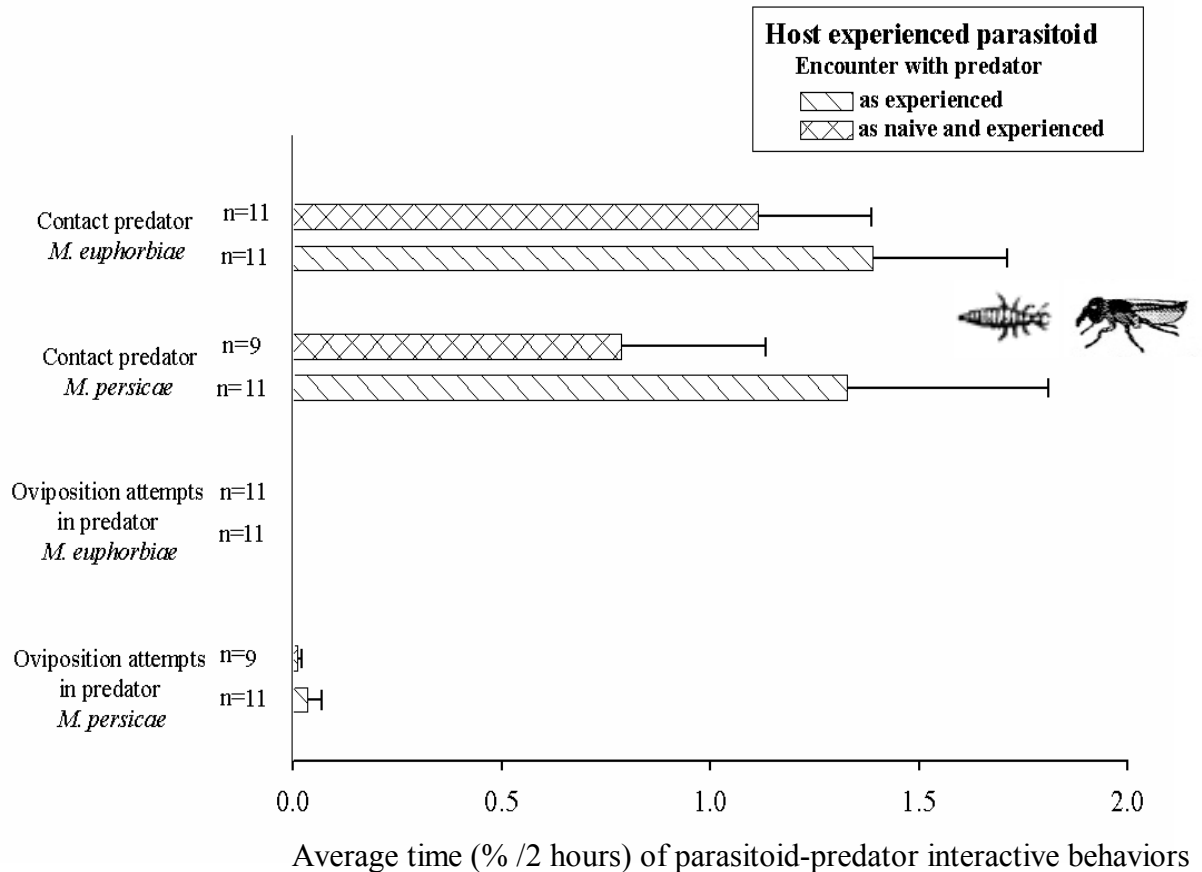


Figure 3.3. Average time (% / 2 hours) (\pm SE) spent in mutual interactive behaviors between predator naïve and predator experienced *Aphelinus abdominalis* and the L2 *Chrysopa carnea* in patches sustaining twelve L3 *Macrosiphum euphorbiae* or *Myzus persicae*.

Predator naïve implies encounter with the predator as host experienced (Figure 3.1).

Predator experienced implies encounter with predator as naïve and host experienced (Figure 3.1).

The parasitoid experience level with the predator had no significant effect on any behavior for both aphid species.

The species of the aphid had no significant effect on the behavior of *Aphelinus abdominalis* parasitoid.

(n) indicates the number of replications per treatment.

Parasitoid prey behavioral repertoire

To standardize the experience level of the females, we have compared the behavioral states (rewarding and non-rewarding) of *Aphelinus* parasitoids, which are aphid experienced but are either predator naïve or predator experienced (Figure 3.1). Those females were allowed to forage in patches with or without L2 *C. carnea*. Our aim is to investigate, 1) the effect of predator learning on the foraging behavior of the *Aphelinus* wasps, and 2) whether a

continuous learning process would keep the *Aphelinus* prey up to date regarding the actual threat of a given L2 *C. carnea* predator.

Rewarding behaviors

Results reveal that predator naïve *Aphelinus* displayed comparable mean frequency as predator experienced *Aphelinus* in the oviposition behavior in patches free from or sustaining *C. carnea* when the extraguild prey was either *M. euphorbiae* (ANOVA $df = 3$, $F = 1.53$, $P = 0.223$) or *M. persicae* (ANOVA $df = 3$, $F = 0.51$, $P = 0.679$) (Figure 3.4). Furthermore, predator naïve *Aphelinus* invested a similar proportion of time to oviposit in *M. euphorbiae* (ANOVA $df = 3$, $F = 1.69$, $P = 0.185$) or in *M. persicae* (ANOVA $df = 3$, $F = 0.47$, $P = 0.703$) as predator experienced *Aphelinus* in patches with or without *C. carnea* (Figure 3.5). Also, predator naïve and predator experienced *Aphelinus* yielded a statistically comparable oviposition success in the presence or absence of *C. carnea* (ANOVA $df = 7$, $F = 1.72$, $P = 0.420$) (Figure 3.6).

In *M. euphorbiae* patches with or without *C. carnea*, predator experienced *Aphelinus* exhibited a similar frequency (ANOVA $df = 3$, $F = 0.53$, $P = 0.661$) (Figure 3.4) and allocation time (ANOVA $df = 3$, $F = 0.63$, $P = 0.603$) (Figure 3.5) as predator naïve *Aphelinus* to the host feeding behavior. Similarly, in *M. persicae* patches bearing or free from *C. carnea*, predator experienced *Aphelinus* host fed as frequently as predator naïve *Aphelinus* (ANOVA $df = 3$, $F = 0.80$, $P = 0.502$) (Figure 3.4) and spent a comparable time with host feeding behavior as predator naïve *Aphelinus* (ANOVA $df = 3$, $F = 0.47$, $P = 0.704$) (Figure 3.5).

Predator experienced *Aphelinus* performed honeydew feeding behavior in patches harboring or lacking *C. carnea* as frequently as predator naïve *Aphelinus* when *M. euphorbiae* (ANOVA $df = 3$, $F = 1.46$, $P = 0.241$) or *M. persicae* (ANOVA $df = 3$, $F = 0.89$, $P = 0.456$) was the extraguild prey (Figure 3.4). Furthermore, in patches with or without *C. carnea*, predator experienced *Aphelinus* invested a similar proportion of time as predator naïve *Aphelinus* in collecting *M. euphorbiae* (ANOVA $df = 3$, $F = 0.94$, $P = 0.430$) or *M. persicae* (ANOVA $df = 3$, $F = 0.85$, $P = 0.475$) honeydew droplets (Figure 3.5).

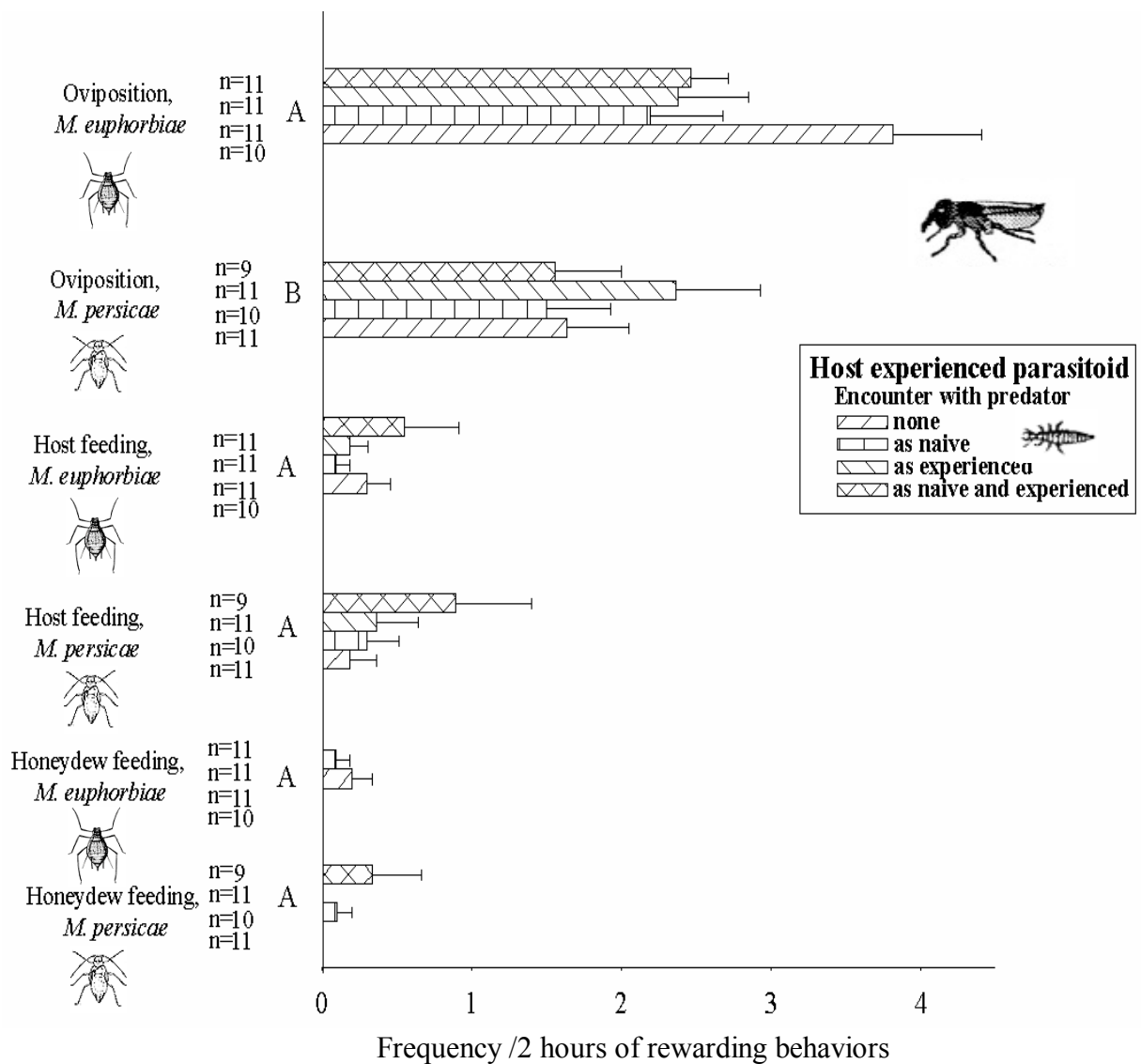


Figure 3.4. Mean frequency / 2 hours (\pm SE) of rewarding behaviors of predator naive and predator experienced *Aphelinus abdominalis* females foraging in patches sustaining twelve L3 *Macrosiphum euphorbiae* or *Myzus persicae* with or without the L2 *Chrysopa carnea* predator.

Predator naïve implies encounter with the predator as host experienced or no encounter (Figure 3.1)

Predator experienced implies encounter with predator as naive and host experienced, or as host experienced (Figure 3.1).

Parasitoid experience level with the predator had no effect on any behavior at a given aphid species.

Capital letters indicate the effect of the aphid species on the behavior of the parasitoid.

(n) indicates the number of replications per treatment.

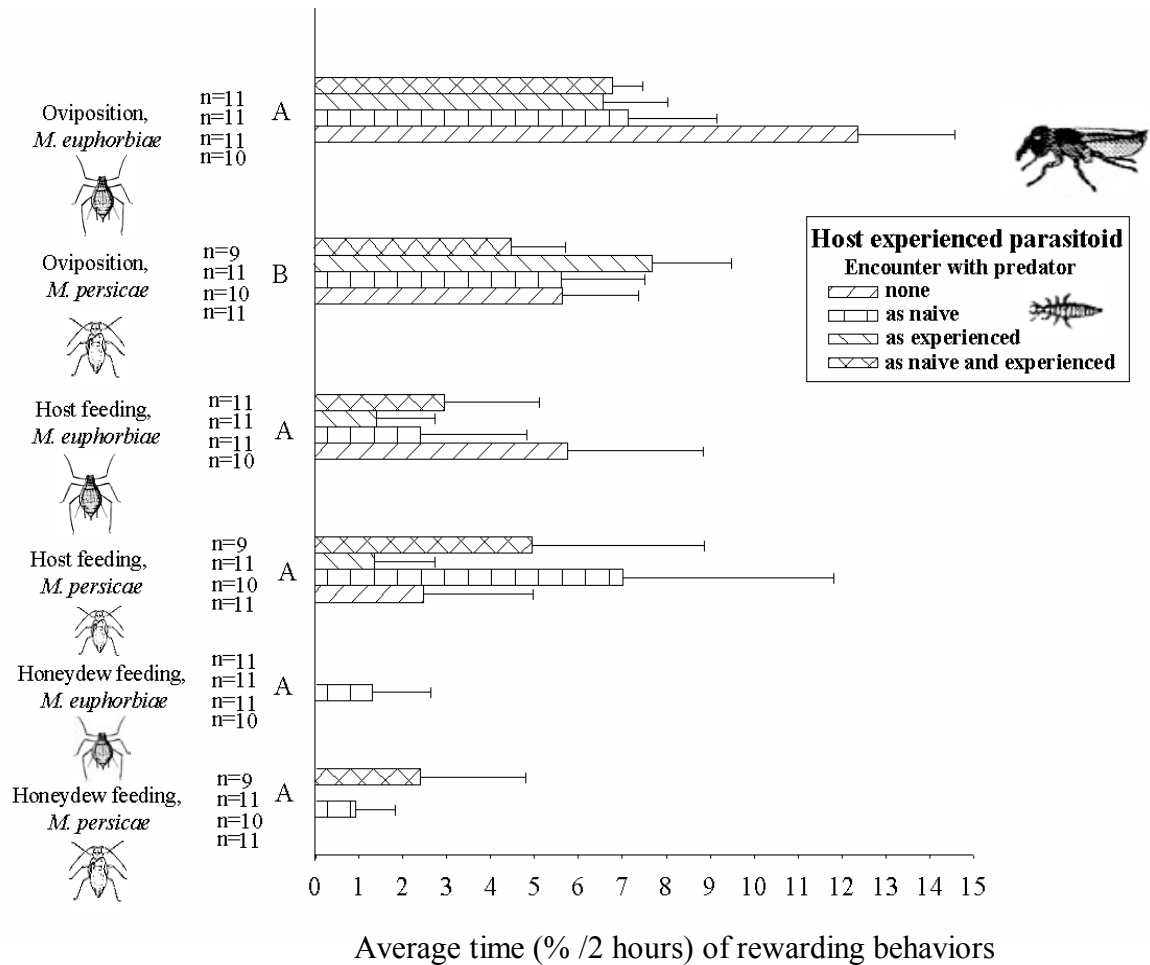


Figure 3.5. Average time (% / 2 hours) (\pm SE) spent by predator naïve and predator experienced *Aphelinus abdominalis* females with rewarding behaviors in patches sustaining twelve L3 *Macrosiphum euphorbiae* or *Myzus persicae* with or without the L2 *Chrysopa carnea* predator.

Predator naïve implies encounter with the predator as host experienced or no encounter (Figure 3.1).

Predator experienced implies encounter with predator as naive and host experienced, or as host experienced (Figure 3.1).

Parasitoid experience level with the predator had no effect on any behavior for both aphid species.

Capital letters indicate the effect of the aphid species on the behavior of the parasitoid.

(n) indicates the number of replications per treatment.

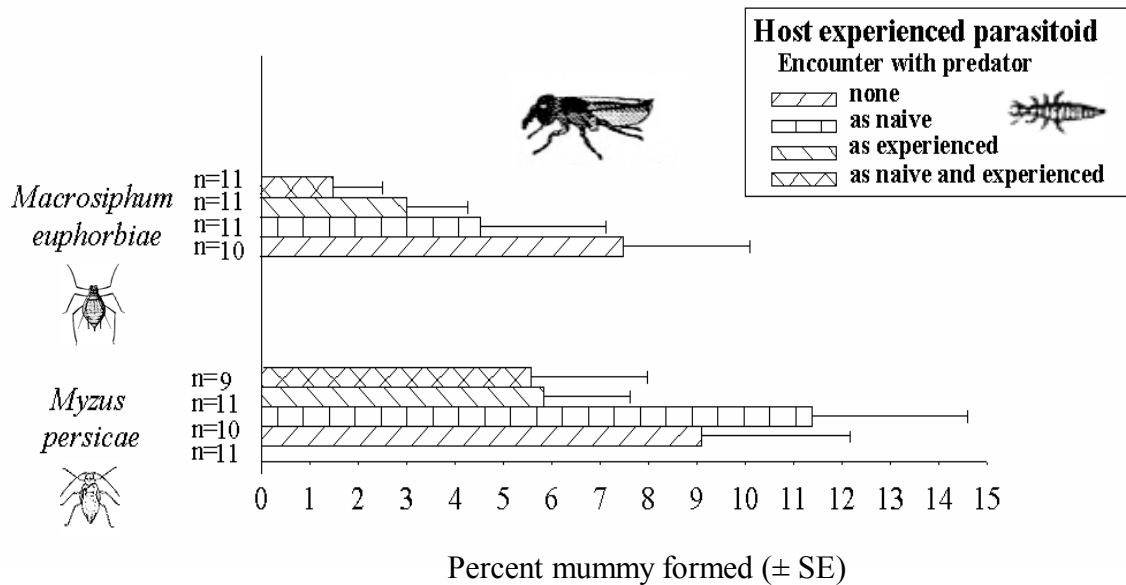


Figure 3.6. Percent mummy formed (\pm SE) by predator naïve or predator experienced female *Aphelinus abdominalis* in patches sustaining twelve L3 *Macrosiphum euphorbiae* or *Myzus persicae* with or without the L2 *Chrysopa carnea* predator.

Predator naïve implies encounter with the predator as host experienced or no encounter (Figure 3.1).

Predator experienced implies encounter with predator as naïve and host experienced, or as host experienced (Figure 3.1).

Predator naïve and predator experienced *Aphelinus* displayed similar oviposition success in *Macrosiphum euphorbiae* and *Myzus persicae* patches with or without *Chrysopa carnea* (ANOVA $df = 7$, $F = 1.72$, $P = 0.420$).

(n) indicates the number of replications per treatment.

Non-rewarding behaviors

The presence of L2 *C. carnea* in *M. euphorbiae* patches affected the searching activity of the foraging *Aphelinus* wasps (ANOVA $df = 1$, $F = 47.88$, $P < 0.0001$) and the proportion of time (ANOVA $df = 1$, $F = 11.13$, $P = 0.0019$) allocated to the searching behavior. Predator naïve and predator experienced *Aphelinus* displayed 2.6 fold higher mean searching frequency (Figure 3.7) and invested 1.4 fold higher proportion of time (Figure 3.8) in searching for *M. euphorbiae* aphids in the patch with *C. carnea* than in the patch without *C. carnea*.

With *M. persicae* as extraguild prey, predator naïve and predator experienced *Aphelinus* exhibited similar searching frequency (ANOVA $df = 3$, $F = 1.46$, $P = 0.241$) (Figure 3.7)

and invested similar proportion of time (ANOVA $df = 3$, $F = 2.54$, $P = 0.071$) (Figure 3.8) in searching behavior in a patch with or devoid of *C. carnea*.

Foraging in the occurrence of *C. carnea* had a significant impact on the mean frequency (ANOVA $df = 1$, $F = 19.25$, $P < 0.0001$) and the time devoted (ANOVA $df = 1$, $F = 20.71$, $P < 0.0001$) to *M. euphorbiae* contact. With *C. carnea* in the patch, predator naïve and predator experienced *Aphelinus* contacted *M. euphorbiae* host 1.5 fold more frequently than predator naïve and predator experienced *Aphelinus* foraging in a *C. carnea* free patch (Figure 3.7). On the other hand, the *Aphelinus* parasitoids exhibited a different scenario regarding the time budget devoted to *M. euphorbiae* contact behavior. Predator experienced *Aphelinus* rivalling with L2 *C. carnea* invested a similar proportion of time as predator naïve *Aphelinus* in *M. euphorbiae* contact behavior in a *C. carnea* free patch. Moreover, predator naïve *Aphelinus* foraging in the presence of *C. carnea* spent a comparable time in *M. euphorbiae* contact as predator experienced *Aphelinus* in a patch lacking *C. carnea* (Figure 3.8).

Predator naïve and experienced *Aphelinus* contacted *M. persicae* in a patch with *C. carnea* as frequently as in a patch lacking *C. carnea* (ANOVA $df = 3$, $F = 2.66$, $P = 0.062$) (Figure 3.7). In contrast, *C. carnea* presence in the patch (ANOVA $df = 1$, $F = 16.52$, $P = 0.0001$) affected the percent duration of *M. persicae* contact behavior. For example, predator naïve *Aphelinus* foraging in a *C. carnea* free patch expended 1.5 fold higher proportion of time in *M. persicae* contact than predator experienced *Aphelinus* confronted with the L2 *C. carnea* (Figure 3.8).

C. carnea sharing the same *M. euphorbiae* extraguild prey with predator naïve or predator experienced *Aphelinus* had no significant effect on the mean frequency (ANOVA $df = 3$, $F = 0.87$, $P = 0.465$) (Figure 3.7) and percent duration (ANOVA $df = 3$, $F = 0.35$, $P = 0.786$) (Figure 3.8) of oviposition attempts in *M. euphorbiae* host.

With *M. persicae* as extraguild prey, being predator naïve or predator experienced had a significant impact on the mean frequency of *Aphelinus* oviposition attempts behavioral state (ANOVA $df = 1$, $F = 8.16$, $P = 0.007$). For instance, predator naïve *Aphelinus* wasps foraging in a patch lacking *C. carnea* displayed 2.8 times more frequent oviposition attempts behavior in *M. persicae* contrasted with predator experienced females competing with L2 *C. carnea* (Figure 3.7). In parallel, predator naïve and predator experienced *Aphelinus* allocated an analogous percent duration of time to oviposition attempts behavior in *M. persicae* patches harboring or free from L2 *C. carnea* (ANOVA $df = 3$, $F = 0.27$, $P = 0.847$) (Figure 3.8).

Predator naive and predator experienced *Aphelinus* in the presence of *C. carnea* paused with similar frequencies as predator naive and predator experienced *Aphelinus* in the absence of *C. carnea* in *M. euphorbiae* (ANOVA $df = 3, F = 2.29, P = 0.093$) or *M. persicae* (ANOVA $df = 3, F = 0.73, P = 0.539$) patches (Figure 3.7). Furthermore, in the presence of *C. carnea*, predator naive and predator experienced *Aphelinus* spent an equivalent percent of the foraging time motionless as in the absence of *C. carnea* in *M. euphorbiae* (ANOVA $df = 3, F = 0.47, P = 0.706$) or in *M. persicae* (ANOVA $df = 3, F = 1.65, P = 0.195$) patches (Figure 3.8).

No female *Aphelinus* departed from *M. euphorbiae* patches. 9.09 % predator naive *Aphelinus* in the presence of *C. carnea*, 20 % predator experienced *Aphelinus* in *C. carnea* free patch and 11.11 % predator experienced *Aphelinus* in a patch with *C. carnea* left *M. persicae* patches.

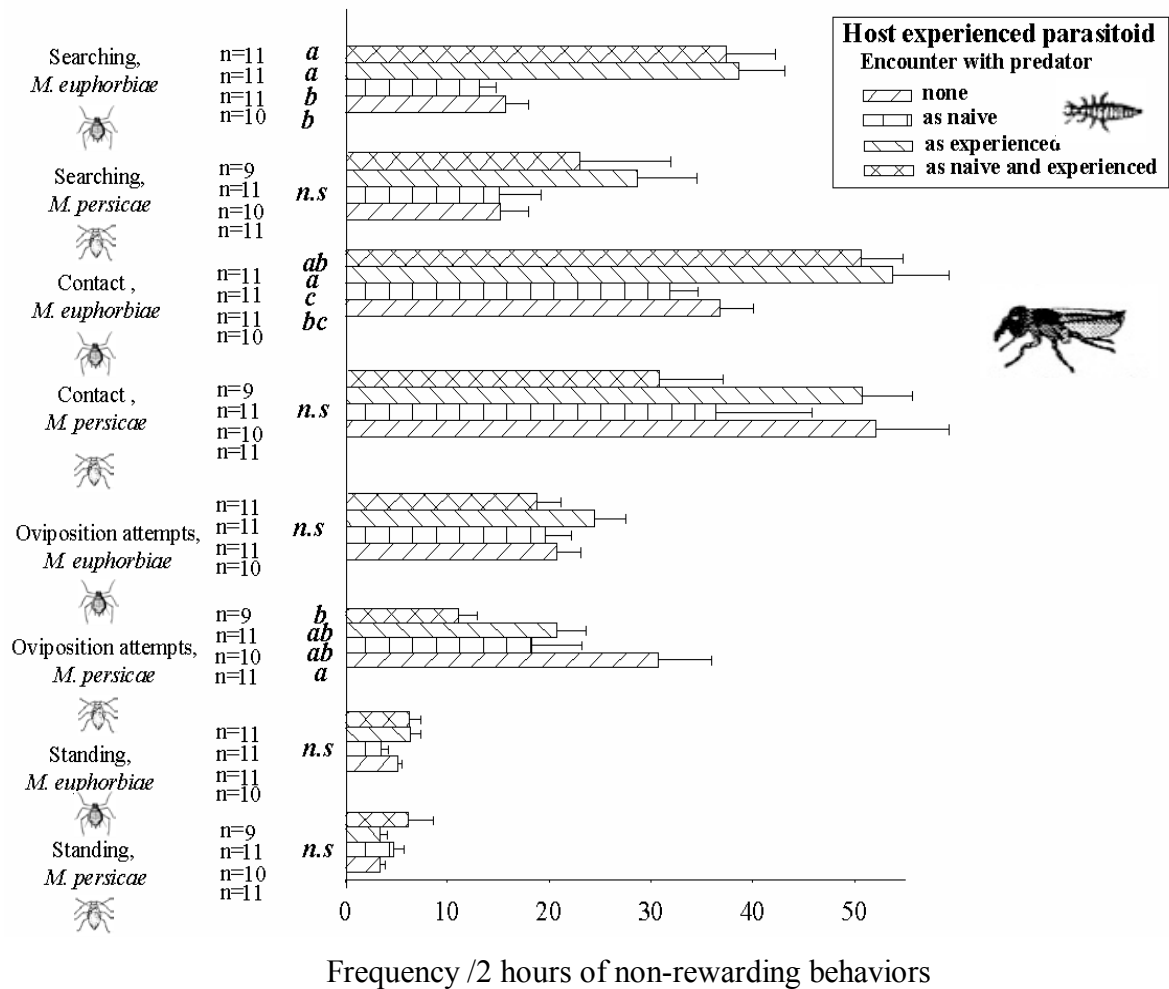


Figure 3.7. Mean frequency / 2 hours (\pm SE) of non-rewarding behaviors of predator naïve and predator experienced *Aphelinus abdominalis* females in patches sustaining twelve L3 *Macrosiphum euphorbiae* or *Myzus persicae* with or without the L2 *Chrysopa carnea* predator.

Predator naïve implies encounter with the predator as host experienced or no encounter (Figure 3.1)

Predator experienced implies encounter with predator as naive and host experienced, or as host experienced (Figure 3.1).

n.s /italic letters indicate non-significant/significant difference between predator naïve and predator experienced *Aphelinus abdominalis* for a given behavior and a given host species.

(n) indicates the number of replications per treatment.

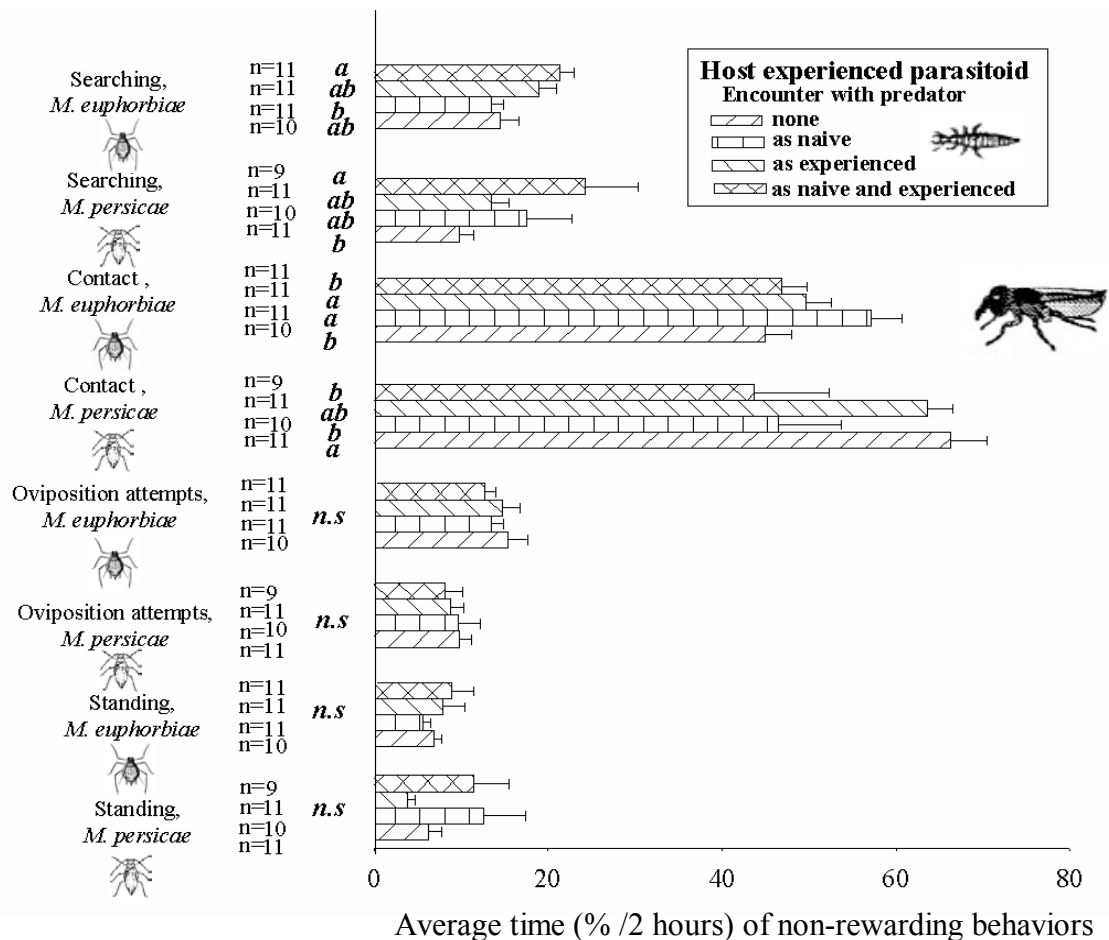


Figure 3.8. Average time (% / 2 hours) (\pm SE) of non-rewarding behaviors of predator naïve and predator experienced *Aphelinus abdominalis* in patches sustaining twelve L3 *Macrosiphum euphorbiae* or *Myzus persicae* with or without the L2 *Chrysopa carnea* predator.

Predator naïve implies encounter with the predator as host experienced or no encounter (Figure 3.1).

Predator experienced implies encounter with predator as naïve and host experienced, or as host experienced (Figure 3.1).

n.s. /italic letters indicate non-significant/significant difference between predator naïve and predator experienced *Aphelinus abdominalis* for a given behavior and a given host species.

(n) indicates the number of replications per treatment.

Effect of extraguild prey species on intraguild interactions

This comparison inspects whether the species of the extraguild prey promotes IGP interactions, therefore *M. euphorbiae* experienced *Aphelinus* were compared to *M. persicae* experienced ones when parasitoid predator interactive behaviors and rewarding behaviors are presented.

Parasitoid predator interactive behaviors

Results reveal that the foraging *Aphelinus* displayed 1.8 fold higher mean frequency of contact predator behavior in *M. euphorbiae* relative to *M. persicae* patches (Figure 3.2). In contrast, the percent duration of contact predator behavior was similar in both patches ($P = 0.301$) (Figure 3.3).

In addition, *C. carnea* exhibited three times higher frequency of predatory contact behavior in *M. euphorbiae* compared to *M. persicae* patches (Figure 3.2). The *Aphelinus* females performed oviposition attempts in predator behavior with similar frequencies in *M. euphorbiae* and *M. persicae* patches ($P = 0.142$) (Figure 3.2). The predator attacked with comparable frequency the *Aphelinus* foraging in *M. euphorbiae* and *M. persicae* patches ($P = 0.904$) (Figure 3.2).

Rewarding behaviors

We found that the *Aphelinus* female exhibits a higher mean frequency (Figure 3.4) and endowed a higher proportion of time (Figure 3.5) to oviposition in *M. euphorbiae* than in *M. persicae*. The *Aphelinus* host fed on *M. persicae* as frequently as on *M. euphorbiae* (Figure 3.4) and allocated comparable proportion of time to performing host feeding behavior in *M. euphorbiae* and *M. persicae* patches. Moreover, the frequency (Figure 3.4) and percent total duration (Figure 3.5) of honeydew feeding behavior were not influenced by the species of the herbivore victim.

3.5. Discussion

Any defensive response (including fleeing or migration) by potential prey is costly (e.g. Dicke and Grostal, 2001) and frequent responses to cues from sources that have no consequences on fitness would be counter adaptive (Dicke and Grostal, 2001). Thus the ability to accurately assess the risk of predation should be beneficial (Helfman, 1989).

In general *Aphelinus* behavioral reactions (non-rewarding and more specifically rewarding ones) were not altered by the presence of the predator in the patch or by experience with L2 *C. carnea*. The parasitoid assessed the values of patches with or free from *C. carnea* of equal suitability. Therefore escape manoeuvres as departure from the patch were minimal. For example, predator naive and predator experienced *Aphelinus* exhibited comparable frequency and time allocated for oviposition, host feeding and honeydew feeding activities in patches free from or bearing *C. carnea*. Furthermore, no reduced oviposition success

was found between patches with or without *C. carnea* or between predator naïve and experienced *Aphelinus*.

We offer three hypotheses to explain this outcome. First, based on her superiority in size, *Aphelinus* female does not validate the relative risk associated with the presence of the L2 *C. carnea* threatening enough to display pronounced antipredator responses such as leaving the patch or depressed oviposition. In some cases, the intensity of the kairomone-mediated antipredator responses may be related to the relative size of the prey compared to the predator (e.g. Kusch et al., 2004; Mathis et al., 2003; Persons and Rypstra, 2001). L2 *C. carnea* is less than 0.8 mm long whereas female *Aphelinus* is about 3 mm long. Those results agree with Persons and Rypstra (2001) who found that *Pardosa milvina* mite tended to avoid substrates previously occupied by a *Hogna helluo* larger than itself, but showed no substrate avoidance if the paper had supported a spider or spiders of equal or smaller size than itself.

On the other hand, Meyhöfer and Klug (unpublished data) put the “size related predation risk” postulation into question. They used L3 *C. carnea* (approximately 13 mm long) on sweet pepper leaf discs sustaining twenty L2 *M. persicae*. One minute after the introduction of either female *Aphidius colemani* (Viereck) or *Aphidius ervi* (Haliday) (Hymenoptera: Aphidiidae) the predator was released and the predator-parasitoid interactions were recorded with video cameras for the following four minutes. Both parasitoids are smaller in size than the L3 *C. carnea*. Meyhöfer and Klug (unpublished data) found that one *A. colemani* and two *A. ervi* left the patch by flight in the presence of L3 *C. carnea*. Running away or short take offs from the leaf were the preferred strategies to increase distance to the predator. Nevertheless, most females were not influenced at all by direct encounters and continued host foraging even in the close vicinity of L3 *C. carnea* (Meyhöfer and Klug, unpublished data). Consequently, one question may be raised: why aren't parasitoids in the current study and in that conducted by Meyhöfer and Klug (unpublished data) adjusting their behaviors to decrease the predation risk?

We propose the second premise. Since on aphidophagous patches the parasitoid is exposed to a variety of information from a cocktail of predators belonging to different orders, it may not be adaptive to the parasitoids to respond to predator cues through sensitization but mostly through associative learning. The learned recognition of a prey exposed to generalist predators is synergized through diet-related chemical labelling of the predator. *A. abdominalis* wasp has proven an ability to learn associatively (Mölck et al., 2000). So the female should enjoy the general capacity to associate an odor to which she innately

responds with predation threat that she learns. This negative association helps to initiate a timely escape that increases her survival rate and thus that of her offspring. In cohort with this supposition, Dicke and Grostal (2001) proposed that when predator kairomones (constituting the learned cues) are not encountered in combination with cues from recently killed conspecifics (as innate cues), the predator cues might not represent a real danger. So, it would be challenging to verify this hypothesis through the exploration of the response of predator naïve *A. abdominalis*, *A. ervi* or *A. colemani* on aphid patches in confrontation with predators fed intra or interspecific mummies. We hypothesize that the predator naïve parasitoids learn to recognize the predator when stimuli from conspecific mummies are paired with stimuli from predators. This kind of research would tackle another little explored domain: do arthropod preys utilize information about enemy presence from heterospecifics mainly when they are sympatric as for example *A. ervi* and *A. colemani*?

Third suggestion, *Aphelinus* females are foraging in a host rich patch, where the abundance of extraguild preys dilutes the threat of IGP. Meyhöfer and Klug (unpublished data) found that the L3 *C. carnea* fed mostly two aphids during the five minutes recording session. Our video observations demonstrated that during the two hours recording, L2 *C. carnea* consumed four aphids maximum. We suggest releasing *Aphelinus* and L2 *C. carnea* antagonists in a patch with half of the prey currently offered and monitor their interactions. *Aphelinus* is a slow moving parasitoid, equipped with an efficient jumping reaction when disturbed. This behavior might constitute an alternative to learning, meaning that the parasitoid needs not to learn to respond to any threatening situation.

In general in most behaviors (for example frequency of searching, contact, and oviposition attempts in *M. persicae* patches), and more specifically in the rewarding ones, predator experienced *Aphelinus* in a patch with L2 *C. carnea* behaved similarly to predator naïve *Aphelinus* in a *C. carnea* free patch. These results suggest that at least with a double exposure to the predator, the number of exposure events does not seem to affect the intensity of the response. We conclude that the female *Aphelinus* seems to use a safety strategy responding to the predator cues with an intensity that matched her risk assessment, following our above mentioned suggestions.

Furthermore, we have inversed the order of exposure to the predator with predator naïve *Aphelinus* in a patch harboring the *C. carnea* predator versus predator experienced females in a *C. carnea* free patch. This interplay did not seem to influence the intensity of the learned response. We found no statistical significance between the behaviors of the mentioned *Aphelinus* wasps in all the rewarding behaviors and for example, in the

frequency and percent duration of oviposition attempts behavior in *M. persicae* and *M. euphorbiae* patches. This result suggests that *Aphelinus* females seemed to rely primarily on the latest of the predator exposure events to respond to a predator threat. Because predation fluctuates in space and time (Ferrari and Chivers, 2006) this response of *Aphelinus* appears to be adaptive. Relying on the most updated information regarding the level of threat associated with a given predator reduces unnecessary costs associated with antipredator behaviors as for instance leaving the patch.

The extraguild prey species played a trivial role in promoting the IGP interactions. In *M. euphorbiae* patches, we found higher frequency of contact predator and predatory contact behaviors. The antipredator response of the aphid explains this outcome. The *M. euphorbiae* aphid retreats its stylets and moves away from the feeding site to escape *C. carnea* attack. This pronounced aphid mobility offers a higher probability of predator-parasitoid encounter in their search for the common resource. Furthermore, it makes *M. euphorbiae* more attractive for oviposition (e.g. Cournoyer and Bovin, 2005; Mackauer et al., 1996; Michaud and Mackauer, 1995; Turlings et al., 1993) as compared to the more quiescent *M. persicae*.

To conclude, our study showed that despite the simultaneous presence of the two protagonists in the same patch, no lethal effects (interspecific killing) of IGP was found. *A. abdominalis* females often approached the predator up to a certain point as “predator inspection” phenomenon (Lima, 1998) but initiated no evasive behaviors. *C. carnea* are active predators with broad habitat domains. Prey facing highly mobile predators may be the least risk averse, considering the fitness costs (energetic and survival penalty) associated with continuous predator avoidance (Bouskila, 2001). Additionally, the wasp has not shown a behavioral alteration due to learning the presence of the L2 *C. carnea* predator. This is not a lack of her ability to express learning of predatory cues. Even tiny, short-lived animals are capable of associative learning (e.g. Papaj and Lewis, 1993). Since not all predators are equally dangerous (Kats and Dill, 1998), the parasitoid has expressed a functional choice dictated by the larval stage of *C. carnea* i.e. risk assessment through previous experience plus information update and the aphid abundance in the patch.

4 Direct and Indirect Interactions Between *Aphelinus abdominalis* and *Chrysopa carnea*: Effects of Intraguild Predation and Prey Antipredator Behaviors

4.1. Abstract

Intraguild predation (IGP) is defined as the killing and eating of species that use similar, often limiting, resources and are thus potential competitors. It constitutes one of primordial factors threatening the success of aphidophagous biological control programs exploiting a cocktail of protagonists. IGP is asymmetric between parasitoids and predators. In addition to the destruction of mummified aphids or killing the adult parasitoid, predators induce behavioral changes in the extraguild prey, which can reduce its survival and reproduction. The cost of antipredator behaviors may not only have negative impacts on the phytophagous prey, but also indirectly affect the inferior parasitoid. Combination of a predator and a parasitoid may exert an additive (proportional to individual enemy effect) or non-additive (greater or less than expected from the individual enemy effects) pest population suppression.

We investigated the interactions between the aphelinid parasitoid *Aphelinus abdominalis* (Dalman) and the chrysopid predator *Chrysopa carnea* (Stephens), natural enemies of *Macrosiphum euphorbiae* (Thomas) and *Myzus persicae* (Sulzer) aphids in microcosms each housing single sweet pepper plant. Our first objective is to determine the intensity and direction of the protagonists' interactions by comparing the combined and individual impacts of both natural enemies over time. The second aim is to assess the direct (mummy destruction) or indirect (through extraguild prey behavioral modification) impacts of IGP. For that we censused aphid densities and recorded the number of mummified aphids (eaten or intact) on days two, five, eight and eleven following treatment application. The treatments are: 1) aphid alone (control), 2) *A. abdominalis* alone, 3) *C. carnea* alone, and 4) *A. abdominalis* and *C. carnea*.

Our results revealed that the parasitoid caused little immediate reduction in *M. euphorbiae* and *M. persicae* population growth over time. Consequently, the aphid density always converged with the control. The predator did not inflict density dependent reduction on both extraguild prey species except eight days following release. After eight days of

interaction, the combined treatment effect was additive and synergistic in reducing *M. euphorbiae* and *M. persicae* population, respectively. In parallel, after eleven days of interaction, pairing both natural enemies was synergistic and antagonistic on *M. euphorbiae* and *M. persicae* suppression, respectively.

No direct impacts of IGP were apparent. The induced antipredator response is prey specific. We found that within five days of interaction, *M. euphorbiae* antipredator behaviors resulted in lower number of mummies formed relative to the mummified *M. persicae*. *M. euphorbiae* induced defensive responses caused a decrease in the aphid population without an increase in the predator weight gain eight days following the antagonists' release. We conclude that in our system behaviorally mediated prey suppression constituted a factor more important than direct IGP in shaping the outcome of *A. abdominalis*-*C. carnea* interactions in *M. euphorbiae* microcosms.

4.2. Introduction

The increased awareness about environmental safety has directed the avenues of scientific research towards the blending of benign, reliable and long lasting tactics to combat pest problems. A possible but still debatable candidate is whether the introduction of multiple natural enemy species leads to more efficient pest suppression than the release of a single species. Enemy impacts often attenuate through a diverse network of reticulate species interactions (e.g. Ferguson and Stiling, 1996; Finke and Denno, 2003; Rossi, 2004). Intraguild predation (IGP), the consumption of one predator by another predator, enhances the reticulate nature of a food web (Finke and Denno, 2003). Predators not only feed on herbivorous prey but also either attack each others symmetrically (e.g. Persons and Rypstra, 2001) or asymmetrically (e.g. Meyhöfer and Hindayana, 2000; Meyhöfer and Klug, 2002), or interfere with another predator's foraging behavior (Lima and Dill, 1990). Predator induced defensive responses are costly (Lima and Dill, 1990; Lima, 1998). A rapidly growing body of research on indirect interactions is building appreciation for the role of non-consumptive effects in multi-species communities (Nelson et al., 2004). Through induced changes in prey behavior, predators can transmit indirect effects upon their prey's resources, competitors and other predators (Nelson et al., 2004). The ultimate result is often relaxed predator pressure and diminished top-down control of shared phytophagous prey (Snyder and Ives, 2001). Between predator and parasitoid guilds, the IGP interaction is always asymmetrical, the parasitoid being the inferior antagonist.

IGP is a ubiquitous phenomenon among aphidophagous systems (Lucas, 2005) subject to attack by specialist and generalist entomophagous arthropods. Because of their lack of specificity, generalists not only feed on the herbivorous preys, but also engage in IGP.

Thus elucidating the impact of how a complex of predators interact to influence herbivore populations is of vital consequence for both population ecology and integrated pest management. If two predators do not interact, then their combined impact on the prey population will be additive and simply equal the sum of their individual impacts (Snyder et al., 2004). If one predator species kills (through IGP) or interferes with another predator's foraging behavior then the enemy interaction is antagonistic and fewer than expected prey will be killed by their combined action (Finke and Denno, 2003; Snyder and Ives, 2001). In contrast, predator species can interact synergistically when the complex of predator species kill more prey in combination than the sum of their individual impacts (Cardinale et al., 2003; Losey and Denno, 1998).

Aphelinus abdominalis (Dalman) (Hymenoptera: Aphelinidae) is a solitary endoparasitoid reported to accept a number of cereal aphid species as hosts, e.g. *Sitobion avenae* (F.) and *Metopolophium dirhodum* (Walker) (Kalina and Stary, 1976), and *Myzus persicae* (Sulzer) and *Macrosiphum euphorbiae* (Thomas) on sweet pepper, aubergine, tomato etc. in greenhouses (e.g. Colombo and Fasce, 1994). *Chrysopa* spp. (Neuroptera: Chrysopidae) have long been noted as predators on a wide array of agricultural pests. In addition, they have many desirable attributes for use in biological control: they inhabit many diverse agroecosystems, they are tolerant to many insecticides (Bartlett, 1964; Lingren and Ridgway, 1967; Rajakulendran and Plapp, 1982; Shour and Crowder, 1980), and they are easily mass reared (Ridgway et al., 1970). Adults are pollinivorous feeding mainly on honeydew from homopteran species and floral nectar (Canard and Principi, 1984). Larval stages have a voracious appetite for aphids and mealybugs. They also attack spider mites, mite eggs, leafhoppers, small caterpillars, and thrips (Henderson and Raworth, 1991). This catholic feeding of *Chrysopa carnea* (Stephens) may also detract it from biocontrol effectiveness due to IGP. *C. carnea* is a well known IG predator (Meyhöfer and Hindayana, 2000; Meyhöfer, 2001; Meyhöfer and Klug, 2002).

In microcosms housing each a single sweet pepper plant, we examined the effect of pairing the predator *C. carnea* and the parasitoid *A. abdominalis* on the population dynamics of the two aphid species *M. euphorbiae* and *M. persicae* over time. We aimed 1) to investigate whether a guild combination suppressed aphid population to a greater extent than a single enemy species and if so, 2) to decode whether suppression was additive (proportional to

individual enemy effects) or non additive (greater or less than expected from the individual enemy effects), 3) to study the direct (IGP) and indirect (through induced antipredator behavior of the prey) effects on the coexistence of both natural enemies and 4) to examine the effect of the extraguild prey species on the IGP interactions.

4.3. Materials and Methods

Rearing

Sweet pepper (*Capsicum annuum* L., cv. “Mazurka”) plants and eggplants (*Solanum melongena* L., cv. “Ecavi”) (Solanaceae) were grown in the nursery of the Institute of Plant Diseases and Plant Protection (Leibniz University of Hannover, Germany), at a temperature of 20°C, 60-70 % rh and 16:8 L:D photoperiod. Aphid cultures were kept in climatic chambers in gauze cages at a temperature of 20 ± 1°C, 16:8 L:D photo phase, and 60 % rh. *M. persicae* was exclusively reared on sweet pepper plants whereas a mixture of sweet pepper and eggplants was offered to *M. euphorbiae* to enhance colony proliferation and alate production. A stock culture of the grain aphid *Sitobion avenae* (F.) feeding on wheat (*Triticum aestivum* L.) (Triticeae) was sustained to generate *A. abdominalis* mummies. After pupation, mummies were harvested and transferred into fine gauze-covered acrylic cylinders that were placed on plastered pots. Emerged wasps were supplied with 15 % sucrose solution at 16:8 L:D cycle, 22 ± 1°C and 90 % rh until their experimental deployment.

Experimental plants

Four to five week old sweet pepper plants were inoculated with twelve to fifteen alate *M. persicae* or *M. euphorbiae* collected from the stock culture. The aphids were confined overnight in a clip cage to generate synchronized progeny. The subsequent day, the alates were removed, and the synchronized nymphs were allowed to develop on a single leaf enclosed in a 15×25 cm mesh bag (PA-132/40 Nylon, with mesh size 132 µm width and nylon size 0.083 mm) supplied by Franz Eckert GmbH (Germany). Five days later, the small bag was removed and the initial aphid density was adjusted to seventy aphids of all instars. The aphid population was allowed to propagate on the entire plant in a microcosm made of two metal wires the length of each is 50 cm. The wires were manipulated to form a tent like skeleton of 40 cm height, which was firmly inserted into the pot soil. This frame served to maintain a 40×80 cm mesh bag with identical mesh quality as previously described. To prevent the escape of aphids and protagonists from the microcosm, the mesh

cage was tightly closed with a rubber band at the bottom end of the pot. Each plant was placed in a 12×2 cm diameter plastic plate offering a tool to water the plant without opening the microcosm. *M. persicae* and *M. euphorbiae* infested plants were placed in two separate climatic chambers at 16:8 L:D regime, $22 \pm 1^\circ\text{C}$ temperature and 60-70 % rh.

Protagonists' treatments

Three to seven day old naïve wasps removed from the stock culture were transferred to the sweet pepper plants each female singly enclosed in a plastic eppendorf cup and placed on the soil inside the microcosm. *C. carnea* predator was supplied by Katz Biotech AG (Germany). One day prior to testing, second larval stages of the predators were singly confined in 30 cm diameter plastic arenas covered with a plastic lid. The lid centre is perforated and replaced with mesh for ventilation. Each larva was offered a mixed diet of about thirty *M. persicae*, *M. euphorbiae* and *S. avenae* aphids. To assess the predator's voracity, each individual was weighted on the test day and the initial weight recorded. Similarly, at the end of each treatment date, the predator when recuperated, was weighted. The *C. carnea* larvae were deposited directly on the soil inside the microcosm using a fine Kolinsky hairbrush.

We established four treatments: 1) control (aphids alone), 2) aphids and one female *A. abdominalis*, 3) aphids and one L2 *C. carnea*, and 4) aphids and a combination one *A. abdominalis* and one L2 *C. carnea*. Each treatment constituted of twenty plants, every plant being a replication. In total, eighty plants per aphid species represented all the treatments. We then censused aphid densities at four different dates: on days two, five, eight and eleven after treatment application. Thus we were able to follow the impact of the *C. carnea* predator on both aphids and the parasitoid over time. Within each date, the plants were subjected to a completely randomized design so that no identical treatments between dates neighbor each others. In treatments where the parasitoid was prevalent, the plant bearing the aphids was kept after census inside the climatic chamber until mummification. After seven days, black mummies appeared. The number of mummies, when observed, was noted. The mummies were kept until parasitoid emergence. Thereafter, they were checked under the binocular and categorized as 1) emerged, with a distinctive hole in the middle or 2) eaten, with two holes on the sides.

Statistical analysis

To evaluate the efficacy of predator alone, parasitoid alone, or combination of the natural enemies on the population build up of a given aphid species per distinct date, treatments with the protagonists were compared to the control or to each others. Population dynamics of aphids over time was followed by comparing the mean number of aphids of a particular treatment at different dates of data collection. To examine treatment merit between aphid species, the percent reduction was corrected for control data using the following formula

$$\% \text{ population reduction} = \frac{\text{Aphid count in control} - \text{Aphid count in treatment}}{\text{Aphid count in control}} * 100$$

The weight gain of the predator was traced by subtracting the initial weight from the final weight recorded at the start and end of the experiment, respectively.

Count data (i.e., numbers of aphids and numbers of mummies formed) and percent population reduction were $\sqrt{x+0.5}$ and arcsine $\sqrt{}$ transformed, respectively, before being subjected to statistical analyses. The data were analyzed using the PROC GLM procedure in SAS to determine single or interaction effects of factors (SAS Institute, 1999). Whenever significant interactions were observed between factors, the level of one factor was compared at each level of the other factor. The significance of individual treatment on aphid population reduction was evaluated by comparing the numbers of aphids under treatments to the one under no natural enemies using Dunnett's two-sided test. When significant factor effects were detected by means of ANOVA, treatments at different levels of the respective factor were compared using Tukey's multiple means comparison procedure. A significance level of $\alpha = 0.05$ was used in all analyses. Data are presented as means \pm SE.

Aphidophagous predators occurring simultaneously in a crop and exploiting the same food resource are likely to interact. Therefore, we examined the consequences of combined versus single release of *A. abdominalis* and *C. carnea* on *M. euphorbiae* and *M. persicae* population reduction. We have opted to scrutinize the protagonists' interactions at days eight and eleven. We hypothesize that by then, the predator enjoys a voracious feeding habit, mummification occurred and thus opportunity for direct effects of IGP is prevalent.

The additive or non-additive effect (synergism or antagonism) of the predator and the parasitoid was assessed by the modified Finney (1964) procedure (McVay et al., 1977; Salama et al., 1984) for probit analysis. The expected additive percent population reduction (Me) of the combined *A. abdominalis*-*C. carnea* treatment was calculated by: $Me = Mn + Mi(1-Mn)$, where Mn and Mi are the observed percent population reduction caused by the

A. abdominalis and *C. carnea* alone, respectively. Results from the chi-square test, $\chi^2 = (M_{ni} - M_e)^2 / M_e$, where M_{ni} is the observed percent population reduction caused by *A. abdominalis*-*C. carnea* combination, were compared to a chi-square tabulated for 1 df, and $\alpha = 0.05$. If the calculated chi-square value exceeded the tabulated value, a non-additive effect (i.e. synergistic or antagonistic) was assumed (Finney, 1964). Furthermore, if the difference $M_{ni} - M_e = D$ had a positive value, a significant interaction was considered synergistic; if D had a negative value, a significant interaction was considered antagonistic.

4.4. Results

Time related combined and independent impacts of *Aphelinus abdominalis* and *Chrysopa carnea* on aphid population growth

To study the time related impact of the natural enemies when used singly or combined on the aphid population growth, we have undertaken two types of comparisons: first, we have assessed the natural enemy treatments' effect on the aphid population density per date of data collection. Second, we have followed the interactions between each natural enemy treatment and the aphid preys throughout the experimental period namely across the dates of data collection.

Treatment effect on aphid density per date of data collection

We examined aphid-*A. abdominalis* population dynamics in microcosms in the presence and absence of *C. carnea*. Results elucidate that the mean number of aphids recorded was significantly affected by the treatment applied and the date of data collection (Table 4.1). In days two and five, none of the natural enemy treatments, single or combined, had *M. persicae* or *M. euphorbiae* densities that differed from the control. Furthermore, the combined or single impact of the protagonists on *M. euphorbiae* or *M. persicae* density was alike (Figure 4.1).

At day eight, the mean number of *M. euphorbiae* detected between the parasitoid and the control treatments was similar. The predator and the combined treatment yielded a significant decline in the aphid population when compared to the control. Furthermore, combining the two protagonists caused the lowest *M. euphorbiae* population growth. *C. carnea* alone treatment resulted in medium aphid suppression. The highest *M. euphorbiae* density was recorded in the parasitoid treatment (Figure 4.1). In parallel, only when *A. abdominalis* and *C. carnea* were combined, *M. persicae* population density was

significantly lower than the control or any of the antagonists alone treatment eight days following natural enemy release (Figure 4.1).

At day eleven, the combined treatment inflicted a higher *M. euphorbiae* population reduction than the control or each single beneficial treatment (Figure 4.1). In contrast, the mean number of *M. persicae* recorded at day eleven was not significantly affected by the natural enemy presence when the beneficial treatments were compared to the control or among each others (Figure 4.1)

Treatment effect on aphid density across dates of data collection


We have investigated the impact of each beneficial treatment on the dynamics of the aphid population throughout the experimental period, i.e. across dates.

Results reveal a significant increase in *M. euphorbiae* density over time in the control treatment. The aphid population growth was slow early in the experiment then reached an intermediate density on days five and eight to attain the highest peak at the eleventh day. In contrast, *M. persicae* displayed a different scenario: the aphid population build up in the control was slow at days two and five but abruptly and significantly increased at days eight and eleven (Figure 4.1).

With the release of the parasitoid singly, a similar outcome in the population dynamics of both extraguild preys was traced: after two and five days of parasitoid-prey interactions, *A. abdominalis* initially depressed the mean aphid numbers but this effect diminished with time, the aphid densities eventually significantly increasing to converge at eight and eleven days after the wasp release (Figure 4.1).

The noteworthy effects of *C. carnea* became apparent at different dates scaled to the generation time of the predator. *C. carnea* presence in *M. euphorbiae* microcosms led to a balanced *M. euphorbiae* population during the course of the experiment and sustained a low *M. persicae* density two and five days following the predator application. A relaxed aphid control was translated with a significantly pronounced raise in the density of *M. persicae* eight and eleven days after using the predator (Figure 4.1).

The combined treatment retained a low *M. euphorbiae* population with overall similar mean aphid densities throughout the trial period (Figure 4.1). *M. persicae* population exhibited a significant linear increase when both protagonists were jointly applied: as the time to collect the data expands from two to eleven day period of the antagonists' interactions, the aphid density build up significantly upsurges (Figure 4.1).

Table 4.1. Summary of ANOVA results for densities of *Macrosiphum euphorbiae* or *Myzus persicae* hosts recorded after treatment applications at different dates of data collection. 

Source of variations	df	<i>F</i>	<i>P</i>
Treatment	3	18.68	<. 0001
Date	3	105.10	<. 0001
Treatment*Date	9	4.29	<. 0001
Host	1	123.08	<. 0001
Treatment *Host	3	2.59	0.056
Date *Host	3	25.58	<. 0001
Treatment*Date*Host	9	2.19	0.028

Treatments applied are aphid alone as control, *Aphelinus abdominalis* alone, *Chrysopa carnea* alone, and combination of *Aphelinus abdominalis* and *Chrysopa carnea*.

The dates of data collection are 2, 5, 8 and 11 days after treatment application.

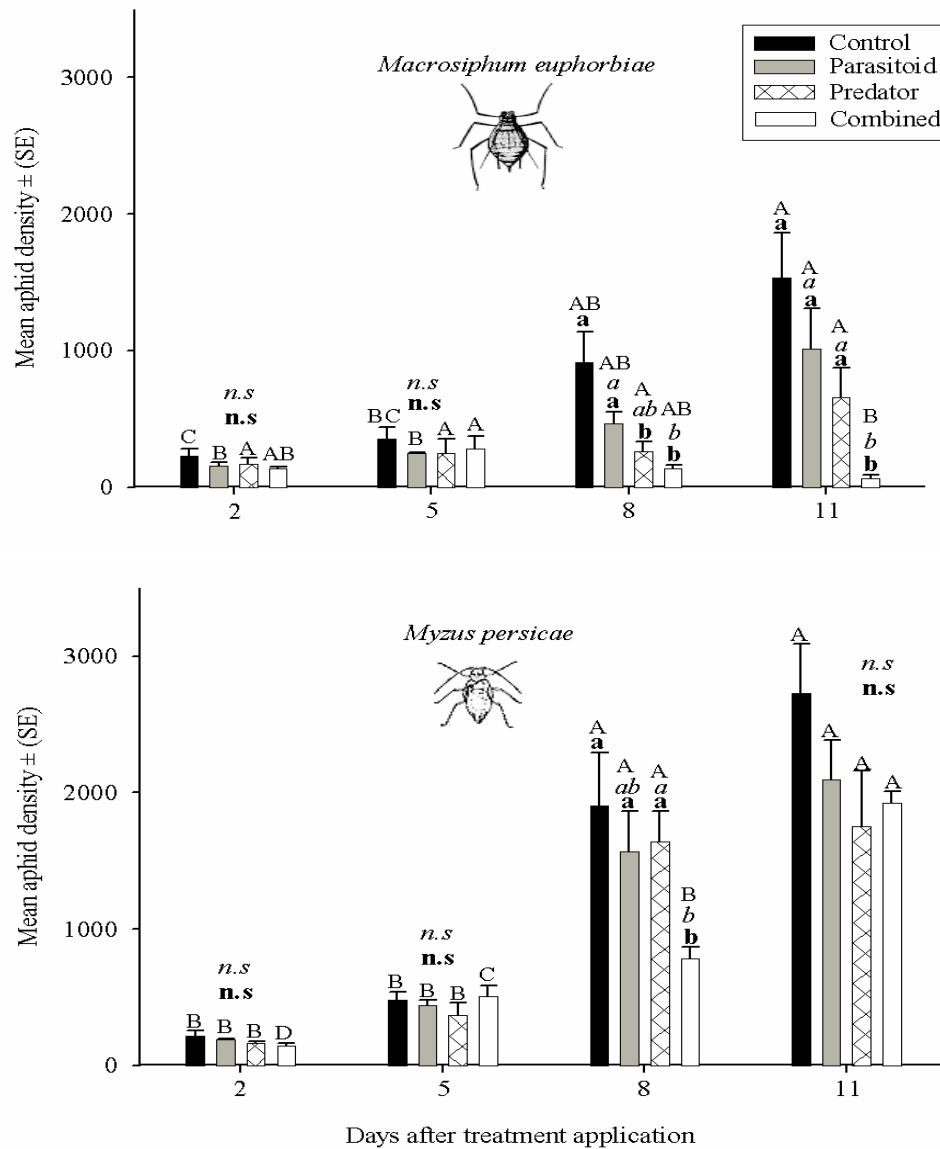


Figure 4.1. Mean (\pm SE) aphid densities collected at different dates (2, 5, 8 and 11 days after treatment application) as affected by different natural enemy treatments.

Bars on a given date for a given aphid species followed by the same small letters do not differ significantly.

Bold small letters serve to compare control vs. treatments with natural enemies.

Italic small letters serve to compare treatments with protagonists among each others.

Bars of similar treatments over different dates per aphid species followed by capital letters do not differ significantly.

Interaction type between the antagonists

Within eight days, the combination of *A. abdominalis* and *C. carnea* was additive for *M. euphorbiae* control (Table 4.2 a) and synergistic for *M. persicae* population reduction (Table 4.2 b).

Within eleven days, the presence of each natural enemy mutually improved the outcome of the other when *M. euphorbiae* was the extraguild prey. Therefore, the effect of the natural enemy combination was synergistic (Table 4.2 c). In contrast, when *M. persicae* was the host, the effect of pairing the two beneficials was antagonistic (Table 4.2 d).

Effect of the extraguild prey species on the performance of the antagonists

We investigated the impact of the extraguild prey species, *M. euphorbiae* or *M. persicae*, on the performance of *A. abdominalis* and *C. carnea* used singly or in combination over time.

Results illustrate that no significant difference in the mean percent aphid population reduction per antagonist treatment between aphid species was detected at days two (ANOVA $df = 5$, $F = 0.21$, $P = 0.956$) and five (ANOVA $df = 5$, $F = 0.68$, $P = 0.645$) after treatment application.

In parallel, the aphid species significantly affects the mean aphid percent population reduction caused by the protagonist treatments eight and eleven days following the beneficials' release (Table 4.3).

After a period of eight days, predation alone yielded three-fold higher mean percent *M. euphorbiae* reduction 60.38 % (± 12.20 SE) relative to *M. persicae* population decline 19.74 % (± 12.09 SE). The interspecific mean percent aphid decline in the parasitoid alone or in the combined treatments was statistically alike (Figure 4.2).

Following an eleven-day period of interactions, the combined treatment resulted in three fold higher mean percent decrease of *M. euphorbiae* 92.85 % (± 4.29 SE) as compared to *M. persicae* 28.88 % (± 10.36 SE). None of the single antagonist treatments exhibited significant means percent reduction between *M. euphorbiae* and *M. persicae* preys (Figure 4.2).

Table 4.2. Interactions between *Aphelinus abdominalis* and *Chrysopa carnea*: effects on *Macrosiphum euphorbiae* and *Myzus persicae*.



a) Additive effect against *Macrosiphum euphorbiae* aphid eight days after treatment application.



Mni	Mn	Mi	Me	χ^2 calculated	χ^2 tabulated
0.7710	0.3354	0.6038	0.7367	0.0016	0.0039

b) Synergistic effect against *Myzus persicae* aphid eight days after treatment application.



Mni	Mn	Mi	Me	χ^2 calculated	χ^2 tabulated	D
0.5308	0.2656	0.1974	0.4106	0.0352	0.0039	0.1202

c) Synergistic effect against *Macrosiphum euphorbiae* aphid eleven days after treatment application.



Mni	Mn	Mi	Me	χ^2 calculated	χ^2 tabulated	D
0.9285	0.4483	0.5518	0.7527	0.0411	0.0039	0.1758

d) Antagonistic effect against *Myzus persicae* aphid eleven days after treatment application.



Mni	Mn	Mi	Me	χ^2 calculated	χ^2 tabulated	D
0.2888	0.2332	0.3896	0.5319	0.1111	0.0039	-0.2431

Mni = The observed percent population decrease caused by the *A. abdominalis*-*C. carnea* combination.

Mn = The observed percent population decrease caused by *A. abdominalis*.

Mi = The observed percent population decrease caused by *C. carnea*.

Me = The expected additive percent population decrease for the combination of the *A. abdominalis*-*C. carnea* treatment.

$D = Mni - Me$ (D is positive implies synergism; D is negative implies antagonism).

χ^2 calculated = $(Mni - Me)^2 / Me$.

χ^2 tabulated ($df = 1; \alpha = 0.05$).

Table 4.3. Summary of ANOVA results of percent population decrease of *Macrosiphum euphorbiae* or *Myzus persicae* hosts as affected by the antagonist treatments and the two dates of data collection.

a) 8 days after treatment application

Source of variations	df	<i>F</i>	<i>P</i>
Host	1	6.00	0.023
Treatment	2	4.75	0.020
Host*Treatment	2	0.98	0.392

b) 11 days after treatment application

Source of variations	df	<i>F</i>	<i>P</i>
Host	1	11.27	0.0030
Treatment	2	2.19	0.136
Host*Treatment	2	2.53	0.104

The antagonist treatments are *Aphelinus abdominalis* alone, *Chrysopa carnea* alone, or a combination of *Aphelinus abdominalis* and *Chrysopa carnea*.

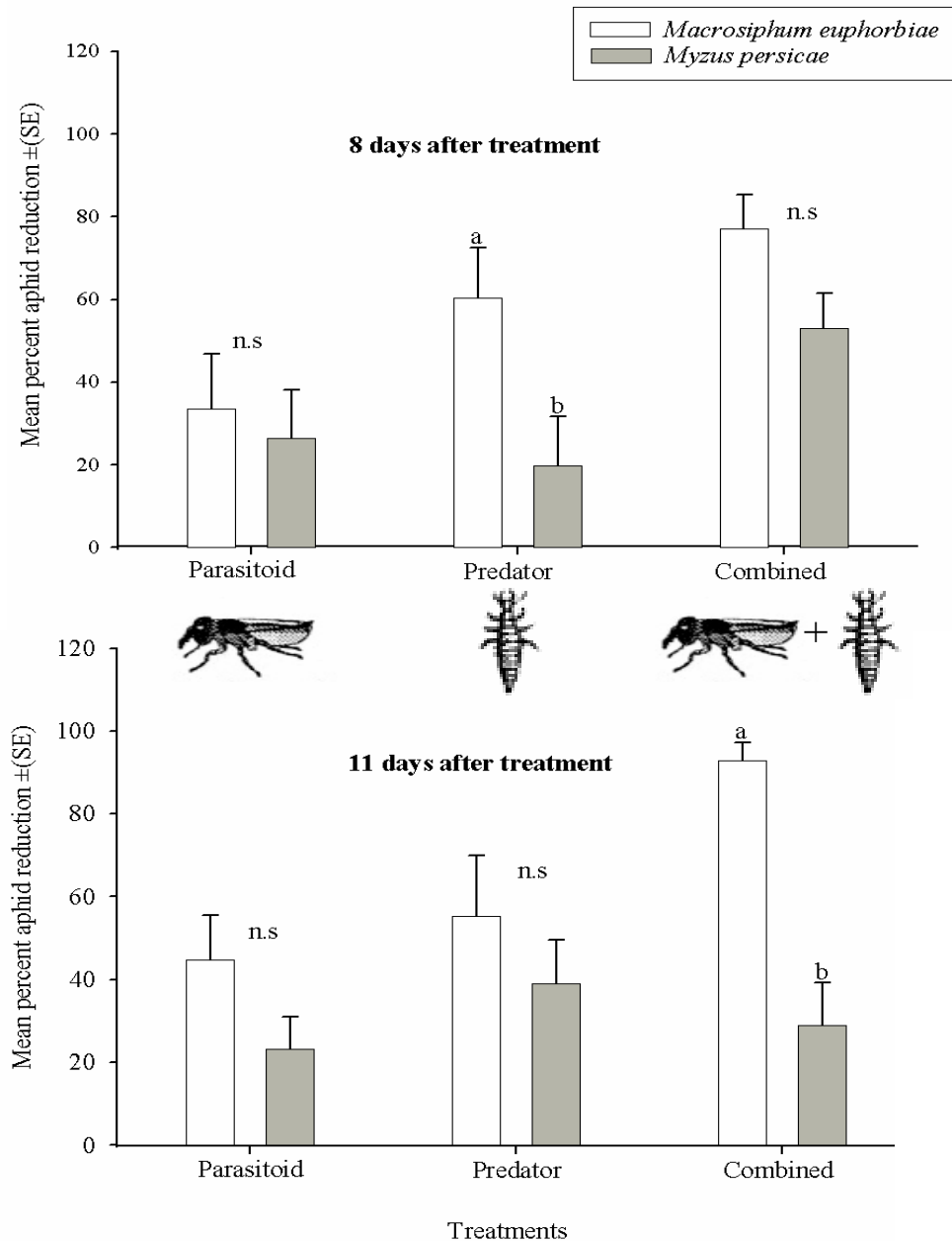


Figure 4.2. Mean percent aphid reduction (\pm SE) as affected by different antagonist treatments, host species and treatment application dates.

For a given date and a given treatment and between aphid species comparison, bars followed by the same small letters are not significantly different (Tukey, $\alpha = 0.05$).

Intraguild predation effects on the parasitoid

We studied whether the combined use of *C. carnea* and *A. abdominalis* could be detrimental over time (within and across dates of data collection) to the parasitoid through direct effect of IGP. Overall, our results revealed that *C. carnea* caused minor mummy destruction through mummy consumption. After eight days of parasitoid-predator

interactions, 0 % eaten and 100 % emerged were recorded with *M. euphorbiae* as extraguild prey, and 2.63 % eaten and 97.36 % emerged with *M. persicae* as herbivore victim. Eleven days following the paired release of the natural enemies, 0 % eaten and 100 % emerged were recorded in *M. euphorbiae* microcosms. 0.45 % eaten and 99.54 % emerged were noted in microcosms housing *M. persicae* aphids.

Within dates

The dates of data collection had a significant impact on the mean number of mummies formed (Table 4.4). The results revealed that the average number of mummified *M. euphorbiae* in microcosms with or without the predator was alike at two, five, eight and eleven days following the combined use of *C. carnea* and *A. abdominalis* (Figure 4.3). Only after eleven day period, the predator's presence has yielded a 1.6 lower mean number of *M. persicae* mummies relative to the parasitoid alone treatment, (53.25 ± 6.94 SE) versus (85.60 ± 10.89 SE), respectively (Figure 4.3).

Across dates

Across dates, a steady increase in the mean number of mummified *M. euphorbiae* was traced in treatments where *A. abdominalis* was foraging alone or paired with the predator (Figure 4.3).

There was a trend in the mean number of mummified *M. persicae* in the parasitoid alone treatment: low at days two and five, intermediate at day eight, and reaching a peak at day eleven following treatment application (Figure 4.3). The combination with the predator has not affected *A. abdominalis* oviposition success in *M. persicae* over time. A tendency was outlined in the mean number of mummies formed: a raise between days two and five, then a decrease at day eight followed by a minor increase at day eleven after the antagonists' release (Figure 4.3).

Effect of extraguild prey species

The species of the extraguild prey had a significant impact on the average number of the mummified aphids formed (Table 4.4). More specifically, results disclose that two days following the antagonists' application, the mean number of mummified *M. persicae* was five fold higher than that of *M. euphorbiae* in the parasitoid alone treatment, and eleven fold higher in the combined treatment (Figure 4.3).

Comparable mean *M. euphorbiae* and *M. persicae* mummy numbers were obtained after *Aphelinus* female has been foraging alone for five days. But in the presence of the predator, 2.75-fold higher mean number of mummified *M. persicae* relative to *M. euphorbiae* mummies was found (Figure 4.3). After eight and eleven days of interactions,

the *Aphelinus* oviposition success in *M. persicae* and *M. euphorbiae* preys was comparable in the presence and absence of the predator (Figure 4.3).

Table 4.4. Summary of ANOVA results of numbers of mummies formed as affected by the treatment, the aphid host and the dates of data collection.

Source of variations	df	<i>F</i>	<i>P</i>
Treatment	1	3.04	0.086
Date	3	17.91	<. 0001
Treatment*Date	3	2.46	0.071
Host	1	22.06	<. 0001
Treatment *Host	1	0.19	0.666
Date *Host	3	0.42	0.740
Treatment*Date*Host	3	0.10	0.962

The treatments are *Aphelinus abdominalis* alone or combined with *Chrysopa carnea*.

The aphid host are *Macrosiphum euphorbiae* or *Myzus persicae*.

The dates of data collections are 2, 5, 8 and 11 days after treatment application.

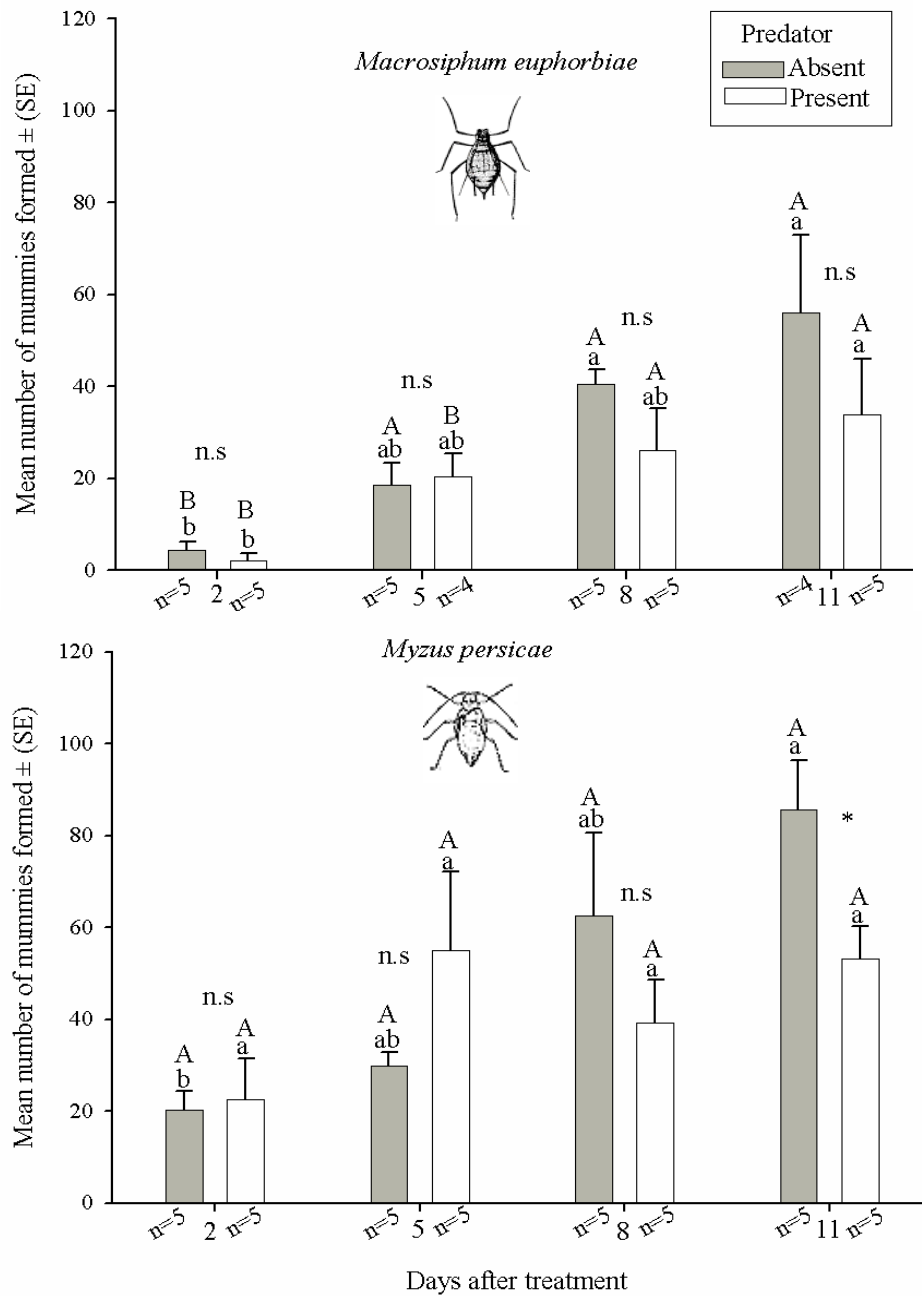


Figure 4.3. Mean \pm (SE) number of mummies formed as affected by the treatment, the dates of data collection and the extraguild prey species.

The treatments are *Aphelinus abdominalis* alone or combination of *Aphelinus abdominalis* and *Chrysopa carnea*.

The dates of data collection are 8 and 11 days after treatment application.

*/n.s. significant/no significant difference between with and without predator treatments at a given date for a given host species.


Bars of a given host species at a given predator level for different dates followed by the same small letters do not differ significantly.

For comparison of the same treatment at a similar date between species, bars followed by capital letters are not significantly different (Tukey, $\alpha = 0.05$).

(n) indicates the number of replications per treatment.

Predation efficiency of *Chrysopa carnea*

To examine the voracity of *C. carnea* over time, we recorded the weight gain of the predator. The predator fed alike in the single or combined treatment (Table 4.5). Therefore, data were pooled and the predator weight gain was analyzed across dates (Table 4.5). *C. carnea* showed a steady increase in weight gain with the date of data collection when both aphid species were inspected (Figure 4.4). The host identity had also a notable effect on the mean predator weight gain (Table 4.5). The consumption of *M. euphorbiae* or *M. persicae* resulted in a similar weight gain after two, five and eleven day foraging period. Within eight days, a diet on *M. euphorbiae* resulted in a significantly lower mean *C. carnea* weight gain relative to a diet on *M. persicae* (Figure 4.4).

Table 4.5. Summary of ANOVA results of *Chrysopa carnea* weight gain as affected by the aphid host, the date of data collection, and the presence or absence of the *Aphelinus abdominalis* parasitoid. 

Source of variations	df	<i>F</i>	<i>P</i>
Host	1	8.39	0.006
Date	3	77.18	<. 0001
Date *Host	3	2.43	0.076
Parasitoid	1	0.34	0.565
Host*Parasitoid	1	0.70	0.407
Date*Parasitoid	3	1.90	0.141
Host*Date*Parasitoid	3	2.58	0.064

The aphid host species is either *Macrosiphum euphorbiae* or *Myzus persicae*.

The dates of data collection are 2, 5, 8 and 11 days after treatment application.

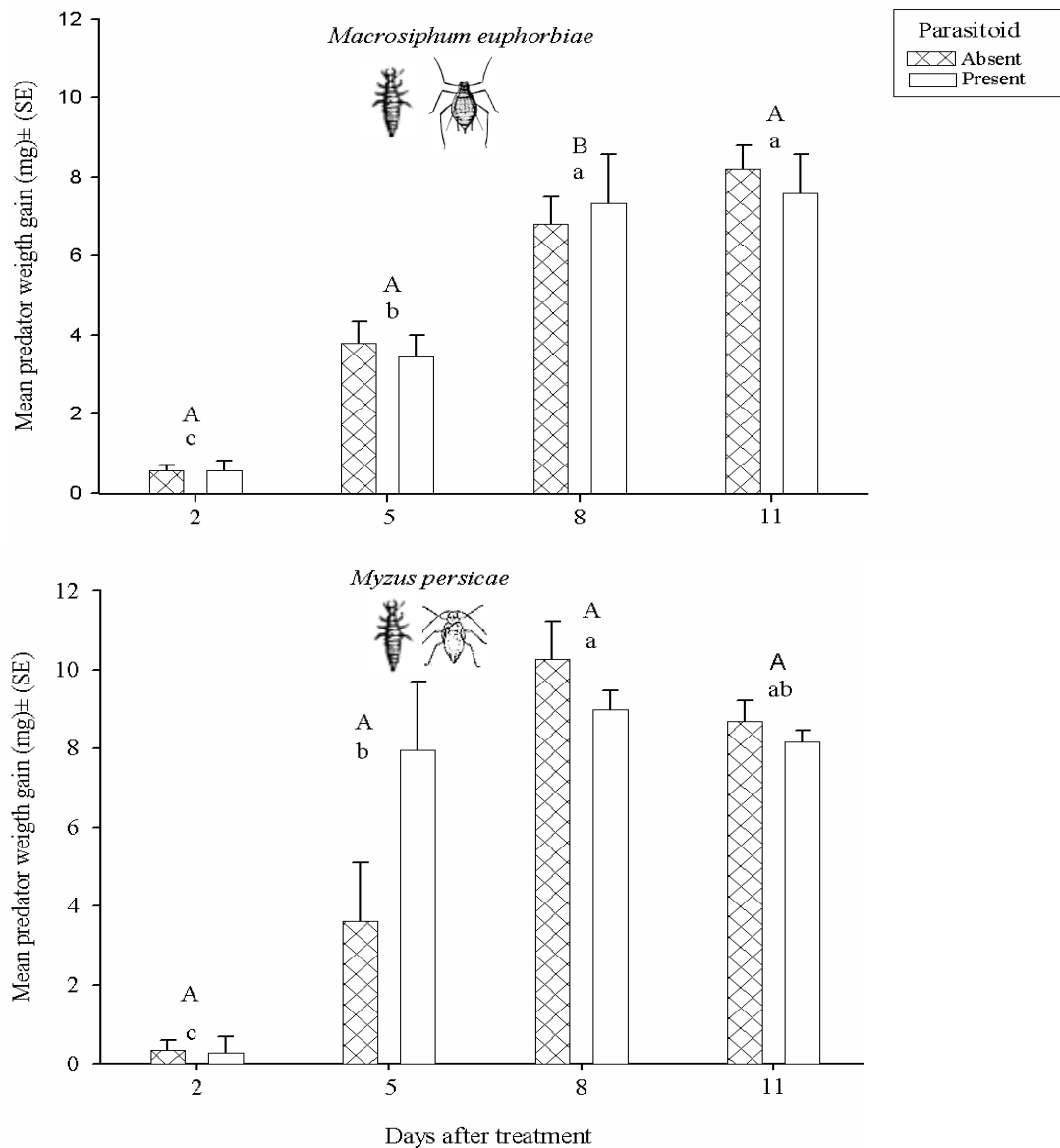


Figure 4.4. Mean predator weight gain (mg) (\pm SE) as affected by the days after treatment application and the species of the aphid host.

Since parasitoid presence or absence does not significantly affect the mean predator weight gain, the data were pooled among treatments (presence and absence of the parasitoid).

Small similar letters for a given date and a given aphid species show non-significant difference in the mean predator weight gain.

Capital letters for a given date show non-significant difference in mean predator weight gain between aphid species (Tukey's test, $\alpha = 0.05$).

4.5. Discussion

In microcosms housing whole sweet pepper plants, we evaluated the individual and combined effects of *A. abdominalis* parasitoid and *C. carnea* predator on *M. euphorbiae*

and *M. persicae* population growth over time. We also investigated the type of interaction (additive or non additive) between the two antagonists. We assessed the direct (IGP through mummy consumption) and indirect (predator induced changes in prey behavior) interactions between the two beneficials. Finally we examined whether the species of the extraguild prey promotes IGP interactions.

Our results revealed that within a foraging period of two, five, eight and eleven days, a single *A. abdominalis* has not prevented the mean *M. euphorbiae* and *M. persicae* densities from increasing steadily and thus converging with the control (Figure 4.1).

Furthermore, in both aphid species, the number of mummified aphids was steady over time in the single or combined antagonist treatments. The dilution effect of the parasitized aphids within a continuously increasing aphid population density offers a potential explanation to this result.

Our findings further demonstrate that after an eight-day period, *C. carnea* reaching the L3 stage, a voracious aphid consumer, inflicted *M. euphorbiae* but not *M. persicae* densities significantly lower than the control (Figure 4.1). Two possible reasons, which are not mutually exclusive, underlie this result: first, the reproductive capacity of each aphid species. *M. euphorbiae* is less fecund than *M. persicae*. The number of nymphs produced per *M. euphorbiae* female varies between thirty to fifty as compared to an average fertility of eighty nymphs per *M. persicae* female. Within eight days, the *M. persicae* population density 1641.6 (\pm 221.74 SE) has reached a six fold higher peak than the *M. euphorbiae* 263.5 (\pm 68.73 SE) one, impractical to be suppressed by a single L3 *C. carnea*. L3 *C. carnea* is capable of consuming up to 200 aphids per week.

Second, the antipredator response of the aphid which is species specific. We have recorded using a multiple video camera set up and analyzed the behavior of *M. euphorbiae* and *M. persicae* in patches with or free from *C. carnea* (Chapter 3). Our video observations demonstrated that given an encounter with *C. carnea*, a *M. euphorbiae* aphid frequently leaves its feeding location and suffers an interruption in its feeding activity. A *M. persicae* aphid remains at its feeding site and suffers consumption.

Under our experimental conditions, the *M. persicae* captured at any location on the sweet pepper plant will buffer the predation effect by arresting the predator at a specific spot of clustered prey availability. This provides other *M. persicae* females an enemy free niche where to sustain the colony. In parallel, *M. euphorbiae* avoiding consumption suffer costs through loss of feeding site and time i.e. lowered energy intake which translates into a reduction in reproductive output. Within eight days, the higher *M. euphorbiae* percent

reduction in comparison to *M. persicae* (Figure 4.2) and the highest mean weight gain of *C. carnea* on *M. persicae* (Figure 4.4) clearly justify this proposed premise.

Eleven days after *C. carnea* release, the predator entered the pupation phase. Therefore, the aphid density in both extraguild prey species was released from control and was comparable to the aphid alone treatment (Figure 4.1).

C. carnea caused lethal or direct (mummy consumption) and indirect or non lethal (lower number of mummy formation) effects on the parasitoids. Those effects are mediated by antipredator behavior of the aphid prey.

After two and five days of parasitoid-predator interactions, our data prove that the predator induced defensive behaviors imposed on the parasitoid fecundity costs. Those were expressed in the lowest mean number of mummified *M. euphorbiae* relative to that of *M. persicae* (Figure 4.3).

Direct IGP effects were minimal. No *M. euphorbiae* consumed mummies were found. The percent of consumed mummified *M. persicae* ranged between 2.63 % within eight days and 0.45 % at eleven day interaction period. This result is explained by the fact that mummies appear seven days following parasitoid release into the microcosms. So starting from day eight, the predator is given the opportunity to prey on mummies. But between day eight and eleven, most of the predators used were already in the pupal stage.

Within eleven days, the *M. persicae* oviposition success was significantly lower in the presence of the predator (Figure 4.3). The possibility that *C. carnea* has killed the parasitoid can be discounted as a factor. The combined treatment was replicated four times. 50 % of the parasitoids were recuperated but 100 % of the plants carried mummies. Perhaps, before pupation, the predator has consumed parasitized *M. persicae* (Meyhöfer and Hindayana, 2000; Meyhöfer and Klug, 2002). *A. abdominalis* prefers the second to third larval stages of aphids. *Chrysopa* like other generalist predators (e.g. Roger et al., 2001) may not exhibit any preference for parasitized or unparasitized aphids at a similar stage of development but may prefer younger nymphs, regardless of whether or not they were parasitized. Furthermore, if there is excess food, *C. carnea* will kill more preys. This excess prey killing of younger nymphs might have negatively impacted the mean number of mummified *M. persicae* formed.

Within eight days, we obtained that *A. abdominalis* and *C. carnea* combination yielded an additive effect on *M. euphorbiae* control and synergistic impact on *M. persicae* population decrease. Within eleven days, treatment combination resulted in a synergistic effect on *M. euphorbiae* decline and an antagonistic outcome on *M. persicae* reduction relaxing the

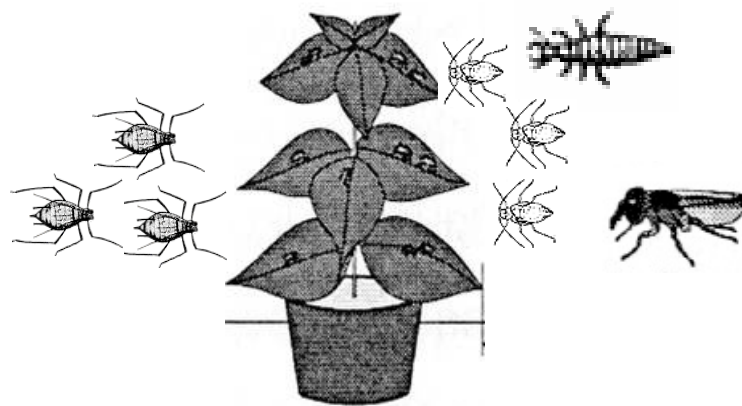
aphid population to build up to a level similar to the control treatment (Figure 4.1). Parasitoid-predator interactions have two different modes of actions, behavioral interactions and trophic interactions (Meyhöfer et al., unpublished data). Both can shape the overall efficiency of the antagonist combination and may contribute to the observed effects in our experiment.

To summarize, the predator has not exerted a density dependent control mainly in *M. persicae* microcosms but caused a reduction in *M. euphorbiae* population growth by costly antipredator behaviors. This has indirectly imposed a reproductive cost on the parasitoid reflected by a lower number of *M. euphorbiae* mummies formed relative to the mummified *M. persicae*.

No IGP through mummy destruction was detected mainly due to the abundance of the extraguild prey diluting the IGP effects (Lucas, 2005; Meyhöfer and Hindayana, 2000) and unfavourable conditions favoring predation on mummies. .

Within eight days our data supports additivity and synergism of predator-parasitoid effects on *M. euphorbiae* and *M. persicae* respectively, while within eleven days, the effects on *M. euphorbiae* were synergistic and on *M. persicae* antagonistic.

Our results show that direct IGP effects, when prevalent, were diluted by the abundant extraguild prey density. Behaviorally induced non-lethal effects of *C. carnea* on *M. euphorbiae* affected the IGP interactions. Hence, the outcome of IGP is not only a function of the interactions between the predatory guilds, but also the extraguild prey species plays an important role.



5 General Discussion

The increased awareness about side effects of synthetic pesticides has focused the avenues of research towards ecologically sound alternatives such as biocontrol agents. In aphidophagous systems, the use of predators and parasitoids as natural enemies offers a promising approach to combat aphid pests.

Arthropods live in a chemical world (Dicke and van Loon, 2000; Vet 1999). Like most animals, foraging parasitoids have to deal with temporal or spatial changes in resources (Vet, 1999). In a challenging environment, flexibility in behavioral response is a necessity and learning provides an adaptive mechanism (Papaj, 1993). The use of general host cues during foraging including an innate reaction to infochemicals seems to be adaptive for carnivores in general, regardless of dietary specialization (Steidle and van Loon, 2003). Learning is not only restricted to the host location process, but also is involved in the improvement of the host handling skills as the parasitoids handle successive hosts.

Parasitoids not only base their foraging decisions on external information e.g. the chemicals they encounter but also on internal information i.e. experienced based information stored in their memory (Vet 1999) and their physiological state (Lewis et al., 1990; Turlings et al., 1993). Integration of these resources of information dictates the behavioral performance. However, food webs of most ecosystems are reticulate and interaction complex (Vet, 1999). Carnivorous species may feed on one another through IGP thus eliminating potential competitors. More specifically, interactions between predators and parasitoids have two outcomes on the inferior guild, i.e. the parasitoid: direct, through IGP and indirect that is behaviorally induced effects of the predators on the herbivore. In aphidophagous systems, IGP is a rule rather than an exception.

With this background, the present study was initiated to explore the potential enhancement of *M. euphorbiae* and *M. persicae* control through the manipulation of *A. abdominalis* learning capacity from three different angles: (i) conditioning the parasitoid on one aphid species and offering her the alternate species (Chapter 2), (ii) combining *A. abdominalis* with L2 *C. carnea* and investigating the risk assessment capacity of the wasp through learning of predatory cues (Chapter 3), (iii) examining the direct (IGP) and indirect (behaviorally induced) effects mediated by the extraguild prey species of *A. abdominalis*-*C. carnea* interactions (Chapter 4).

Host switching trial between *M. persicae* and *M. euphorbiae* revealed no significant effect on *A. abdominalis* mummy production. In addition, the *Aphelinus* wasps handled efficiently the switched aphid species after being trained on a second one (Chapter 2).

In our experiment, we have displaced any potential pre-imaginal learning due to rearing the *Aphelinus* females on *S. avenae*. It would be relevant to follow in a host-switching experiment, for example, how suitable are the nymphs of *M. persicae* for parasitoids originating from mummified *M. euphorbiae* and vice versa. Such finding will tackle the fitness costs issue resulting from host switching, a domain unexplored in the current study.

Mölk et al. (2000) found in a wind tunnel choice test, with a *M. euphorbiae* infested pepper and an infested aubergine plant as odor sources, that female *Aphelinus* trained on one of the offered plant host combinations significantly preferred the odor of the learnt plant host complex to that of the different plant host complex.

Since generalist antagonists use general chemical cues present in all their hosts or food plants, an interesting question might be investigated with a wind tunnel set up: do the synomones emitted by the pepper plant as a response to *M. persicae* herbivory share a chemical similarity with those produced by *M. euphorbiae* feeding as key indicators of host presence from distance? Such results not only reveal the importance of *Aphelinus* host experience background on host location, but also provide an insight into how the parasitoid might deal with plant signals induced by two of her aphid hosts.

If those findings are encouraging, the phenomenon of associative learning in *A. abdominalis* parasitoids may be exploited for purpose of biological control (Duan and Messing, 1999; Grasswitz, 1998; Prokopy and Lewis, 1993). Hence, it may be possible to condition on single aphid species mass-reared parasitoids prior to their release in the target area. When done properly, this may increase strongly the searching efficiency of the released insect such that control will be more effective (e.g. Papaj and Vet, 1990) and a mixed infestation of both aphid preys will be successfully managed.

Results from chapter 3 indicate that in IGP interactions associative learning helps the *A. abdominalis* females to offset unnecessary escape behavior by providing accurate information about the current predation risk. The behavioral responses and oviposition success of predator experienced *Aphelinus* were similar to those of predator naïve wasps in patches with or without the L2 *C. carnea*. *A. abdominalis* inspected the predator through “contact predator” behavior and in some cases attacking the predator with the ovipositor. Nevertheless, *Aphelinus* exhibited no defensive behavior (e.g. patch leaving or depressed oviposition). Further experiments exploiting the associative learning capacity of the wasps

should focus on *Aphelinus* reaction to multiple information sources e.g. direct that is presence of the predator and indirect namely conspecific alarm pheromones resulting from *C. carnea* consumption of mummified aphids. Consequently, *Aphelinus* is expected to display more pronounced antipredator behavior than her reaction to a single source of information.

The exchange between L2 and the voracious L3 *C. carnea* feeding on mummified aphids is also important. This type of experiment permits to answer the following questions: can *Aphelinus* determine the degree of threat posed by *C. carnea* based on the concentration of the conspecific alarm pheromone? Is there a threshold of alarm cues likely to represent threat above which the females respond and below which they display no or minor response? Results of such studies verify the parasitoid's capacity to relate potential risk to recent experience.

A final step would be to examine the persistence of the learned response in the parasitoid's memory and project this response over timescales relevant to predation risk in the natural environment.

Since there is a flexible interplay between predator and prey, there is a continuum of ways that a prey responds to different stages of the same predator species.

Results of chapter 4 reveal that the IGP interactions between the same *Aphelinus* individual and the *C. carnea* predator growing over the experimental time scale can be negative (antagonistic) or positive (additive or synergistic). Direct predation of *C. carnea* on the aphid prey imposes an immediate cost on the foraging *Aphelinus* (loss of an oviposition or a host feeding opportunity or mummy destruction). The abundance of extraguild prey diluted the direct IGP effects of *C. carnea* on *A. abdominalis* but not the indirect ones mediated by the antipredator behavior of the aphid (Chapter 4). The aphid defensive behavior is species specific (Chapters 3 and 4).

Therefore, a complete understanding of the *A. abdominalis*-*C. carnea* interactions requires an appreciation of the behaviorally induced non lethal effects of the predator on each of the extraguild preys. On one hand, demonstrating the longer term dynamical consequences of non-consumptive predator effects will await longer-term experiments spanning multiple generations of the predator, parasitoid and the prey. On the other hand, within the time scale set for this study, an experiment with different parasitoid: predator densities relative to the 1:1 ratio used here could create stronger interactions in a more competitive situation.

In conclusion, this study showed that the adaptive value of learning in *A. abdominalis* revolves around two points: first, improved skills in host handling behavior, which is a credit for future exploitation for biological control purposes. Second, a flexible predation risk assessment, shedding some light on the role of learning of cues associated with active predators. Such information helps to assess the efficiency of coexistence of multiple natural enemy species and the costs for the success of aphidophagous biological control approaches.

References

- Abramsky, Z., Strauss, E., Kotler, B.P., Riechman, A. 1996. The effect of barn owls (*Tyto alb*) on the activity and microhabitat selection of *Gerbillus allenbyi* and *G. pyramidum*. *Oecologia* 105, 313-319.
- Bartlett, B. R. 1964. Toxicity of some pesticides to eggs, larvae, and adults of the green lacewing, *Chrysopa carnea*. *Journal of Economic Entomology* 57, 366-369.
- Bjorksten, A., Hoffmann, A.A. 1995. Effect of pre-adult and adult experience on host acceptance in choice and non-choice tests in two strains of *Trichogramma*. *Entomologia Experimentalis et Applicata* 76, 49-58.
- Bleeker, M.A.K. 2005. Associative learning in two closely related parasitoid wasps: a neurological approach. PhD Thesis, Wageningen University, The Netherlands, pp.133.
- Bouchard, Y., Cloutier, C. 1984. Honeydew as a source of host-searching kairomones for the aphid parasitoid *Aphidius nigripes* (Hymenoptera: Aphidiidae). *Canadian Journal of Zoology* 62, 1513-1520.
- Bouskila, A. 2001. A habitat selection game interactions between rodents and their predators. *Annales Zoologici Fennici* 38, 55-70.
- Brodeur, J., Rosenheim, J.A. 2000. Intraguild interactions in aphid parasitoids. *Entomologia Experimentalis et Applicata* 97, 93-108.
- Budenberg, W.J. 1990. Honeydew as a contact kairomone for aphid parasitoids. *Entomologia Experimentalis et Applicata* 55, 139-147.
- Budenberg, W.J., Powell, W., Clark, S.J. 1992. The influence of aphids and honeydew on the leaving rate of searching aphid parasitoids from wheat plants. *Entomologia Experimentalis et Applicata* 63, 259-264.
- Canard, M., Principi, M.M. 1984. Life histories and behavior. In: Biology of Chrysopidae. Canard, M., Semeria, Y., New, T.R., (Eds.), The Hague, The Netherlands, pp 57–100.
- Cardinale, B.J., Harvey, C.T., Gross, K., Ives, A.R. 2003. The biodiversity and biocontrol: emergent impacts of a multi-enemy assemblage on pest suppression and crop yield in agroecosystem. *Ecology Letters* 6, 857-865.

- Chittka, L., Thomson, J. D. 1997. Sensori-motor learning and its relevance for task specialization in bee. *Behavioral Ecology and Sociobiology* 41, 385-398.
- Chittka, L., Gumbert, A., Kunze, J. 1997. Foraging dynamics of bumblebees: correlates of movements within and between plant species. *Behavioral Ecology* 8, 239-249
- Chivers, D.P., Wisenden, B.D., Smith, J.F. 1996. Damselfly larvae learn to recognize predators from chemical cues in the predator's diet. *Animal Behavior* 52, 315-320.
- Coll, M., Izraylevich, S .1998. When predators also feed on plants: effect of competition and plant quality on omnivore-prey population dynamics. *Annals of the Entomological Society of America* 90, 154-161.
- Colombo, M., Fasce, D. 1994. Integrated control of spring crops of tomato in protected cultivation. *Informatore Agrario* 50, 37-41.
- Cournoyer, M., Boivin, G. 2005. Short distance cues used by the adult parasitoid *Microctonus hyperodae* Loan (Hymenoptera: Barconidae, Euphorinae) for host selection of a novel host *Listronotus oregonensis* (Coleoptera: Curculionidae). *Journal of Insect Behavior* 18, 577-591.
- Darwin, C. 1876. Cross and self fertilization in the vegetable kingdom. Murray, London, pp. 487.
- Dicke, M., van Loon, J.J.A. 2000. Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. *Entomologia Experimentalis et Applicata* 97, 237-249.
- Dicke, M., Grostal, P. 2001. Chemical detection of natural enemies by arthropods: an ecological perspective. *Annual Review of Ecology and Systematics* 32, 1-23.
- Dixon, A.F.G. The Biology of Aphids. Institute of Biology's Studies in Biology No. 44. Arnold E. Limited, London, pp. 58.
- Duan, J.J., Messing, H. 1999. Effects of origin and experience patterns of host acceptance by the opiine parasitoid *Diachasmimorpha tryoni*. *Ecological Entomology* 24, 284-291.
- Dukas, R. 1995. Transfer and interference in bumblebee learning. *Animal Behavior* 49, 1481-1490.
- Dukas, R. 1999. Costs of memory: ideas and predictions. *Journal of Theoretical Biology* 197, 41-50.

- Dukas, R., Duan, J. J. 2000. Potential fitness consequences of associative learning in a parasitoid wasp. *Behavioral Ecology* 11, 536-543.
- Ferguson, K.I., Stiling, P. 1996. Non-additive effects of multiple natural enemies on aphid populations. *Oecologia* 108, 375-379.
- Ferrari, M.C.O., Chivers, D.P. 2006. Learning threat-sensitive predator avoidance: how do fathead minnows incorporate conflicting information? *Animal Behavior* 71, 19-26.
- Finke, D., Denno, R. 2003. Intraguild predation relaxes natural enemy impacts on herbivore populations. *Ecological Entomology* 28, 67-73.
- Finney, D., 1964. Probit Analysis. Cambridge University Press, London, UK, pp. 318.
- Foster, S.P., Hackett, B., Mason, N., Moores, G.D., Cox, D.M., Campbell, J. Denholm, I. 2002. Resistance to carbamate, organophosphate and pyrethroid insecticides in the potato aphid (*Macrosiphum euphorbiae*). *Proceedings of the Brighton Conference - Pests and Diseases* 2, 811–816.
- Gandolfi, M., Mattiacci, L., Dorn, S. 2003. Preimaginal learning determines adult response to chemical stimuli in a parasitic wasp. *Proceedings of the Royal Society of London B* 270, 2623-2629.
- Georghiou, G.P., Lagunes-Tejada, A. 1991. The occurrence of resistance to pesticides in arthropods. An index of cases reported through 1989, FAO, Rome.
- Godfray, H.C. 1994. Parasitoids, Princeton University Press, pp. 473.
- Grasswitz, T.R., Paine, T.D. 1993. Effect of experience on in-flight orientation to host-associated cues on the generalist parasitoid *Lysiphlebus testaceipes*. *Entomologia Experimentalis et Applicata* 68, 219-229.
- Grasswitz, T.R. 1998. Effect of adult experience on the host-location behavior of the parasitoid *Aphidius colemani* (Viereck) (Hymenoptera: Aphidiidae). *Biological Control* 12, 177-181.
- Green, R.F., Ayal, Y. 1998. A simple Markov model for the assessment of host patch quality for foraging parasitoids. *Oecologia* 116, 456-446.
- Grostal, P., Dicke, M. 1999. Direct and indirect cues of predation risk influence behavior and reproduction of prey: a case for acarine interactions. *Behavioral Ecology* 10, 422-427.

- Heie, O.E., 1967. Studies on fossil aphids (Homoptera: Aphidoidea), especially in the Copenhagen collection of fossils in Baltic amber. *Spolia Zoologica Musei Hauniensis* 26, 1-273.
- Heimpel, G., Rosenheim, J.A. 1995. Dynamic host feeding by the parasitoid *Aphytis melinus*: the balance between current and future reproduction. *Journal of Animal Ecology* 64, 153-167.
- Helfman, G.S. 1989. Threat-sensitive predator avoidance in damselfly-trumpetfish interaction. *Behavioral Ecology and Sociobiology* 24, 47-58.
- Henderson, D.E., Raworth, D.A. 1991. Beneficial insects and common pests on strawberry and raspberry crops. Agriculture Canada Publication 1863/E, Ottawa.
- Henry, L.M., Gillespie, D.R., Roitberg, B. 2005. Does mother really know best? Oviposition preference reduces reproductive performance in the generalist parasitoid *Aphidius ervi*. *Entomologia Experimentalis et Applicata* 116, 167-174.
- Hindayana, D., Meyhöfer, R., Scholz, D., Poehling, H. M. 2001. Intraguild predation among the hoverfly *Episyrphus balteatus* de Geer (Diptera: Syrphidae) and other aphidophagous predators. *Biological Control* 20, 236-246.
- Honěk A, Jarosik, V., Lapchin, L., Rabasse, J.M. 1998. Host choice and offspring sex allocation in the aphid parasitoid *Aphelinus abdominalis* (Hymenoptera: Aphelinidae). *Journal of Agricultural Entomology* 15, 209-221.
- Howard, R., Garland, A.J., Seaman, L.W. 1994. Diseases and pests of vegetable crops in Canada. An illustrated compendium. pp 395.
- Kalina, V., Stary, P. 1976. A review of the aphidophagous Aphelinidae (Hymenoptera: Chalcidoidea), their distribution and host range in Europe. *Stud. Ent. Forest.* 9, 143-170.
- Kats, L.B., Dill, L.M. 1998. The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience* 5, 361-394.
- Kerguelen, V., Carde, R. T. 1998. Flight toward a learned odor and factors inducing landing of female *Brachymeria intermedia* (Hymenoptera: Chalcididae), a parasitoid of the gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae). *Journal of Insect Behavior* 11, 221-234.

- Kortet, R., Hedrick, A. 2004. Detection of the spider predator, *Hololena nedra* by naïve juvenile field crickets (*Gryllus integer*) using indirect cues. *Behavior* 141, 1189-1196.
- Kotler, B.P., Brown, J.S., Hasson, O. 1991. Factors affecting gerbil foraging behavior and rates of owl predation. *Ecology* 72, 2249-2260.
- Krebs, J.R., Davies, N.B. 1987. An Introduction to Behavioral Ecology. Blackwell Scientific Publications, Oxford, pp. 420.
- Kupfermann, I. 1991. Learning and Memory. In: Principle of Neural Science. Kandel, E.R., Schwarz, J.H., Jessel, T.M. (Eds.), Appleton and Lange, Prentice Hall International Inc, Norwalk CT, pp. 997-1008.
- Kusch, R.C., Mirza, R.S., Chivers, D.P. 2004. Making sense of predator scents: investigating the sophistication of predator assessment abilities of fathead minnows. *Behavioral Ecology and Sociobiology* 55, 551-555.
- Le Ralec, A., Curty, C., Wajnberg, E. 2005. Inter-specific variation in the reactive distance of different aphid-parasitoid associations: analysis from automatic tracking of the walking path. *Applied Entomology and Zoology* 40, 413-420.
- Lewis, W.J., Tumlinson, J.H. 1988. Host detection by chemically mediated associative learning in a parasitic wasp. *Nature* 331, 635-636.
- Lewis, W.J., Vet, L.E.M., Tumlinson, J.H., van Lenteren, J.C. Papaj, D.R. 1990. Variations in parasitoid foraging behavior: Essential element of a sound biocontrol theory. *Environmental Entomology* 19, 1183-1193.
- Lewis, W.J., Martin, W.R. 1990. Semiochemicals for use with parasitoids: status and future. *Journal of Chemical Ecology* 16, 3067-3089.
- Licht, T. 1989. Discriminating between hungry and satiated predators: the response of guppies (*Poecilia reticulata*) from high and low predation sites. *Ethology* 82, 238-243.
- Lima, S.L., Dill, L.M. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology* 68, 619-640.

- Lima, S.L. 1998. Stress and decision making under the risk of predation: recent developments from behavioral, reproductive, and ecological perspectives. *Advances in the Study of Behavior* 27, 215-290.
- Lingren, P. D., Ridgway, R. L. 1967. Toxicity of five insecticides to several insect predators. *Journal of Economic Entomology* 60, 1639-1641.
- Losey, J.E., Denno, R.F. 1998. Positive predator-predator interactions: enhanced predation rates and synergistic suppression of aphid population. *Ecology* 79, 2133-2152.
- Lucas, E. 2005. Intraguild predation among aphidophagous predators. *European Journal of Entomology* 102, 351-364.
- Mackauer, M., Michaud, J.P., Völkl, W. 1996. Host choice by aphidiid parasitoids (Hymenoptera: Aphidiidae): host recognition, host quality and host value. *Canadian Entomologist* 128, 959-980.
- Magurran, A.E., Girling, S.L. 1986. Predator recognition and response habituation in shoaling minnows. *Animal Behavior* 34, 510-518.
- Mathis, A., Murray, K.L., Hickman, C. 2003. Do experience and body size play a role in response of larval ringed salamanders, *Ambystoma annulatum*, to predator kairomones? Laboratory and field assays. *Ethology* 109, 159-170.
- McVay, J. R., Gudauskas, R. T., Harper, J. D. 1977. Effects of *Bacillus thuringiensis* nuclear-polyhydrosis virus mixtures on *Trichoplusia ni* larvae. *Journal of Invertebrate Pathology* 29, 367-372.
- Meng, R., Janssen, A., Nomikou, M., Zhang, Q., Sabellis, M.W. 2006. Previous and present diets of mite predators affect antipredator behavior of whitefly prey. *Experimental and Applied Acarology* 38, 113-124.
- Mery, F. Kawecki, T.J. 2005. A cost of long term memory in *Drosophila*. *Science* 308, 1148.
- Meyhöfer, R., Hindayana, D. 2000. Effects of intraguild predation on aphid parasitoid survival. *Entomologia Experimentalis et Applicata* 97, 115-122.
- Meyhöfer, R. 2001. Intraguild predation by aphidophagous predators on parasitized aphids: the use of multiple video cameras. *Entomologia Experimentalis et Applicata* 11, 77-87.

- Meyhöfer, R., Klug, T. 2002. Intraguild predation on the aphid parasitoid *Lysiphlebus fabarum* (Marshall) (Hymenoptera: Aphidiidae): mortality risks and behavioral decisions made under the threats of predation. *Biological Control* 25, 239-248.
- Meyhöfer, R., Klug, T. (submitted) Comparative study on the behavior of *Aphidius colemani* and *Aphidius ervi* in confrontations with predators.
- Michaud, J.P., Mackauer, M. 1994. The use of visual cues in host evaluation by aphidiid wasps. I. Comparison between three *Aphidius* parasitoids of the pea aphid. *Entomologia Experimentalis et Applicata* 70, 273-283.
- Michaud, J.P., Mackauer, M. 1995. The use of visual cues in host evaluation by aphidiid wasps. II. Comparison between *Ephedrus californicus*, *Monoctonus paulensis*, and *Praon pequodorum*. *Entomologia Experimentalis et Applicata* 74, 267-275.
- Minks, A.K., Harrewijn, P. 1988. World Crop Pests; Aphids, their Biology, Natural Enemies and Control, Vol. B., Elsevier, Amsterdam, The Netherlands, pp. 384.
- Mirza, R.S., Ferrari, M.C.O., Kiesecker, J.M., Chivers, D.P. 2006. Responses of American toad tadpoles to predation cues: behavioral response thresholds, threat-sensitivity and acquired predation recognition. *Behavior* 143, 877-889.
- Mölck, G., Micha, S.G., Wyss, U. 1999. Attraction to odor of infested plants and learning behavior in the aphid parasitoid *Aphelinus abdominalis*. *Journal of Plant Diseases and Protection* 106, 557-567.
- Mölck, G., Pinn, H., Wyss, U. 2000. Manipulation of plant odor preference by learning in the aphid parasitoid *Aphelinus abdominalis* (Hymenoptera: Aphelinidae). *European Journal of Entomology* 97, 533-538.
- Murdoch, W.W. 1994. Population regulation in theory and practice. *Ecology* 75, 271-287.
- Nelson, E.H., Matthews, C.E., Rosenheim, J.A. 2004. Predators reduce prey population growth by inducing changes in prey behavior. *Ecology* 85, 1853-1858.
- Noldus Information Technology. 1997. The Observer, Support Package for Video Analysis. Reference Manual, Version 4.0 for Windows Edition, Wageningen, The Netherlands.

- Nurindah, B., Cribb, W., Gordh, G. 1999a. Experience acquisition by *Trichogramma australicum* (Girault) (Hymenoptera: Trichogrammatidae). *Australian Journal of Entomology* 38, 115-119.
- Nurindah, B., Cribb, W., Gordh, G. 1999b. Effects of physiological condition and experience on oviposition behavior of *Trichogramma australicum* (Girault) (Hymenoptera: Trichogrammatidae) on eggs of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Australian Journal of Entomology* 38, 109-119.
- Papaj, D.R., Vet, L.E.M. 1990. Odor learning and foraging success in the parasitoid *Leptoplina heterosoma*. *Journal of Chemical Ecology* 16, 3137-3150.
- Papaj, D.R., Lewis, A.C. 1993. Insect Learning: Ecological and Evolutionary Perspective. Chapman & Hall, New York, pp. 398.
- Papaj, D.R. 1993. Afterward: learning adaptation and the lessons of O. In: Insect Learning: Ecological and Evolutionary Perspective. Papaj, D., Lewis, A.C. (Eds.), Chapman & Hall, New York, pp. 374-386.
- Persons, M.H., Rypstra, A. 2001. Wolf spiders show graded antipredator behavior in the presence of chemical cues from different sized predators. *Journal of Chemical Ecology* 27, 2493-2504.
- Pettersson, J., Ninkovic, V., Glinwood, R., Birkett, M.A., Pickett, J. 2005. Foraging in a complex environment-semiochemicals support searching behavior of the seven spot ladybird. *European Journal of Entomology* 102, 365-370.
- Polis, G.A, Myers, C.A., Holt, R.D. 1989. The ecology and evolution of intraguild predation: potential competitors that eat each other. *Annual Review of Ecology and Systematics* 20, 297-330.
- Powell, W., Pennacchio, F., Poppy, G.M., Tremblay, E. 1998. Strategies involved in the location of hosts by the parasitoid *Aphidius ervi* Haliday (Hymenoptera: Braconidae: Aphidiidae). *Biological Control* 11, 104-112.
- Prokopy, R.J., Lewis, W.J. 1993. Application of learning to pest management, In: Insect Learning: Ecological and Evolutionary Perspective. Papaj, D., Lewis, A.C. (Eds.), Chapman & Hall, New York, pp. 308-341.
- Rajakulendran, S. V., Plapp, F. W. Jr. 1982. Comparative toxicities of five synthetic pyrethroids to the tobacco budworm (Lepidoptera: Noctuidae), an ichneumonid

- parasite, *Carnpoletis sonorensis*, and a predator, *Chrysopa carnea*. *Journal of Economic Entomology* 75, 769-772.
- Ridgway, R. L., Morrison, R. K., Badgley, M. 1970. Mass rearing a green lacewing. *Journal of Economic Entomology* 63, 834-836.
- Roger, C., Coderre, D., Vigneault, C., Boivin, G. 2001. Prey discrimination by a generalist coccinellid predator: effect of prey age or parasitism? *Ecological Entomology* 26, 1365-2311.
- Roitberg, B. D., Mangel, M., Lalonde, R., Roitberg, C. A., van Alphen, J.J.M., Vet, L.E.M. 1992. Seasonal dynamic shifts in patch exploitation by parasitic wasps. *Behavioral Ecology* 3, 156-165.
- Roitberg, B.D., Reid, M.L., Chao, L. 1993. Choosing hosts and mates: the value of learning. In: *Insect Learning: Ecological and Evolutionary Perspective*. Papaj, D., Lewis, A.C. (Eds.), Chapman & Hall, New York, pp. 174-194.
- Rossi, M.N. 2004. Evidences of non-additive effects of multiple parasitoids on *Diatraea saccharalis* Fabr. (Lep., Crambidae) populations in sugarcane fields in Brazil. *Journal of Applied Entomology* 128, 88-94.
- Salama, H. S., Foda, M. S., Zaki, F. N., Moawad, S., 1984. Potency of combinations of *Bacillus thuringiensis* and chemical insecticides on *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Journal of Economic Entomology* 77, 885–890.
- SAS Institute Inc. 1999. SAS Institute Inc., SAS OnlineDoc®, Version 8, Cary, NC, USA.
- Schmitz, O.J., Krivan, V., Ovadia, O. 2004. Trophic cascades: the primacy of trait mediated indirect interactions. *Ecology letters* 7, 153-163.
- Shaltiel, L., Ayal, Y. 1998. The use of kairomones for foraging decisions by an aphid parasitoid in small host aggregations. *Ecological Entomology* 23, 319-329.
- Shour, M. H., Crowder, L. A. 1980. Effects of pyrethroid insecticides on the common green lacewing. *Journal of Economic Entomology* 73, 306-309.
- Sih, A. 1992. Prey uncertainty and the balancing of antipredator and feeding needs. *American Naturalist* 139, 1052-1069.
- Snyder, W.E., Ives, A.R. 2001. Generalist predators disrupt biological control by a specialist parasitoid. *Ecology* 82, 705-716.

- Snyder, W.E., Ballard, S.N., Yang, S., Clewenger, G.M., Miller, T.D., Ahn, J.J., Hatten, T.D., Berryman, A.A. 2004. Complementary biocontrol of aphids by the ladybird beetle *Harmonia axyridis* and the parasitoid *Aphelinus asychis* on greenhouse roses. *Biological Control* 30, 229-235.
- Steidle, J.M.L. 1998. Learning pays off: influence of experience on host finding and parasitism in *Lariophagus distinguendus*. *Ecological Entomology* 23, 451-456.
- Steidle, J.M.L., Steppuhn, A., Reinhard, J. 2001a. Volatile cues from different host complexes used for host location by the generalist parasitoid *Lariophagus distinguendus* (Hymenoptera: Pteromalidae). *Basic and Applied Ecology* 2, 45-51.
- Steidle, J.M.L., Lanka, J., Müller, C., Ruther, J. 2001b. The use of general foraging kairomones in a generalist parasitoid. *Oikos* 95, 78-86.
- Steidle, J.M.L., Steppuhn, A., Ruther, J. 2003. Specific foraging kairomones used by a generalist parasitoid. *Journal of Chemical Ecology* 29, 131-143.
- Steidle, J.M.L., van Loon, J.J.A. 2003. Dietary specialization and infochemical use in carnivorous arthropods: testing a concept. *Entomologia Experimentalis et Applicata* 108, 133-148.
- Thiel, A., Hoffmeister, T.S. 2004. Knowing your habitat: linking patch-encounter rate and patch exploitation in parasitoids. *Behavioral Ecology* 15, 419-425.
- Turlings, T.C.J., Wäckers, F.L., Vet, L.E.M., Lewis, W.J., Tumlinson, J.H. 1993. Learning of host-finding cues by hymenopterous parasitoids. In: *Insect Learning: Ecological and Evolutionary Perspective*. Papaj, D., Lewis, A.C. (Eds.), Chapman & Hall, New York, pp. 51-78.
- van Baaren, J., Boivin, G. 1998. Learning affects host discrimination behavior in a parasitoid wasp. *Behavioral Ecology and Sociobiology* 42, 9-16.
- Vet, L.E.M., Groenewold, A.W. 1990. Semiciochemicals and learning in parasitoids. *Journal of Chemical Ecology* 16, 3119-3135.
- Vet, L.E.M., Wäckers, F.L., Dicke, M. 1991. How to hunt for hiding hosts: the reliability-detectability problem in foraging parasitoids. *Netherlands Journal of Zoology* 41, 202-213.

- Vet, L.E.M, Dicke, M. 1992. Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology* 37, 141-172.
- Vet, L.E.M., Lewis, W.J., Cardé, R.T. 1995. Parasitoid foraging and learning. In: Chemical Ecology of Insects. Cardé, R.T., Bell, W. (Eds.), Chapman & Hall, New York, pp. 65-101.
- Vet, L.E.M. 1999. From chemical to population ecology: infochemical use in evolutionary context. *Journal of Chemical Ecology* 25, 31-49.
- Vigianni, G. 1984. Bionomics of the Aphelinidae. *Annual Review of Entomology* 29, 257-276.
- Vinson, S.B. 1976. Host selection by insect parasitoids. *Annual Review of Entomology* 21, 109-133.
- Vinson, S.B. 1998. The general host selection behavior of parasitoid hymenoptera and comparison of initial strategies utilized by larvaphagous and oophagous species. *Biological control* 11, 79-96.
- Wäckers, F.L. 1994. The effect of food deprivation on the innate visual and olfactory preferences in the parasitoid *Cotesia rubecula*. *Journal of Insect Physiology* 40, 641-649.
- Wäckers, F.L., Bonifay, C., Lewis, W.J. 2002. Conditioning of appetitive behavior in the hymenopteran parasitoid *Microplitis croceipes*. *Entomologia Experimentalis et Applicata* 103, 135-138.
- Wajnberg, E. 1989. Analysis of variations of handling time in *Trichogramma maidis*. *Entomophaga* 34, 397-408.

Acknowledgements

Reaching this page in my thesis is not the fruit of my own strength; it is a gift from the Celeste Love Who wanted me to search without finding, to search again and finally to find what I will no more lose...

My first word of gratitude and infinite love goes to my Celeste light, support and guide, Mother Mary, and Celeste friends for being omnipresent with me and in me.

I express my unlimited love and gratitude to my spiritual fathers, father Walid Nkoula and Propst Klaus Funke. Both of them were united to form the heart that pumped in my veins patience, perseverance and faith. From each one of them I have learned how to get dissolved in the Love without losing my integrity. Without their prayers I could have reached nowhere.



I am in debt to my supervisor Dr. Rainer Meyhöfer for his understanding, strong back up in difficult moments and patience in dealing with my deficiencies. But more, much more, for an outstanding value that he taught me, more relevant than IGP or statistics, a value that enriches my being: humility.

I have a deep word of thanks to Prof. Dr. Hans-Michael Poehling who accepted me in this working group and mostly for his generous help when needed.

I owe Prof. Dr. Felix Wäckers my heart-felt gratitude for introducing me to Dr. Meyhöfer at the start of my studies in Hannover and for accepting being one of my examiners.

Some people are met in life but they are not easily forgotten, because they enter the heart to remain: Lemma who carried on his shoulders the heavy burden of the statistical analysis of this “high level data”, Ernest (Ako) who took over after Lemma, “le bon Samaritain” and knew with his extreme delicacy how to heal most of the wounds and Jenny. I thank her for her fidelity, friendship, extreme consciousness while performing hard jobs and wisdom. When we had to make critical decisions, she was with me to choose the best.

I have a special word of thanks to Michael, not only for correcting the German abstract of my thesis or for exchanging statistical advices, but for the warm smile that he used to give for free....it made lot of difference.

I thank Maria (petite etoile) for her precious heart, Julia (bumblebee) for the purity of her heart, Sr. Salwatricze and Sr. Maria Armella for their friendship in the Lord. I know that even if we are apart, we are together....

A warm word goes to Bogusia who does not know now that she has a special place in my heart....but she will know....

Dr. Gunnar Mölck is especially acknowledged for scientific advices and for supplying the *Aphelinus* parasitoid and the wind tunnel (data not presented in this thesis) and the aphids in case of shortage. I thank Thomas for accompanying me to Kiel and being a valuable expert in reconstructing the taken apart parts of the wind tunnel.

Dr. Gemechis Dilba is particularly appreciated for statistical consultancy.

I am very much thankful to Mr. Henning Schmidt (attaché culturel) for being a strong intermediate between the DAAD and me.

I would like to thank the DAAD for granting me the scholarship and generously financing my four years of study.

I am truly in debt for the wonderful father Alex and mother Louise that God gave me: if I am to be born again and have the opportunity to choose my parents, I would have definitively chosen both of them.

I thank my grandparents Renee and Albert and my uncle Elias for their invaluable prayers, warm love and priceless care.

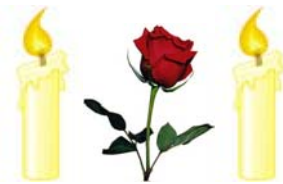
My deepest love goes to my sister Rebecca and her husband Paul for their love, friendship, extreme understanding of my soul, support and prayers, especially Nahoul.



I owe a word of thankfulness to my brothers and sisters in Kfarhabab's school guided by our mother superior mama Nohad: Marie Louise, Najwa, Imad, Emma, Dr. Alain, Akel, Viviane, Inaam...in this school I learnt how to find what I will no more lose...

I thank Nevrik from the depth of my heart....

My last word is to You my Jesus: thank You for the bad before the good, for the pain before the joy, for the failure before the success. Everything in this ephemeral life is an expression of Your love, that's why I have infinite reasons to tell you: I love You.....



Curriculum Vitae

Personal data

Full Name	Chantal Jazzar
Sex	Female
Date and place of Birth	1 st of January, 1972, Tripoli, Lebanon
Marital status	Single
Language	Arabic (mother tongue), French and English (fluent), German (modest)
Email	jazzar@ipp.uni-hannover.de , or cjazzar@hotmail.com

Education

2002–2006: PhD student at Leibniz University of Hannover, Faculty of Horticulture, Germany.

1999-2001: Doctoral Student at the University of British Columbia (UBC), Vancouver, British Columbia, Canada.

1995-1999: MSc in plant protection at the American University of Beirut (AUB), Beirut, Lebanon.

1991–1995: BSc in Plant Sciences at AUB, Lebanon.

1995: Diploma of Agricultural Engineering at AUB, Lebanon

1985–1991: Secondary School at Saints Coeurs Kfarhabab, Lebanon.

1979–1985: Primary School at Saints Coeurs Kfarhabab, Lebanon.

Career after graduation

September 2000-January 2001. Teaching assistant at UBC, Vancouver, Canada. Responsibilities included preparing and teaching introductory entomology laboratories, helping students to recognize the major morphological features of insects, collect, preserve and identify insects to the level of orders and families, in addition to marking the final written laboratory exam and the insect collection (of minimum 25 specimens).

January-12 May 1999. Research assistant at AUB. Responsibilities included the investigation of *Melia azedarach* (L.) aqueous mature fruit extracts with the fungal biocontrol agent *Paecilomyces fumosoroseus* to manage the third to fourth instar nymphs of the sweetpotato whitefly *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae).

February-August 1996. Graduate assistant at AUB. Responsibilities included performing priming experiments on different tomato and wheat varieties.

October-February 1996. Graduate assistant at the AUB. Responsibilities included preparing and teaching introductory entomology laboratories, helping students to recognize the major morphological features of insects, collect, preserve and identify insects to the level of orders and families, in addition to preparing and marking the final written laboratory exam.

Project grant/fellowship awards

Scholarship awarded by the German Academic Exchange Service (DAAD) for my PhD study (2002–2006).

Faculty of Agricultural Sciences, International Graduate Tuition Scholarship, Summer Session 2000, UBC.

Faculty of Graduate Studies, International Graduate Tuition Scholarship, Winter Session 2000-2001, UBC.

Publications

Jazzar, C., Hammad, E-A.F. 2004. Management of the sweetpotato whitefly on tomato using specialized biocontrol agents. *Journal of Applied Entomology* 128, 188-194.

Jazzar, C., Hammad, E-A.F. 2003. The efficacy of enhanced aqueous extracts of *Melia azedarach* L. leaves and fruits integrated with the *Camptotylus reuteri* releases against the sweetpotato whitefly nymphs. *Bulletin of Insectology*, Vol LVI (2).

Jazzar, C., Hammad, E-A.F. 9-21 May 1999. Poster presentation Participation in the “World Neem Conference”, The University of British Columbia, Vancouver, B.C., Canada. Effect of semiqualitative aging classes of *Melia azedarach* L. on *Bemisia tabaci* (Gennadius) and a predatory mirid.