



WAGENINGEN UNIVERSITEIT/
WAGENINGEN UNIVERSITY
LABORATORIUM VOOR ENTOMOLOGIE/
LABORATORY OF ENTOMOLOGY

**‘Effects of variation in plant defenses on the
behaviour and the development of the parasitoid
Cotesia glomerata’**



Vincent Van Gogh, The Blue Cart

No: 07.13
Naam/Name: Georgios Prekatsakis
Periode/Period: 20/09/2006-20/03/2007
Supervisor 1: Dr. T. Bukovinszky
Supervisor 2: E. Poelman
Examinator: Prof. Dr. M. Dicke

Table of contents

Preface.....	3
Abstract.....	4
1. Introduction	
1.1. General introduction: Modern pest management and ecological theory.....	5
1.2. General ecology: Bottom-up versus top-down forces.....	5
1.3. Behavioural ecology.....	7
1.4. Developmental physiology.....	9
2 Materials and methods	
2.1 Studied organisms	
2.1.1. Parasitoids.....	11
2.1.2. Caterpillars.....	11
2.1.3. Plants.....	12
2.2. Behavioural study set-up.....	13
2.2.1. Behavioural Observations.....	14
2.2.2. Data analysis.....	15
2.3 Developmental study set-up	
2.3.1. Experiment 1.....	15
2.3.2. Experiment 2.....	16
2.3.3. Data analysis.....	16
3 Results	
3.1 Behavioural study	
3.1.1 Percentage of parasitism.....	18
3.1.2 Frequency of non host parasitism.....	18
3.1.3 Time spent in patches.....	19
3.1.4 Time allocation per behaviour in patches.....	20
3.1.4.1 Mean duration per behaviour.....	21
3.1.4.2 Percentage time allocated per behaviour.....	21
3.2 Developmental study	
3.2.1 Encapsulation rates as affected by different <i>Brassica</i> lines and larval developmental stage.....	23
3.2.2 Encapsulation rates as affected by different <i>Brassica</i> lines and induced defenses.....	25
4. Discussion	
4.1. Behavioural study.....	27
4.2. Developmental study.....	30
5. Synthesis.....	32
6. References.....	34
7. Appendix: Statistical results.....	38

Preface

This report was the outcome of a research that I have never thought to work on till the September of this academic year. As an agronomist and a farmer, till recently, ecological theories were nothing more than “theories”. Observing organisms to interact deeply changed my perception on nature and agriculture. I have “evolved”. Lot of people contributed in my research, each of them is equally necessary, therefore valuable. I would like to thank my examiner Marcel Dicke, who helped me to avoid molecular studies and gave me the opportunity to meet and work with two excellent young scientists, my two supervisors, Tibor Bukovinszky and Eric Poelman. The cooperation with both Tibor and Eric was a nice experience that I’ll never forget. Moreover I would like to thank my colleague Andre Camp, with whom I shared many of the difficulties of our common experiment. I feel also the need to express my acknowledgements to the researcher of the entomological lab Rieta Gols which apart from providing me important information, in some cases she actively helped in the experiments. Finally, but not least, I deeply appreciate the help and the support of all the “silent heroes” of the entomological lab and unifarm which provided the insects and took care of my plants as if it was their own experiment. I must admit that observations in the greenhouse sometimes become tiresome. Special thanks to Pit de Man which had always something nice to say. His words were a relief.

The whole of nature accessible to us forms a system, an interconnected totality of bodies, and by bodies we understand here all material existencesIn the fact that these bodies are interconnected is already included that they react on one another, and it is precisely this mutual reaction that constitutes motion. It already becomes evident that matter is unthinkable without motion

Engels, Dialectics of Nature

Abstract

The present research aimed at gaining knowledge on the processes that shape the defensive potential of plants. A behavioural and a developmental study were carried out. In the former the effect of mixed herbivore infestation of plants (a host and a non host) with different densities and ratios on the foraging behaviour of the parasitoid wasp *Cotesia glomerata* (Hymenoptera: Braconidae) was investigated, based on an analysis of the recorded behavioural attributes. In the developmental study the effect of plant chemistry (different glucosinolate levels and profiles among two wild and one domesticated *Brassica* lines) on the ability of *Pieris rapae* (Lepidoptera: Pieridae) to encapsulate eggs of *C. glomerata* was examined. Interesting results derived from both studies. Regarding the recorded behaviour of the parasitoid, female wasps in all the different treatments allocated their time mainly in searching, walking and grooming. Host chemistry, triggered the research of the parasitoids resulting in significant time losses and non host parasitism in patches where hosts were removed before the observation. Foraging efficiency expressed as percentage of parasitism of the available hosts in a patch was not negatively influenced by the presence of non hosts. Based on the obtained results it can be speculated that *C. glomerata* takes her foraging decisions based on an adjustable strategy rather than a specific mechanism. Concerning the part of the developmental study, it was clear that the wild *Brassica* lines reduced the encapsulation potential of the herbivore which was even lower when defenses were induced by previous feeding of another herbivore. Considerably low encapsulation rates were measured in 1st instar caterpillars. The observed lack of correlation between larval weights and encapsulation rates suggested that Brassica chemistry affected the immune system of the herbivore both directly (toxic effect of chemical substances on herbivore's ability to mount an encapsulation reaction) and indirectly (degraded suitability of herbivore as a host). Although the obtained results embody a certain value of complexity and in any case lack of generality, they offer a fertile soil for argumentation on how ecological knowledge might be of great use in the management of agroecosystems.

1. Introduction.

1.1. General introduction: Modern pest management and ecological theory.

Mankind has been for thousands of years in a constant struggle to control hostile environmental forces. Among them, agricultural pests, directly related to the survival of the agricultural community have been one of the most inconvenient. Yet, people based on ecological knowledge gained from experience and accumulated through generations managed to efficiently deal with the “potential” enemies of their cultivations (Altieri et al., 1983). Modern world left no place for these practices. Technical solutions laid on the concept “that processes can be reduced into their simplest elements” provided a cure for everything (panacea). A defective part will be replaced, an annoying organism will be eliminated; modern world is simple (Callicott, 1988). At least this was initially thought. This rather “therapeutical approach” had both economical and ecological consequences. The use of synthetic pesticides, apart from its huge cost, poses several risks to the human health and the environment. Around 26 million people are poisoned per year (UNEP, 1997 cited in Paoletti and Pimentel, 2000), whereas acute as well as chronic illnesses were attributed to the effect of agrochemicals on human health (Paoletti and Pimentel, 2000). Moreover, degradation of surface and ground water quality by the irrational use of pesticides is a threat not only for the present but also for the future generations as they are not always broken down easily. Natural communities were, as it was expected, also victims of this anthropogenic activity. Numerous studies have been attributed to the devastating effect of organochlorine insecticides on the reproduction of birds and amphibians (Paoletti and Pimentel, 2000).

Pest management practices are still in our days far away from integrating ecological theories and fundamental research, although the dynamic complexity of an agroecosystem became profound. It has been well documented that components of agricultural systems (as what happens with any natural, economical or social system) are closely interwoven and in any case cannot be described, understood or treated in an isolated context. Interactions among these components have been evolved in time and space through a dialectic relationship making their properties a constant process rather than a state (Levins and Wilson, 1980). Ecological information about the characteristics of pests and their relationships with the other parts of the ecosystem will provide the tools for an efficient and sustainable control of our food competitors.

1.2. Ecology: Bottom up versus top down forces.

Plants in their effort to protect themselves against herbivores have evolved numerous defensive mechanisms. These involve both direct and indirect defenses. The first ones have been described also as bottom-up control and include both physical plant characteristics such as the plant structure, hairs, trichomes, waxes and spines or chemical plant properties such as the production of toxins, digestibility reducers, antinutritive compounds and repellents (Price et al., 1980; De Moraes et al., 2000). Indirect defenses (top-down control) refer to plant traits that facilitate natural enemies to locate and subsequently parasitize or predate their herbivore hosts (Kessler and Baldwin, 2001). Attraction of natural enemies may take place through the rewarding effect of food sources (e.g. nectar), the provision of shelter (e.g. leaf domatia) or the production of volatile compounds following the attack of herbivores that may help natural enemies to locate herbivores (Price et al., 1980; Vet and Dicke, 1992).

Top-down and bottom-up effects can not be isolated, thus not necessarily harmonically cooperate to enhance plant defensive potential (Hunter and Price, 1992). Parasitoids may be regarded as mutualists to plants suffering from herbivores; yet plant responses are complex. As noted by Price et al. (1980) a “good intrinsic defense frequently results in negative impact on enemies”. Parasitoids responding to the help signals of plants (Dicke et al., 1988) can sometimes be confronted in a rather hostile way by their supposed mutualists (Hunter, 2003). Constraints like hairs or trichomes may significantly impede parasitoid’s searching efficiency whereas the cascading effect of sequestration of toxic compounds by their immature progeny (developing in herbivores feeding of toxic plant tissues) may deleteriously affect parasitoid’s reproductive success (Hunter, 2003). Of course, a synergistic effect of plant defenses cannot be excluded. Enhanced parasitoid development has been suggested for host species fed on plants that are considered to provide them protection against encapsulation, through their negative effect on the herbivore’s immune system (Cheng, 1970 cited in Barbosa et al., 1982).

A thorough understanding of trophic relationships apart from the interactions among the different trophic levels should also encompass all the interactions occurring within each level (horizontal interactions) which in turn may indirectly or directly influence top-down or bottom-up impacts. A species can alter the effect that another species has on a third one, through changing its density (density mediated indirect effect) or by modifying its behaviour (trait mediated effect) (van Veen et al., 2005).

As was above described and can be seen in the figure 1. a complex suite of direct and indirect interactions within different trophic levels will shape herbivore and carnivore populations, determining the total outcome of plant defenses. Studies on tritrophic systems aim to unravel this complexity. In the following research two different attributes of ecological interactions were examined: a behavioural and a developmental. In the first one the effect of mixed herbivore populations (represented by different densities and distributions of a host herbivore and a non host herbivore) on the searching efficiency of a parasitoid is investigated whereas in the developmental study it is tested the effect of plant chemistry (different glucosinolate profiles and contents among one domesticated and two wild populations of Brassicas) on the encapsulation ability of a herbivore.

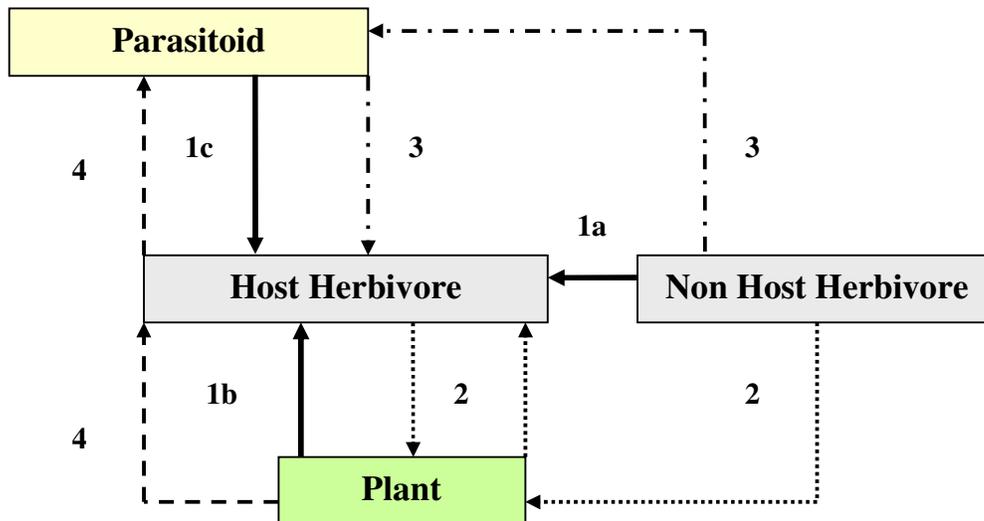


Fig. 1. Representations of interactions within a tritrophic system, focused on the effects on the host herbivore. 1a) direct competition, 1b) direct top down effects, 1c) direct bottom up effects, 2) induced defenses 3) apparent competition 4) effect of plant quality on the parasitoid (adapted from Gripenberg and Roslin, 2006)

1.3. Behavioural ecology

A large body of work has been elaborated on the reliability-delectability problem that parasitoids face when they forage for their hosts (Vet and Dicke, 1992; De Moraes et al., 2000). Parasitoids will primarily use chemical cues to locate their hosts. These cues are in fact chemically derived information (semiochemicals) which can act intraspecifically (pheromones) or interspecifically (allelochemicals). Host derived cues although reliable indicators of the herbivore's presence are not appropriate for long distance assessment due to their low abundance. A solution to this problem is the use of herbivore induced plant volatiles (HIPVs) by natural enemies. These are synomones¹, that are produced by the plant when it is attacked by a herbivore (Vet and Dicke, 1992). However, searching in nature does not take place in a uniform environment, but plant and host distributions and densities show large spatial and temporal heterogeneity. In many cases parasitoids will encounter complex environments containing host and non host populations in different spatial distributions and densities. The derived chemical information from the herbivores feeding is not always complete, therefore unreliable (Vos et al., 1998; Vos et al., 2001). Studies on how foraging behavior of parasitoids is affected by such "unreliable" ecological contexts are limited (Vos et al., 2001).

The effects of lack of specificity of plant information on foraging efficiency of parasitoids have been studied in the *Brassica- Pieris* sp. - *Cotesia glomerata* system. *C.glomerata* had an equal preference (choice tests) for mechanically, host and non host damaged leaves (Steinberg et al., 1993, Wiskerke and Vet, 1994; Vos et al., 2001), whereas in a previous research (Steinberg et al., 1992) it was found that infochemicals derived from the herbivore host (*Pieris brassicae*) or its by-products

¹ Synomones are chemicals that are beneficial both for the emitter and the receiver

were not so attractive to this parasitoid wasp. This described lack of information discreteness would probably lead to significant waste of time during foraging. Taking into account that most of the parasitoid species are time limited (females die before they have laid all their eggs), time is often considered as the determinant factor of reproductive success (Rosenheim, 1999). Yet, the effect of diversified host and non host populations on the population dynamics of a parasitoid is not necessarily detrimental (van Veen et al., 2005). Parasitoid community may become more diverse through the increase of infochemicals-mediated weak trophic links (Vos et al., 2001). Such effects have barely been studied on the way that a parasitoid allocates its foraging time inside a patch. (mainly initial encounters have been studied from this perspective).

Exploitation of host patches by parasitoids has been described as a process related to host density (Walde and Murdoch, 1988). It was suggested that parasitoids will exploit a patch based mainly² on the intensity of the chemical stimulus derived from the host (kairomones), as well as on the number of host encounters (Waage, 1979; Driessen et al. 1995). Through this perspective it is expected that the presence or interference of hosts with non hosts to have a profound effect on parasitoid's searching efficiency. In this case the different herbivore species apart from being direct competitors (for foliage) interact indirectly by changing the behaviour of the natural enemy (trait mediated indirect effect).

Our study system included the parasitoid *Cotesia glomerata* and two herbivore species: a host (*Pieris rapae*) and a non host (*Mamestra Brassicae*). Our hypothesis was that both densities as well as ratios of herbivore populations (hosts and non hosts) would influence the decisions (patch depletion, time allocation within a patch, ovipositions) of the forager. We were interested not only on the expression of these effects (e.g. how fast or to what extent a patch is depleted) but also on the underlying mechanisms (rationale) that govern the way that the females decide. Several behaviours (fly, search, walk, search, stop, attack, oviposition host, oviposition non host) in different experimental contexts were recorded in order to understand the nature of the interactions among the two herbivore species and the natural enemy. A stepwise dynamic analysis was followed. In the first step the research was carried out simultaneously and in cooperation with the MSc student Andre Kamp. Andre's work mainly focused on the effect of mixed herbivore populations on the searching efficiency (e.g. percentage of parasitism, time spent in a patch) of the forager, by comparing the parasitism under three different herbivore's densities and ratios within a patch: patches containing only hosts, patches containing only non hosts, patches containing equally distributed hosts and non hosts. My research focused on isolating the effect of host and non host herbivore presence/absence from the cues associated with the herbivore itself and its damage on the behaviour of the parasitoid. This was carried out also by comparing patches of different herbivores' densities and ratios:

In the first three treatments, the forager faced the same number of hosts; however the chemical and psychical context may differ:

- I) plants infested with 5 hosts and 5 non hosts
- II) plants infested with 5 hosts and 5 non hosts; the latter ones were removed before releasing the wasps. Same type of damage compared to the first treatment is expected but non hosts are absent in this case.

² Numerous other parameters such as: the contacts with competitors or already parasitized hosts, the time elapsed between last visit in a patch and the physiological state of the parasitoid may influence patch exploitation decisions (van Alphen et al., 2003).

- III) plants infested with 10 hosts; 5 of them were removed before releasing the wasps. Different type of damage compared to both two first treatments is expected whereas compared to the first treatment non hosts are absent in this treatment.

The following two treatments represent clean populations (a kind of control) of 10 herbivores (hosts or non hosts).

- IV) plants infested with 10 hosts. Type of damage is similar to the third treatment but availability of hosts is doubled.
- V) plants infested with 10 non hosts. A pure unfruitful (in terms of host, physical and chemical presence) environment for the forager.

As in the first step of our research (first five treatments) the presence of a non host herbivore seemed to have a significant, though complex effect (wasps stayed long enough, parasitized a proportionally higher number of hosts compared to only host patches whereas in some cases parasitized also non hosts) the second step was designed to as possible unravel this complexity. Two treatments were added:

- VI) plants infested with 5 non hosts and 5 hosts; the latter ones removed before releasing the wasps. A question that can be addressed through this treatment is whether wasps would still sting *M. brassicae* even in the absence of their host (is parasitoid confused by host cues or the actual presence of hosts is necessary?).
- VII) plants infested with 8 non hosts and 2 hosts. In this case it was investigated up to what extent searching efficiency of the parasitoid is ratio hosts/non hosts dependent.

1.4. Developmental physiology

Apart from their effect on the foraging behaviour of adult parasitoids, host plant characteristics may also influence the growth and the development of the immature parasitoid that feeds on the host herbivore tissues (Kester and Barbosa, 1991; Turlings and Benrey, 1998). In this case the effect of plant chemistry on the third trophic level is either direct (exposure of parasitoid larva to plant chemicals inside the host haemolymph) or indirect as mediated by host development (plant chemistry affects the suitability of the host herbivore as a host) (Turlings and Benrey, 1998).

Among the most important internal immune defenses of insects to parasitoids is the physiological mechanism of encapsulation. The specific mechanism refers to a cellular response of insects to foreign organisms in which specialized reaction cells (haemocytes) circulating in insects haemocoel will surround and isolate any material alien to the insect's physiology (Vinson and Iwantsch, 1980). Plant chemistry is regarded to play an important role on the herbivore's ability to encapsulate eggs (Benrey and Denno, 1997). Low nutrient levels, starvation or production of toxic secondary metabolites of the host plant may negatively affect herbivore's encapsulation potential (Turlings and Benrey, 1998). Benrey and Denno (1997) investigated encapsulation rates of *Cotesia glomerata* eggs, as affected by the diet of its host (*Pieris rapae*). Significant differences were found, suggesting higher encapsulation rates on the plant species on which the host larvae was developing more rapidly. Therefore, in this case it can be speculated that low nutritional quality or presence of toxins in plant tissues indirectly benefited parasitoids through suppression of the host's herbivore immune system.

Host plant species and cultivars often vary in their secondary chemistry. Glucosinolates are secondary plant metabolites occurring in the Capparales order,

which includes numerous crops of agricultural interest such as oilseed rape, cauliflower, broccoli, mustard and cabbage. Glucosinolates are hydrolyzed by myrosinase enzymes when plant tissue is damaged by pests or pathogens leading to the production of D-glucose, sulphate and several cyanide compounds (Larsen, 1981 cited in Siemens and Mitchell-olds, 1998). Glucosinolate degradation products have been reported to affect the behaviour and the physiology of insect pests. They have been observed to act as attractants, feeding and oviposition stimulants mostly for specialized herbivores and their natural enemies (Renwick et al., 1992; Raybould and Moyes, 2002; Loon and Schoonhoven, 1999) whereas in many other insects (which it have not evolved resistance) they have a deterring or even harmful effect (Raybould and Moyes, 2002). Different chemical profiles as well as concentrations of glucosinolates can be found among wild and cultivated populations within *Brassica oleracea* species (Rosa E.A.S, 1999). It has been documented that herbivores respond to variation in glucosinolate levels (Raybould and Moyes ,2002).

In this study we examined in a tritrophic context the effect of three *B.oleracea* lines (one cultivated and two wild ones), that differ in their glucosinolate profile and content, on the ability of *P. rapae* to encapsulate eggs of the parasitoid *C. glomerata*. Our hypothesis is that the wild cabbage relatives with increased glucosinolate levels in their tissues will negatively influence the encapsulation ability of *P.rapae*. Moreover, given the fact that earlier host stages are preferable by the specific parasitoid (Brodeur and Geervliet, 1992; Brodeur and Vet, 1995) it is expected that host's defenses, as expressed by the encapsulation mechanism, are reduced in early larval instars. Furthermore, trying to encompass horizontal interactions (apart from bottom up effects) in our study system, it is speculated that feeding damage by another herbivore (apart the parasitized one), will enhance plant defenses, resulting in even higher negative effect on the host herbivore encapsulation potential. Two different, though quite similar, experiments will try to validate these three hypotheses. In both of them encapsulation rates are correlated to *Brassica* lines, whereas in the first and the second one the effect of host age (1st or 2nd) and previous induced defense by another herbivore are respectively tested. Caterpillars, before being dissected, will be weighted and the obtained weights will be integrated in the analysis in order to investigate if there is any correlation among larval performance and encapsulation rates.

2. Materials and methods.

2.1. Studied organisms

2.1.1. Parasitoids

Parasitoids are among the most intensely studied organisms in ecology due their importance in regulating the populations of herbivores (Godfray and Shimada, 1999). “Insect parasitoid” was described by Eggleton and Gaston, (1990) as “An organism that develops within an insect, extracts nourishment from it and kills it as a direct or indirect result of its development”. Adult parasitoids are free living, whereas only the immature stages are parasitic; the host is used as a feeding container that is mercenarily modified by the parasitoid (Vinson and Iwantch, 1980). Life cycle of parasitoids is typical of the “holometabolous” insect groups having four different stages: egg, larva, pupa and adult. Different parasitoid species may attack different host stages. However, parasitization of adults is less encountered. Parasitoids may develop within (endoparasitoids) or on their hosts (ectoparasitoids). Based on the host – parasitoid relationships, parasitoids can be categorized into: 1) idiobionts, which are the parasitoids that do not allow further growth and movement of their hosts after parasitization and 2) koinobionts that allow movement and development of their hosts long after the initial parasitization. Moreover, based on the number of parasitoids that emerge per host, solitary (one individual per host) and gregarious parasitoids (more than one individuals per host) can be distinguished (Godfray, 1994).

Cotesia glomerata (Hymenoptera: Braconidae) is a gregarious, koinobiont larva endoparasitoid that attacks early instar (preferably) larvae of several pierid species³, being rather specified on *Pieris brassicae* (Lepidoptera: Pieridae). Soon after emerging from their cocoons adult females mate and begin to search for their host. *C. glomerata* may lay up to 40 eggs in its caterpillar host (Laing and Levin, 1982). The larvae will feed on the host’s haemolymph and fat body and approximately 15-20 days after parasitisation the mature larvae will egress through the host side to pupate on its outer surface or close to it (Laing and Levin, 1982). The preference of *C. glomerata* to oviposit in younger instars of their hosts has been correlated to the reduced immune system (encapsulation ability).of these stages (Bordeur and Geervliet, 1992). *C. glomerata* wasps used in the experiments were reared on *P. rapae* larvae infested on Brussels sprouts. Parasitoid cocoons were collected in Petri dishes and placed in emergence cages that were in turn placed in a climate controlled chamber at 23 °C, 70 % RH and 16:8 L:D photoregime in the absence of hosts and plants. Emergence cages were supplied with wet cotton fabric and droplets of honey.

2.1.2. Caterpillars

Cabbage caterpillars are responsible for significant economical losses in many *Brassica* crops (Mc Kinlay, 1992). In our study system three different caterpillar species will be used: the Small White butterfly [*Pieris rapae* (Lepidoptera: Pieridae)], the Large White butterfly [*Pieris brassicae* (Lepidoptera: Pieridae)] and the Cabbage moth [*Mamestra brassicae* (Lepidoptera: Noctuidae)].

³ *C. glomerata* has been reported (experimentally; not from direct observations in its natural environment) to attack also species from other families in which its progeny in most of the cases does not develop mainly due to encapsulation (Laing and Levin, 1982).

P. rapae attack species belonging in the Brassicaceae family including both wild and cultivated cabbage, charlock and garlic mustard. *P. rapae* gives 2 generations per year. The first one will emerge from overwintered pupae in spring and causes insignificant damage, whereas the second one will emerge at the beginning of the summer and in fact is considered the harmful one. Eggs are laid individually in the underside leaf surface of many different plants and the emerged larvae will feed on the heart of Brassicas. Yet, the crop in many cases is not totally destroyed but it becomes rather unmarketable (Mc Kinlay, 1992; Porter, 1997)

P. brassicae is also specified in Brassicas attacking wild and cultivated cabbage, cauliflower and nasturtium. *P. brassicae* also gives 2 generations per year (in warm climates may give 3-4). Adults of the first generation will appear in April and May. Eggs are laid in batches on the underside of the leaves. Plants close to shelters and hedgerows will be preferred. Larvae mainly after the second moulting start to greedily feed on outer leaves of the host plants. After approximately 30 days larvae will abandon their hosts looking for a shelter to pupate. From these pupae the second generation will emerge during July and August and as in the case of *P. rapae* is regarded to be the more damaging (Mc Kinlay, 1992; Porter, 1997).

M. brassicae is a rather polyphagous caterpillar feeding on wild and cultivated Brassicas, tobacco, lettuce, beets, onions, garden flowers e.t.c.. As the two previous Lepidoptera has two generations per year. The first adults will appear at the end of May, emerged from overwintered soil pupae. Eggs are singly but closely deposited on the under surface of leaves forming neat groups. Caterpillars (mainly young stages) nibble the leaf surface and it is considered that the economic damage will primarily derive due to frass contamination rather than leaf consumption. After 5 weeks of moulting they burrow into the soil where they pupate. These pupae will give rise late in the summer to a new generation of moths (Mc Kinlay, 1992; Porter, 1997).

Larvae of *P. rapae*, *P. brassicae* and *M. brassicae* were obtained from a laboratory rearing on Brussels sprouts (*Brassica oleracea gemmifera* cv. Cyrus) in a climate room of Wageningen University (WUR) at 21 °C, 60 % RH and 16:8 L:D photoregime. 2nd instars of each species have been used to infest the plants..

2.1.3. Plants

Brassica crop species (family:Brassicaceae) are important sources of edible plant parts (roots, stems, leaves, flowers, seeds) whereas many of them are also recognized for their ornamental value. Brassicaceae secondary chemistry has been fairly studied and although quite a few substances (protease inhibitors, saponins, anthocyanins) have been isolated for their defensive action there is a little doubt that the most important of all are the glucosinolates (van Dam et al., 2004). Glucosinolates and their breakdown products (glucosinolates are hydrolysed by an enzyme called myrosinase, which is considered to coexist with glucosinolates in almost all the *Brassica* species) have been reported for their antifungal (Phipps, 1990), bactericidal (Chew, 1988), nematicidal (Potter et al., 1999) properties as well as for their role in mediating plant-herbivore-natural enemies interactions (Raybould and Moyes, 2001; Harvey et al., 2003). The glucosinolate content and pattern differs among plant parts and apart from the cultural practices and the growing environments are also subject to the large genetic variation of *Brassica* species. Different glucosinolate levels have been used even to characterize cultivars. It has been also reported wild *Brassica* populations to have increased glucosinolate levels compared to their domesticated relatives (Rosa E.A.S, 1999).

Brassica oleracea L., a spring perennial known to grow wild in the European Atlantic coasts, has been fully domesticated after its introduction in the Mediterranean basin giving rise to numerous cultivated forms (kales, cabbages, kograbi, cauliflower, broccoli, calabrese) (Gómez-Campo and Prakash, 1999). For the behavioural studies white cabbage has been used (*B. oleracea capitata* cv. Christmas Drumhead). In the developmental study one cultivated cabbage variety of Brussels sprouts (*Brassicae oleracea gemmifera* cv. Cyrus) and two wild *B.oleracea* lines (*B. oleracea* “Kimmeridge”, *B. oleracea* “Old Harry”) with different glucosinolate levels⁴ were used. For the wild populations seeds originated from natural wild populations of *B. oleracea* that grow along the Atlantic coastlines of southern England, about 10 kilometres distance from each other. Plants were reared in a greenhouse compartment at 21 °C, 70 % RH and 16:8 L:D photoregime in pots (0.7 lt) filled with potting soil (Lentse potgrond). Foliar nutrient solutions [concentration 3 g/l, KristalonTM (16% N, 6 % P, 20% K, 3% Mg)] were provided in a weekly basis in order all experimental plants to be robust and healthy before the conduction of the treatments.

2.2. Behavioural study set-up

Observations on the foraging behaviour of *Cotesia glomerata* were conducted in a greenhouse compartment at 21 °C and 70 % RH. Additional illumination was obtained with the use of mercury lamps. Parasitoids used were naïve (i.e. no oviposition experience or contact with hosts and plant material), 4-7 day old, mated females. They were provided with water and honey *ad libitum*. Responsive females were collected from the rearing cage by using a Brussels sprout leaflet damaged by larvae of *P. rapae* (all larvae feeding on the leaf were carefully removed previously its use) and transferred inside Petri dishes to the greenhouse compartment where the observation took place. Parasitoids would pass an adaptation period of at least one hour to the new environment before released. In this compartment a tent made of white sheets was set in order to offer the appropriate contrasting background for the flying parasitoids. Each treatment consisted of four 8 weeks aged Brussels sprouts plants (*Brassicae oleracea gemmifera* cv. Cyrus) with the same type of infestation (plants were infested with caterpillars 24 hours before the observation), placed on a table within the tent and shaping a square of 50 cm each side (fig.2.). The release site of the wasps was set at 15 cm height from table’s surface and at a distance of 50 cm from the two nearest experimental plants (fig2.). After each observation the plants that were visited by the parasitoid, were replaced by a new one. In each experimental day 2-3 different treatments took place whereas the time that these treatments were conducted (morning or afternoon) was changing between days. The 7 different treatments were the following:

- I) plants infested⁵ with 5 hosts and 5 non hosts
- II) plants infested with 5 hosts and 5 non hosts; the later ones were removed just before the release

⁴ Total glucosinolate content of the wild *Brassica* lines was higher than the one of the cultivated *Brassica* line whereas also glucosinolate chemical profiles differed considerably among cultivated and wild *Brassica* lines (Gols R., personal communication).

⁵ Caterpillars were carefully spread on the surface of only one leaf of each plant with small paintbrushes 24 hours before the observation. Attention was given in order the chosen leafs of each treatment to be of similar physiological condition and age. For each caterpillar species different brushes were used.

- III) plants infested with 10 hosts; 5 of them were removed just before the release
- IV) plants infested with 10 hosts
- V) plants infested with 10 non hosts
- VI) plants infested with 5 non hosts and 5 hosts; the later ones were removed just before the release
- VII) plants infested with 8 non hosts and 2 hosts

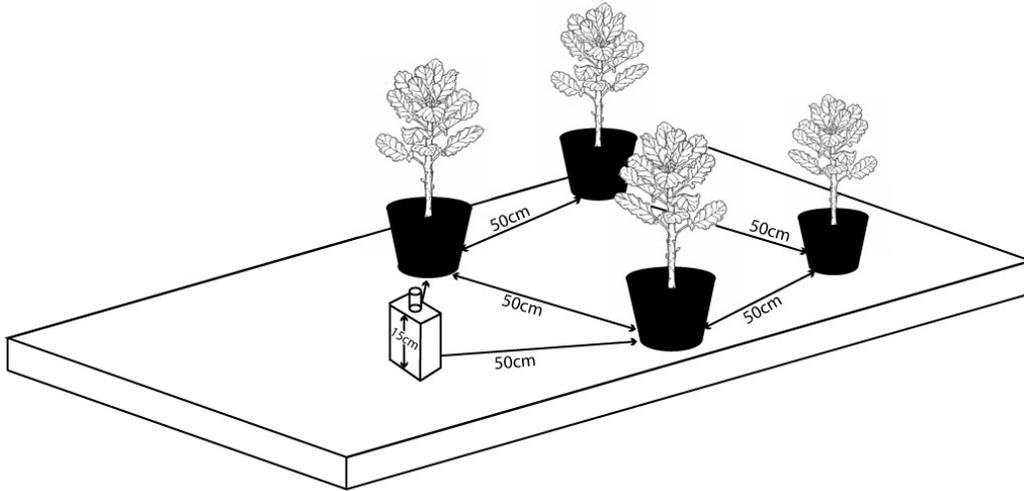


Fig. 2. Schematic representation of experimental set-up

2.2.1. Behavioural Observations

Foraging behaviour of the wasps was recorded on a portable microcomputer (Psion Workabout Pro) using the software (The Pocket Observer). The exact behavioural parameters that were recorded correspond to the ones of Wiskerke and Vet (1994) and are described in table 1.

Each observation started when the parasitoid left the releasing point and ended after the parasitoid abandoned the first visited plant for another plant where it landed and stayed for at least one minute or less in the case that managed earlier to oviposit. In any case observations were terminated 1 hour after the parasitoid's landing on the first patch. As in many cases the parasitoids after their release flew to other places than the leaf surface (e.g. tent, floor, table) or even stayed at the releasing point, it was regarded that if the parasitoid in a time period of 5 minutes would not have visited any patch (plant) than the observation should be rejected.

Table 1. Description of the recorded behaviours

Type of behaviour	Description
Fly	Parasitoid is airborne
Walk	The wasp moves rather quickly on the leaf surface without turning around. The antennae move up and down touching alternately the leaf surface.
Search	The wasp reveals strong arrestment behaviour: it decreases its speed and persistently turns around on the place where holes or feces of the herbivore can be found. The antennae are bent and swept along the leaf surface..
Groom	It refers to any cleaning activity that takes place by the wasp, from drawing the antennae through her forelegs, brushing the thorax with the midlegs and the abdomen, ovipositor and wings with the hindlegs.
Stop	The wasp stands motionless, except some antennal movement, which in any case does not touch the leaf surface.
Attack host	The wasp initially touches its potential target with the antennae, than draws her abdomen between her legs and raises her antennae and wings.
Attack non host	The wasp initially touches its potential target with the antennae, than draws her abdomen between her legs and raises her antennae and wings.
Oviposit into a host larva	The antennae and wings are raised. She inserts her ovipositor in a host larva.
Oviposit into a non host larva	The antennae and wings are raised. She inserts her ovipositor in a non host larva.

2.2.2. Data analysis

Percentages of parasitized hosts have been compared by a Generalized Linear Model with a logit link function and binomial distribution for errors. For a pairwise comparison of treatment groups linear contrasts have been carried out. Analysis was performed using the GENMOD procedure of SAS 8.2 (SAS Institute Inc.). Frequency of non host parasitism, total time spent in patches and time allocated per behaviour in the different patches were analyzed using non-parametric tests. Kruskal-Wallis tests were used to validate significance of differences among patches followed by multiple comparisons using pairwise Mann-Whitney U tests and a sequential Bonferroni correction to correct for type I errors. For this analysis the SPSS statistical software package (SPSS Inc.) was used. Differences were found significant at $P < 0.05$.

2.3. Developmental study set-up

2.3.1. Experiment 1

Larvae of *P. rapae* were obtained by placing 2 plants of each examined *B. oleracea gemmifera* line (Cyrus, Old Harry and Kimmeridge) in an oviposition cage with *P.*

rapae butterflies. After 24 hours the oviposited plants were taken out from the cages and were kept in a climatic room at 21 °C, 60 % RH and 16:8 L:D photoregime till larvae emergence. Newly emerged larvae from each line were used to infest plants from the corresponding line. Specifically 8 larvae were placed in 16 plants of each cultivar. Plants were divided in two teams of 8 plants. In the first team larvae were allowed to feed on the plants for 24 hours (1st instar larvae) and then were parasitized whereas in the second team larvae were parasitized after 48 hours (2nd instar larvae). Each plant from each team was identified with a number and a description of the team that belongs. For the parasitization, *C. glomerata* wasps, prompt to oviposit (irrespective of their age) were used. Parasitized larvae were transferred after parasitization again to their plants of the team that were initially developed (cultivar and stage before parasitization; not necessarily the exact same plants), in which they were reared for 2 days before dissection takes place. Just before dissecting them, larvae were weighted and as in the case of plants, identified (each of them represents an experimental unit). All dissections were made using a stereo microscope. Encapsulated eggs were recognized based on the white substance (“capsule”) that was formed around individual or mostly group of eggs

2.3.2. Experiment 2

In the second experiment the process of *P. rapae* larvae obtainment was same as the first one. However, 3 days before infestation with *P. rapae* 8 plants from each of the 3 Brassicae lines were infested with 6-8 first instar *P. brassicae* larvae to induce plant defenses. In parallel 8 plants of the Cyrus line and 8 plants of the Old Harry line stayed uninfected (plants of Kimmeridge were unavailable, by mistake). Infestation with *P. rapae* was similar with the first experiment (apart from the fact that in the second experiment 15-20 larvae per plant were used, to compensate the larvae mortality due to physical factors that was observed in the first experiment). After infestation with *P. rapae*, *P. brassicae* larvae in the induced plants were removed to avoid physical interference. Parasitization in this experiment took place in all treatments 3 days after the infestation (late 2nd instar) as infesting early host ages during the first experiment yielded relatively few encapsulations (specially for 1st instar) and in this experiment the effect of host age is not tested. The rest of the procedure (identification of plants and caterpillars, dissection) is identical to the first experiment.

2.3.3. Data analysis

Differences in weights between the different treatments in both experiments were validated with one-way ANOVA tests followed by Tukey- HSD procedures. The SPSS statistical software package (SPSS Inc.) was used. Differences were found significant at $P < 0.05$. To compare encapsulation rates of eggs, the probability that a parasitized caterpillar contained an encapsulated egg has been modeled using logistic regression. For this the logit of the probability of encapsulation has been used $\{\text{logit} = p / (1-p)\}$ and the odds ratios for encapsulation were computed when larvae fed on the different *Brassica* lines. A second analysis of the effects of *Brassica* lines included the weights of caterpillars at dissection as a covariate. The comparison of main effects (type 3 analysis) was based on likelihood ratio statistics and the pairwise comparisons were carried out by requesting linear contrasts of treatment groups. For the analysis

the GENMOD procedure has been used with a logit link function and a binomial distribution of errors SAS 8.2 (SAS Inst. Ink.)

3. Results

3.1. Behavioural study

3.1.1. Percentage of parasitism

Among different patches differences can be observed in the percentage of parasitized hosts ($\text{Chi}^2 = 10.40$, $\text{df} = 4$, $P = 0.0343$). Wasps exploited more intensively the patch of 10h5hrem parasitizing almost half of the available hosts. The magnitude of exploitation was significantly higher in this case compared to the environments of 5h5nhrem ($P = 0.040$) and 10host ($P = 0.063$) but not to 5h5nh ($P = 0.4293$) and 8h2nh ($P = 0.1971$). In the patch of 10 hosts percentage of parasitism was even lower compared to 5h5nh ($P = 0.0273$). No significant differences among the treatments of mixed herbivore populations (5h5nh, 5h5nhrem, 8nh2h) were found.

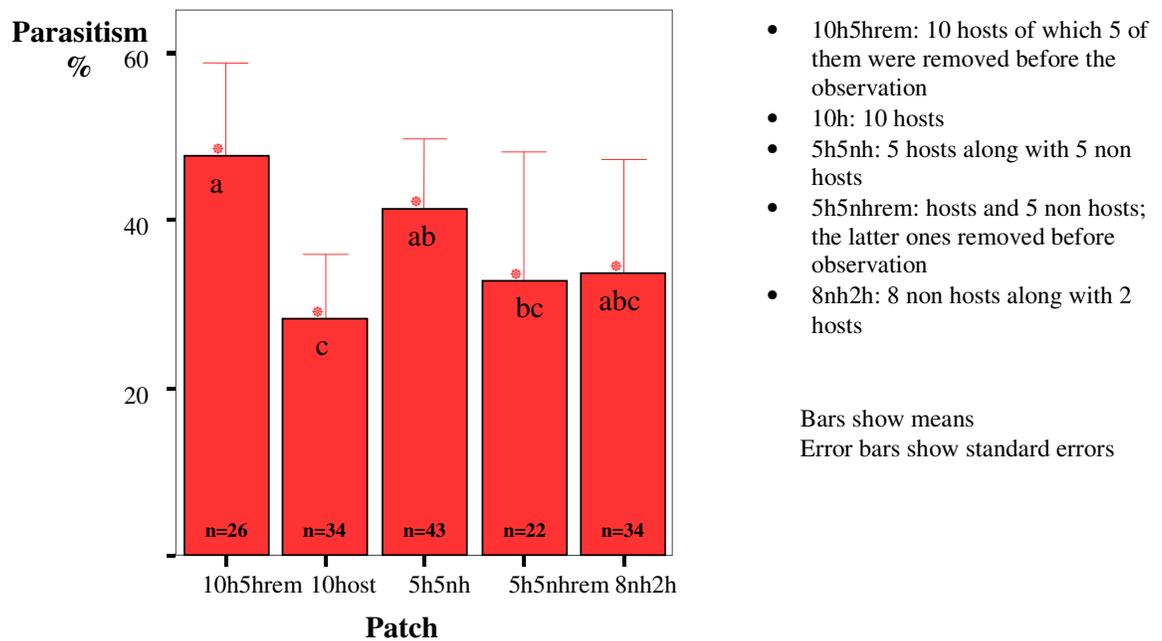


Fig.3.1 Percentage of parasitized hosts among the available hosts in different patches. Numbers in the columns correspond to the observations conducted in each patch. Means followed by different letters are significantly different.

3.1.2. Frequency of non host parasitism

Wasps in our experiment parasitized non hosts and although the frequency of this phenomenon was rather low, differences among the different non host patches were statistically validated (Kruskal-Wallis test, $\text{Chi}^2 = 11.507$, $\text{df} = 3$, $P = 0.009$). In patches of solely non hosts (10nh) not even one parasitism was observed. However, compared to patches in which just 2 hosts were available and in which non host parasitism occurred, differences were not significant (Mann-Whitney U test, $P = 0.097$). Non host parasitism was significantly higher in patches in which hosts were removed before the observation (5h5nhrem) compared to mixed herbivore

patches of only 2 hosts (Mann-Whitney U test, $P=0.016$) but not to the ones of equal host and non host herbivore populations (Mann-Whitney U test, $P=0.239$).

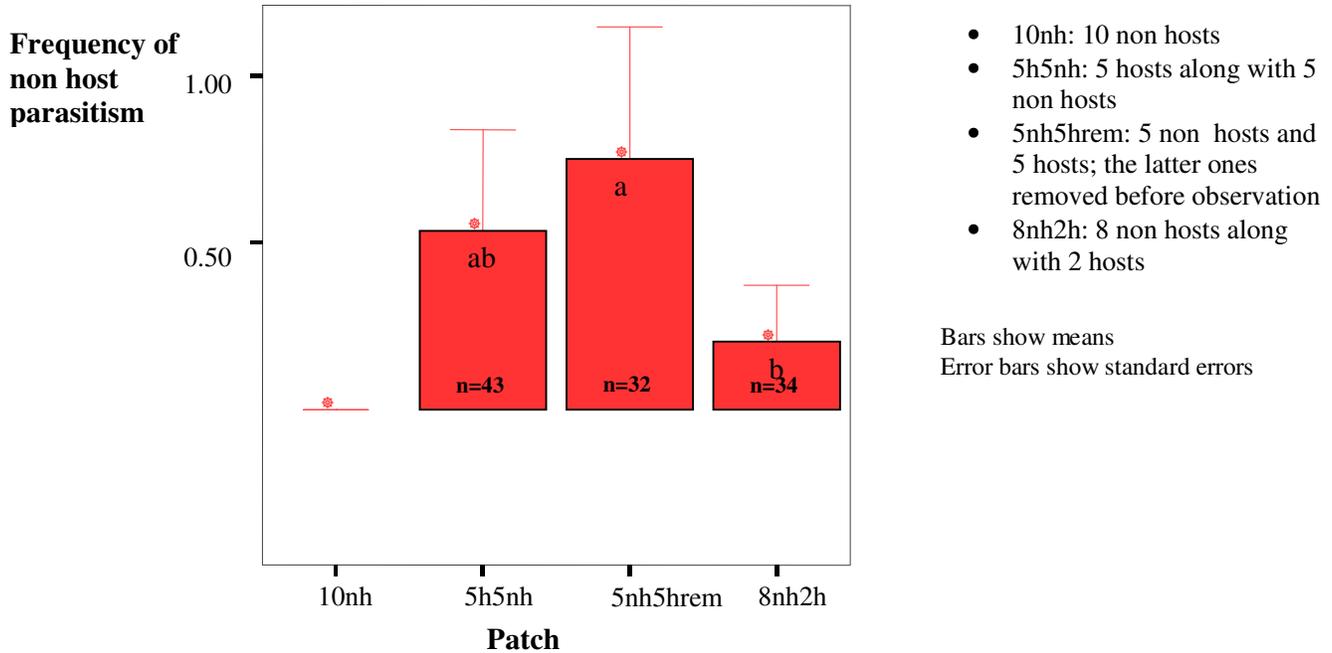


Fig. 3.2 Frequency of non host parasitism in different non host patches. Numbers in the columns correspond to the observations conducted in each patch. Means followed by different letters are significantly different.

3.1.3. Time spent in patches

Regarding the total time that wasps spent in a patch, significant differences have been observed among the different patches (Kruskal-Wallis test, $\text{Chi}^2= 37.654$, $\text{df}= 6$, $P= 0$) (fig 3.3). It was clear that in 10nh patches they stayed significantly less compared to all the other patches. In observations with equal populations of 5 hosts (5h5nh, 5h5nhrem, 5nh5hrem), even when these hosts were removed before the observation (5nh5hrem), wasps residence times did not differ significantly. However wasps stayed more in 10h5hrem compared to 8nh2h (Mann-Whitney U test, $P= 0.026$) and 5h5nhrem (Mann-Whitney test U test, $P= 0.047$). Time spent in patches of 10 hosts was not significantly different compared to the rest of the patches; except of course the 10 non host patches.

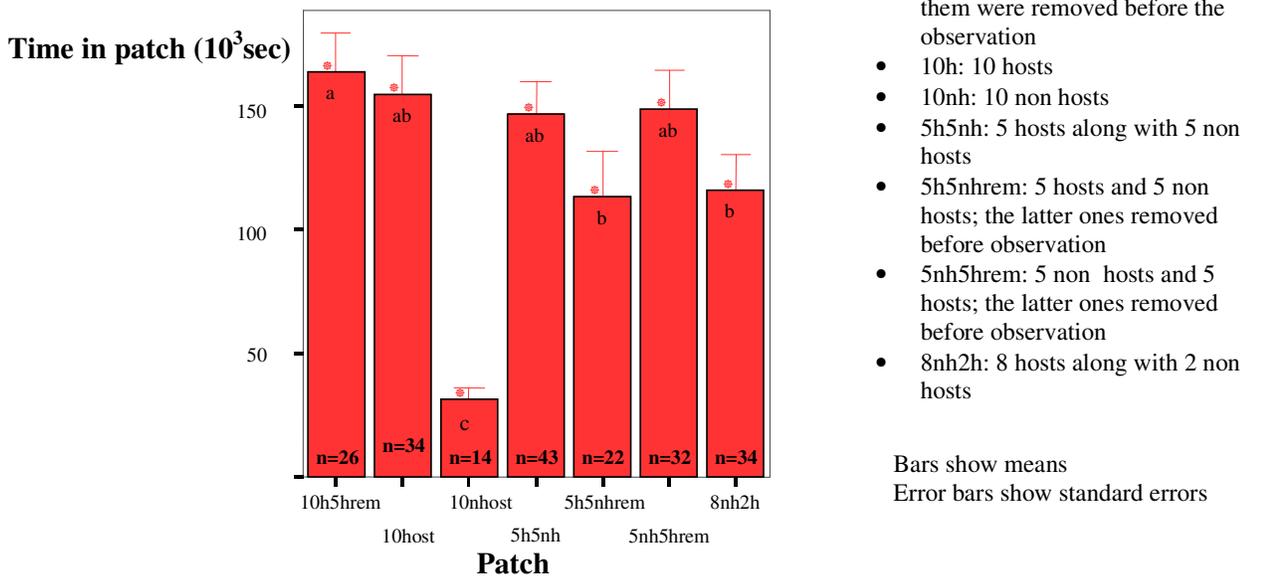


Fig. 3.3 Time spent by wasps in different patches. Numbers in the columns correspond to the observations conducted in each patch. Means followed by different letters are significantly different.

3.1.4. Time allocation per behaviour in patches

3.1.4.1. Mean duration per behaviour

Wasps dedicated a substantial amount of their time in a patch for flying, grooming and searching and less time for stopping and walking (Fig. 3.4).

Flying duration differed among the different patches (Kruskal-Wallis test, $\chi^2=16.471$, $df=6$, $P=0.11$). Wasps spend more time flying in patches where non hosts were present during the observation (5nh5hrem, 8nh2h, 5h5nh). However, differences in flying duration among these patches and the ones that contain only hosts (10h5hrem, 10host) were not significant. When the non hosts were removed (5h5nhrem) the wasps flew considerably less time compared to the patches in which the hosts were removed (5nh5hrem). Females flew significantly less compared to almost all the other treatments (except 5h5nhrem) in patches with only non hosts (10nh) (Table 3.1).

Searching durations were also found to be influenced by the type of the patch (Kruskal-Wallis test, $\chi^2=16.471$, $df=6$, $P=0.11$). Wasps spend a considerable amount of time searching in mixed herbivore population patches where the hosts were removed before the observation (5nh5hrem). This amount of time was significantly higher compared to the patches in which non hosts were removed before the observation (5h5nhrem) and to patches of relatively high number of non hosts (8nh2h, 10nh). In any case in patches of 10 non hosts wasps spend a dramatically lower amount of time for searching (Table 3.1).

Grooming was another time consuming behaviour in which differences among patches were statistically validated (Kruskal-Wallis test, $\chi^2=40.541$, $df=6$, $P<0.001$).

Females grooming activity was relatively longer in patches of 10h5hrem, 10hosts and 5h5nh. Yet, the differences were significant only compared to patches of low number or total absence of non hosts (8nh2h and 10nh respectively). Likewise searching, also grooming is by far less practiced in patches of 10 non hosts.

Although significant differences can be found among patches in the amount of time that females allocate for stopping (Kruskal-Wallis test, $\text{Chi}^2 = 12.718$, $\text{df} = 6$, $P = 0.048$) and walking (Kruskal-Wallis test, $\text{Chi}^2 = 66.135$, $\text{df} = 6$, $P < 0.001$) it is almost profound that wasps do not devote considerable part of their foraging time in both behaviours. What can be seen (Table 1), is that when wasps foraged in patches containing solely hosts they stop for a higher amount time and that in patches of just 2 hosts this time is almost negligible. Regarding walking duration, time spent for this behavior in patches where relatively to hosts high number of non hosts can be found (10nh, 8nh2h, 5nh5hrem) is rather unconsidered (Table 1).

Table 1. Mean observation times (\pm standard error) per behaviour¹

Patch	10h5hrem	10host	10nhost	5h5nh	5h5nhrem	5nh5hrem	8nh2h
Fly	403.57 ^{ab} (31.22)	391.54 ^{ab} (44.43)	223.49 ^c (33.11)	414.36 ^{ab} (41.84)	326.81 ^{bc} (42.29)	475.46 ^a (47'.37)	440.57 ^{ab} (51.47)
Search	432.81 ^{ab} (49.56)	450.57 ^{ab} (54.25)	44.36 ^c (9.94)	449.84 ^{ab} (46.73)	272.12 ^b (42.01)	608.65 ^a (75.35)	335.05 ^b (46.24)
Groom	490.51 ^a (68.10)	462.09 ^a (59.07)	24.09 ^c (9.01)	449.59 ^a (60.12)	364.98 ^{ab} (78.24)	357.23 ^{ab} (50.65)	302.71 ^b (48.13)
Stop	151.41 ^a (83.59)	105.36 ^{ab} (45.95)	18.47 ^{bc} (10.95)	20.64 ^{abc} (6.48)	19.69 ^{bc} (5.,78)	19.8 ^{bc} (8.15)	9.61 ^c (2.44)
Walk	66.53 ^{ab} (13.75)	63.16 ^{ab} (12.1)	4.02 ^c (1.81)	41.46 ^b (8.37)	97.97 ^a (20.81)	4.63 ^c (1.22)	10.31 ^c (4.55)

1) Means within a row followed by the same letter are not significantly different according to Mann-Whitney *U* test ($P < 0,05$)

3.1.4.2. Percentage time allocated per behaviour

In Fig. 3.4 percentagewise representation of time allocated per behaviour in different patches can be seen. Wasps fly by far more, compared to the other patches, when they search for hosts in a non host environment. In 10nhost patches approximately 72 % of their total time in a patch is spent on flying. Moreover, it seems that their tendency to fly decrease as number of hosts (presence and host by products) increase. So, they flew longer in 8h2nh compared to 5nh5hrem and 5h5nhrem. In turn, the latter ones gave longer flying activities compared to “clean environments” where only hosts can be found (10h, 10h5hrem); yet differences in this case were not so big (Fig 3.4).

In contrast to flying, searching occupies only a small proportion of wasps foraging time (13 %) in patches of only non hosts (10nh). The presence of hosts or their by products in a patch, even in a rather low analogy to non hosts, seems to trigger the searching activity of females. In the patch of 8nh2h the percentagewise time allocated to searching was more than twofold higher compared to the 10nh patch. Moreover in the 5nh5hrem patch (where hosts were removed before the observation; only their by-products could be found) searching was more intense compared to all the other patches concerning 39 % of the total time spent in a patch. In the rest of the patches, searching varied among 25-30 % of the total time (Fig 3.4).

Similarly to searching, wasps spent a low percentage of their time (6 %) to grooming in patches of only non hosts (10nh). In the same way (as with searching), also the presence of hosts and their by products seems to enhance cleaning activities. In patches of 8nh2h the time allocated to searching was around 20 % of the total time, whereas the longer percentages were evaluated approximately 28 % for patches of 10 hosts (Fig.3.4).

The rest of the behaviours, although differences existed among patches, received in general a considerably low amount of time proportionally to the total time spent in a patch (Fig. 3.4).

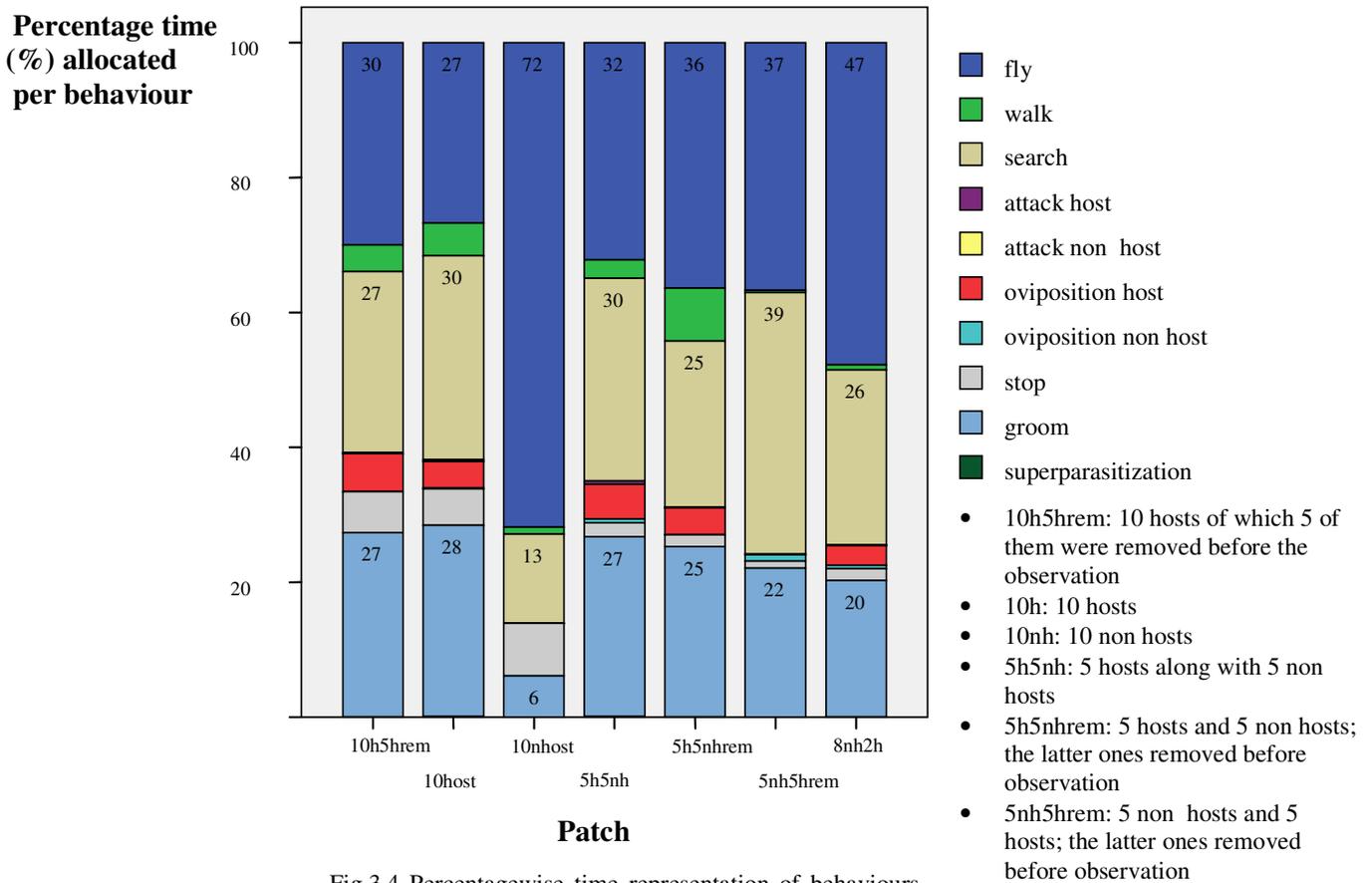


Fig.3.4 Percentagewise time representation of behaviours observed in different patches. Numbers in columns of different colours express % percentages of the total time spent in a patch.

3.2. Developmental study

3.2.1. Encapsulation rates as affected by different *Brassica* lines and larval developmental stage

Parasitoids' ability to encapsulate varied significantly among the different treatments. Responsible for this variation was the plant genotype ($\text{Chi}^2= 15.98$, $\text{df}=2$, $P=0.003$), the host instar ($\text{Chi}^2= 82.15$, $\text{df}=1$, $P<0.0001$) as well as the interaction between the latter ones ($\text{Chi}^2= 7$, $\text{df}=2$, $P=0.030$). 1st instar caterpillars managed to encapsulate a small proportion of the laid eggs that in the most extreme case (1st instar Cyrus) didn't exceed 10 % (Fig. 3.5). This was significantly higher only compared to the almost minimal encapsulation that could be found in the 1st instar Old Harry. In the 2nd instar encapsulation rates were considerably different among the *Brassica* lines. So, caterpillars fed on Cyrus encapsulated approximately 55 % of the parasitoid eggs, whereas in Old Harry and Kimmeridge levels of encapsulation reached in average 42 % and 21 % respectively. The significance of the interaction among *Brassica* lines and larval instars indicates that plant genotype didn't affect encapsulation similarly in the different instars. It can be seen (Fig 3.5), that although 2nd instar Old Harry larvae gave substantially higher encapsulation rates than 2nd instar Kimmeridge larvae (almost double), this was not the case in the first instar.

Including weight as a covariate suggested that a part of the variation found among treatments should be attributed to larval weight ($\text{Chi}^2= 27.64$, $\text{df}=1$, $P<0.0001$). Yet, the significant effects of plant genotype ($\text{Chi}^2= 9$, $\text{df}=2$, $P=0.0011$) and its interaction with larval instar could still be found ($\text{Chi}^2= 8.29$, $\text{df}=2$, $P=0.0159$) This didn't apply for the sole effect of larval instars, which after weight statistical inclusion, was found to be insignificant ($\text{Chi}^2= 3.32$, $\text{df}=1$, $P=0.0685$). From Fig 3.5 and 3.6, it can be seen that weights are not always correlated, with encapsulation rates. Particularly, 2nd instar Old Harry larvae weighted less than 2nd instar Kimmeridge but had, as was above referred, considerably higher encapsulation ability. However, the encapsulation superiority of 2nd instar Cyrus matched with quite well to its increased weight whereas the small differences in 1st instar larvae encapsulation were not observed in weights.

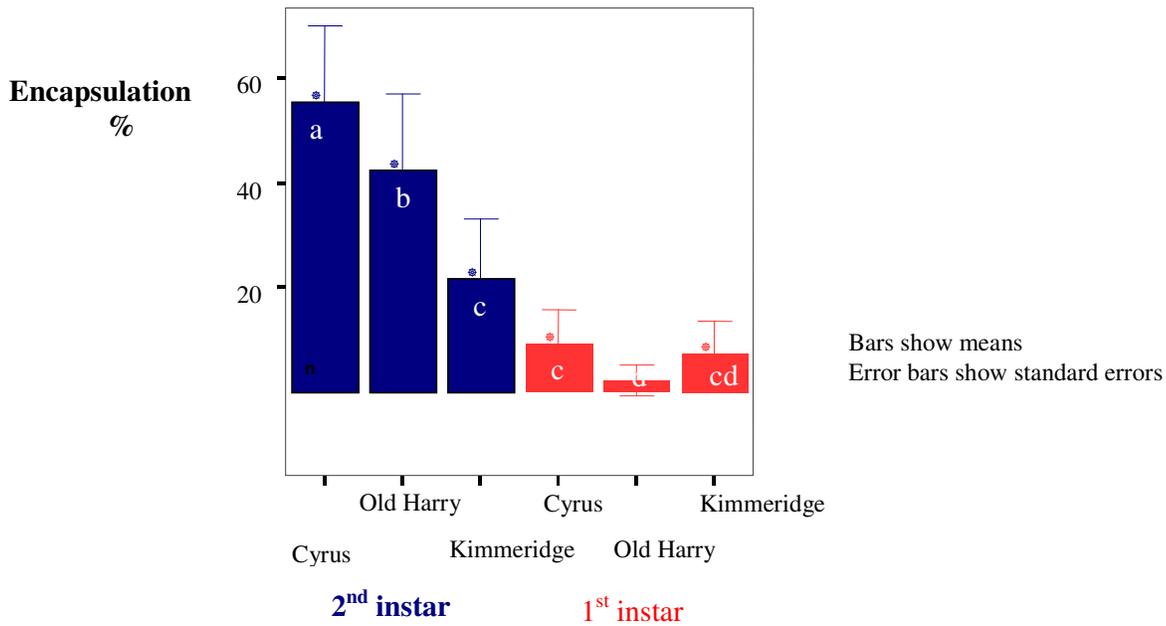


Fig. 3.5 Percentages of encapsulated *C. glomerata* eggs in 1st and 2nd instar *P. rapae* larvae on 3 different *Brassica* lines. Means followed by different letters are significantly different.

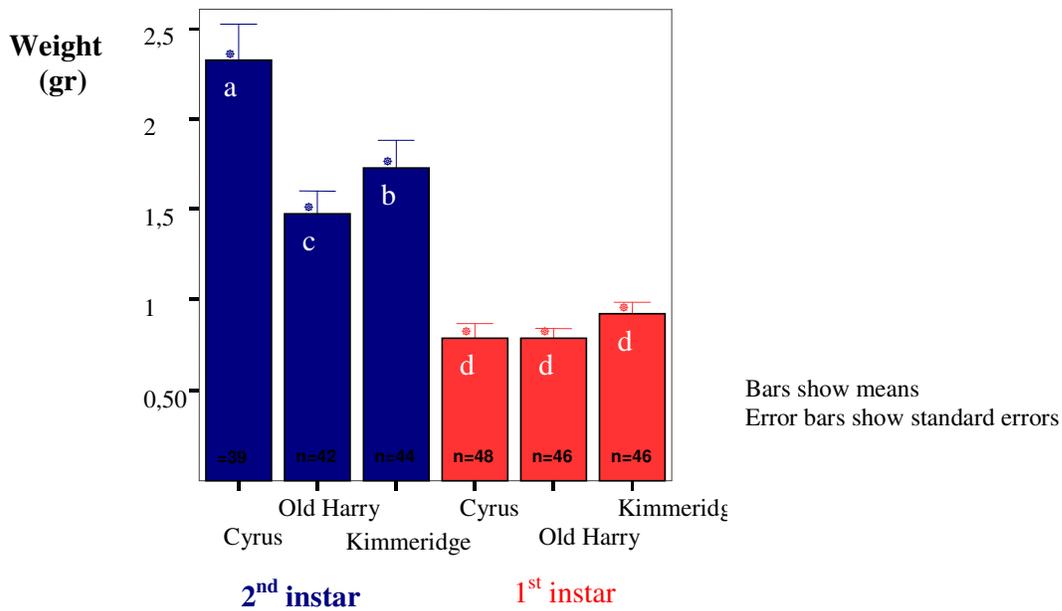


Fig 3.6 Average weights of parasitized 1st and 2nd *P. rapae* larvae on 3 different *Brassica* lines and induction of defences by *P. Brassicae*. Means followed by different letters are significantly different.

3.2.2. Encapsulation rates as affected by different *Brassica* lines and induced defenses.

Encapsulation rates of *C. glomerata* eggs by *P. rapae* larvae were different among treatments ($\text{Chi}^2 = 32.61$, $\text{df}=4$, $P < 0.0001$). Both for Old Harry and Cyrus, induced defenses lowered significantly the encapsulation potential (Fig. 3.5). However caterpillars fed on Cyrus managed to encapsulate a higher proportion of eggs compared to the rest of the treatments of the same always induced or not condition. Significant differences could not be found between induced and untreated Old Harry as well as between induced Kimmeridge and induced Old Harry (Fig. 3.5).

Further analysis (including weight as a covariate) showed that differences among treatments could partly at least be explained by larval weight ($\text{Chi}^2 = 34.27$, $\text{df}=1$, $P < 0.0001$) and its interaction with the different treatments ($\text{Chi}^2 = 13.12$, $\text{df}=4$, $P = 0.0107$). However, the effect of plant treatment on encapsulation rates remained significant ($\text{Chi}^2 = 17.31$, $\text{df}=4$, $P = 0.0017$). The significance found in the interaction between plant treatment and caterpillar weight suggested that larval weight did not influence encapsulation rates the same way in the different plant treatments. This can also be seen in the two figures (Fig. 3.5, Fig. 3.6). So, although weights were not strongly different between uninduced Cyrus and Old Harry and between control and induced Cyrus (Fig. 3.8.), encapsulation rates were significantly different (Fig. 3.7.). Moreover, when weight was included as a covariate, induced Cyrus gave the highest encapsulation compared almost to the rest of the treatments (except induced Kimmeridge) but significant differences among the other treatments were not statistically validated.

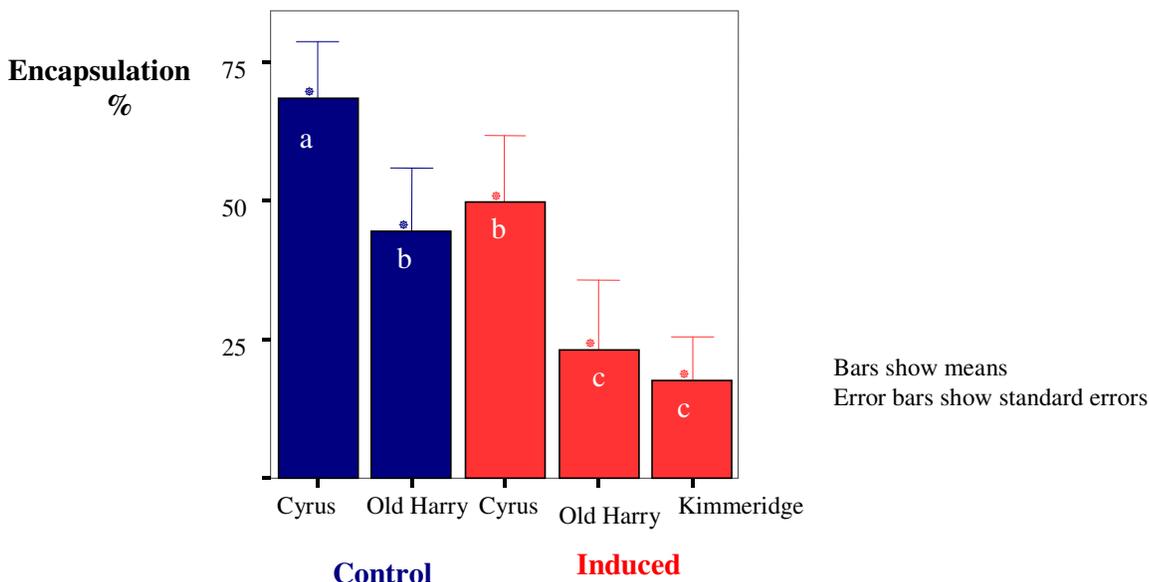


Fig. 3.7 Percentages of encapsulated *C. glomerata* eggs in *P. rapae* larvae on 3 different *Brassica* lines and induction of defences by *P. Brassicae*. Means followed by different letters are significantly different.

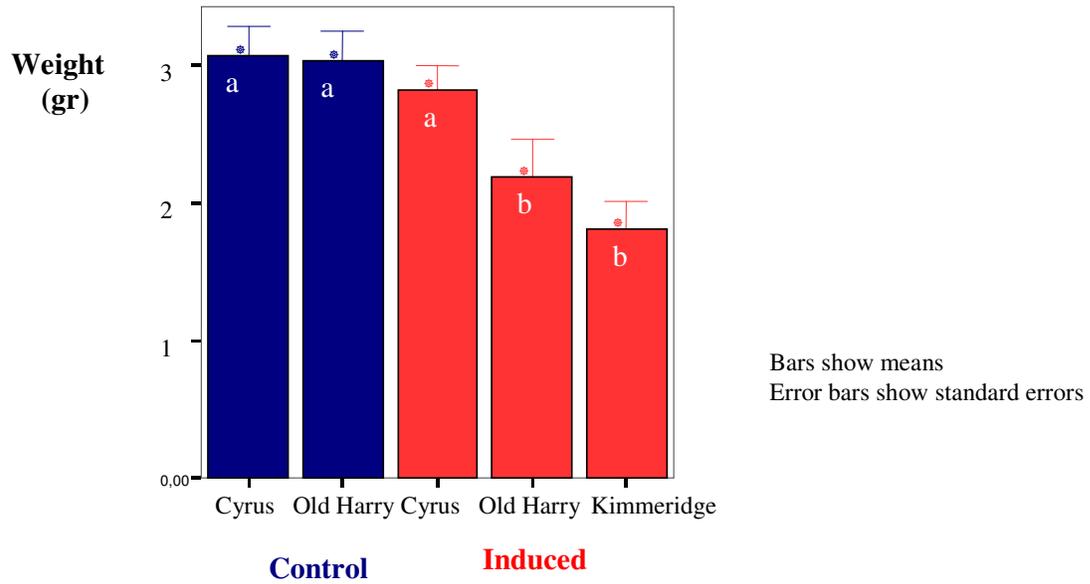


Fig 3.8 Average weights of parasitized *P. rapae* larvae on 3 different *Brassica* lines and induction of defences by *P. Brassicae*. Means followed by different letters are significantly different.

4. Discussion

4.1. Behavioural study

The aim of this research was to shed light on the way that densities and ratios of mixed herbivore populations affect the decisions of a foraging parasitoid. We found complex effects of presence and density of host and non-host related cues on parasitoid foraging behaviour.

C. glomerata left relatively fast upon arriving in a patch free of hosts or their by-products (feces, exuviae, silk) (Fig.3.3). In the rest of the cases (patches were at least host by-products could be found) differences in patch leaving tendencies among various patches were less profound. Although the highest number of hosts that could be found among the patches (10 hosts) increased wasps' tendency to stay in a patch, this effect was significantly different compared only with patches of just 2 hosts but not with patches of 5 hosts. Moreover, when hosts were removed before the observation (so only the host by products were available) the wasps didn't abandon the patch significantly earlier compared to patches where hosts were around (Fig.3.3), indicating a strong effect of host-related cues⁶ on the leaving tendencies of the parasitoid. This is not surprising; kairomones concentrations for example are used by parasitoids to adjust their leaving tendencies from host patches for optimal exploitation of host patches (Waage, 1979; Driessen, 1995). To what extent this effect of host/non host-related cues was ratio dependent cannot be easily deduced from the results of this experiment. It has been referred that different concentrations or ratios of the same compounds may lead to different expressions of a specific behaviour (Elkinton and Karde, 1984 cited in Wiskerke and Vet, 1994). This was not exactly the case in our experiment. As was above mentioned when 2 hosts were present a considerably longer stay was observed compared to the total absence of hosts. Yet, as 10 host patches had a similar effect as almost all the patches containing hosts or their by-products, staying is likely not to be a linear function of host-related cues. Therefore it can be speculated that it is not only concentration but simply the presence of host-related cues that makes already a large impact on parasitoid behaviour. Vos et al., 1998 referring to unpublished data, also on *C. glomerata*, observed that leaving tendencies among environments with a big variation of feeding damage in leaves (1 to 8 hosts) was not different.

Parasitoids showed different behaviours while searching in patches of varying herbivore composition and density (Fig 3.4). It was recorded that wasps shared their time in a patch mainly in flying, searching and grooming. In patches poor in hosts (or their by- products) females flew more and searched less. On the other hand parasitoids intensively searched for hosts in patches where only the host by products could be found (Fig 4). This is in accordance with what was previously stated on the dominant effect of host kairomones. Parasitoids in many cases are triggered by host cues even in the absence of host larvae (Ohara et al, 2003, Wang and Keller, 2002). Moreover,

⁶ In our experiment it was not possible to isolate cues directly related to herbivore from plant cues originating from herbivore damage. So, in terms of clarity when I explain the results of comparisons of the different chemical contexts I should refer to infochemical mixtures rather than herbivore kairomones. However, the reviewed literature (Lewis and Martin, 1990, Vet and Dicke, 1992) as well as the results of our experiments clearly showed the dominant role of kairomones over such short distances and for the same plant species. Therefore the use of the term host/non host related cues instead of infochemical mixtures is considered not to act misleadingly.

the physical presence of non hosts did not reduce searching times. In fact, as with total resident times, removing non hosts (5 hosts-5 non hosts removed) decreased time spent in searching compared to the patch in which hosts were removed (5 non hosts - 5 hosts removed) (Fig. 3.4). It seems that these reproductive foragers are irritated when the expected reward is missing and searching is prolonged.

Regarding the magnitude of patch depletion within the different patches it can be concluded that patch exploitation is not related to host density. Wasps in any of the studied patches did not parasitize more than half of the available hosts, irrespectively of the wide range of this availability (2- 10 hosts) (Fig.3.1). Presence of non hosts did not negatively influence the foraging efficiency of the females. *C. glomerata* was able to find hosts even in patches with a low proportion of hosts (2 hosts-8 non hosts), and in some of the cases that she was prompt to stay and search these relatively poor patches (there were cases that she left fast without any oviposition; personal observation) she managed to parasitize all of the available hosts. In fact it can be speculated that the physical presence of non hosts enhanced in a way parasitism activity, as although patches of 10 hosts-5 hosts removed didn't led to significantly higher parasitism rates to compared to patches of 5 hosts-5 non hosts, this was true when they were compared to patches of 5 hosts - 5 non hosts removed. However, as the differences between the two last ones (5 hosts-5 non hosts, 5 hosts-5 non hosts removed) are very small it might be risky to suggest a positive effect of non hosts' presence on parasitism. Underexploitation, mainly of high density patches, has been frequently reported in researches conducted both in the lab and the field (Waalde and Murdoch, 1988; Matsumoto et al, 2004). This kind of density independent parasitism is regarded to be the result of numerous factors concerning parasitoids idiosyncrasy such as limitation in eggs and time, physiological characteristics, gregariousness (Costamagna, 2004), interference with their competitors (Visser et al, 1999), risks imposed by their natural enemies (Strong, 1989) and mortality risks for their offspring (Weisser et al., 1994).

Including in our analysis the frequency of observed non host parasitism provided some useful understanding on how the patch quality is assessed and exploited by *C. glomerata*. It was observed that when hosts and their by products were totally absent, non host parasitism did not occur. Females parasitized non hosts more frequently in patches where hosts were removed before the observation (5 non hosts-5 hosts removed) compared to patches of 2 hosts and 8 non hosts but not to patches of equal presence of hosts and non hosts (5 hosts-5 non hosts) (Fig. 3.2).It may be suggested that wrong oviposition decisions are related to the strength of host chemical stimuli and not with the actual physical presence of hosts. The specific parasitoid in previous researches was found willing (after being excited by feces of its host) to oviposit in many "unusual" hosts (among them was also *Mamestra Brassicae*) although if this would mean a waste of its laid eggs (Kitano, 1968 cited in Sato and Ohsaki 2004). Moreover, emphasis should be given on the fact that the used wasps were inexperienced. *C. glomerata* has been described to have an adaptive foraging strategy based on the attained in the patch experience (Wiskerke and Vet, 1994; Vos et al, 1998). This strategy involves risks and the subsequent mistakes, based on which strategy is regarded to be improved.

Several mechanistic explanations of parasitoids' foraging behaviour have been proposed. Validating any of them through the findings of our experiment was not part of the aims of our research and therefore the way our data are analyzed do not support

this procedure (a proportional hazard model⁷ would be more suitable). However a brief description of the central ideas of these mechanistic views and to what extent are consistent to our results will be attempted. Waage (1979) proposed a patch exploitation mechanism which connected the effect of host density and oviposition. Parasitoid when they enter a patch have a basic tendency to stay (responsiveness) based on kairomones intensity (host density related). As time proceeds this tendency decreases but as it is above a certain threshold parasitoids that reach the end a patch come back. According to Waage (1979), events like ovipositions increase parasitoids' responsiveness (incremental mechanism). Driessen (1995) on the other hand suggested another approach on the effect of ovipositions; parasitoids after the initial assessment of host population, their responsiveness decrease after each oviposition. Vos et al. (1998) proposed that each of these mechanisms could be adaptive depending on the heterogeneity of host distribution in the patches. So, in a uniform environment it is expected that a count down mechanism would perform better, whereas incremental mechanisms would be more adaptive in habitats with variable patch quality. However this means that parasitoids are well informed on the availability both in terms of quantity and quality of hosts in the habitat where they forage. Taking into account the informational constraints that parasitoids face within or between patches Vos et al. (1998) suggested an "adjustable termination rate" model in which the females dynamically adjust their decisions based on "bad" or "good" experiences. In our experiment patches in which ovipositions did not occur at all but kairomones concentration was set high enough (5 non hosts-5 hosts removed) parasitoids stayed almost equal times compared to patches of similar kairomone concentration but in which oviposition took place (5 hosts-5 non host removed) (Fig.2., Fig.4.) indicating in this case a non incremental mechanism. On the other hand, there wasn't clear evidence that ovipositions enhanced patch leaving decisions as the 5 hosts-5 non hosts removed had approximately the same percentage of parasitism and not significantly higher patch staying compared to 2 hosts- 8 non hosts (Fig.2. Fig.4.). Probably, considering the polyphagous character of *C.glomerata* (Laing and Levin, 1982), its adapting foraging behaviour as shown by previous researches (Wiskerke and Vet, 1994; Vos et al, 1998) and the complexity of our results an adjustable strategy rather a simple model should be followed by this parasitoid.

Trying to understand what is inside the mind of a parasitoid is more than ambitious. In our experiment different densities and ratios of hosts and non hosts in different patches were offered for exploration to individual females. Certain behavioural trends were observed. Host related cues stimulated the research of the foragers resulting in significant time losses (egg waste also due to non host parasitism) in patches where hosts were removed before the observation. However, foraging efficiency (as can be assessed by percentage of parasitism) was not considerably influenced by densities or ratios. Underexploitation was observed in all the type of patches whereas the presence of non hosts didn't reduce parasitism rates. A specific mechanism, based on which *C.glomerata* takes her decisions, was not evident. Ovipositions did not necessarily increased parasitoids' tendency to stay. And wasps' residence times in patches of similar chemical characteristics (kairomones concentrations irrespectively of hosts' presence) did not differ significantly. Yet, on the other hand ovipositions did not enhance patch leaving decisions. Probably more experiments will be necessary to

⁷ Proportion hazard models are regression models used mainly in medical and biological sciences to model the effect of different covariates on a hazard rate (e.g. survival).

elucidate behaviours and come closer to mechanisms; if they are any. In any case future experimental work on the effect of mixed herbivore populations should also include the influence of parasitoids' previous experiences as well as field work.

4.2 Developmental study

The results of the developmental study showed a clear effect of genotypic and phenotypic variation within *Brassicae oleracea* and host age on the ability of *Pieris rapae* to encapsulate eggs of *Cotesia glomerata*.

Wild *Brassica* lines negatively influenced the encapsulation rate of *P. rapae* (Fig. 3.5, Fig. 3.7) which was even more reduced when defenses were induced by previous feeding of another herbivore (Fig. 3.7). Caterpillar age, had an influence on encapsulation rates as lower encapsulation percentages were measured in younger than in older instars. Specifically, first instars were in all *Brassica* lines rather inefficient to encapsulate the eggs of their enemy (Fig. 3.5). However, their efficiency significantly increased in the second instar and if we take into account the results of the second experiment (only third instars were tested) encapsulation was even more enhanced in the third instars (Fig. 3.7). Moreover, based on the results of the first experiment it can be referred that encapsulation was not necessarily positively correlated with larval weight (only in Cyrus) (Fig. 3.5, Fig. 3.6). 2nd instar Old Harry caterpillars had lower weights than 2nd instar Kimmeridge ones but encapsulation rates were twofold higher in the first ones.

Caterpillars' weights were unfavorably affected by the high in glucosinolate levels *Brassica* lines and by the induced condition as was recorded in the second experiment (Fig. 3.8). Yet, according to the obtained results only part of the reduced encapsulation rates can be attributed to the poor condition (as expressed by low weight) of larvae. The weight of untreated Old Harry caterpillars in average didn't significantly differed from the average weight of untreated Cyrus caterpillars whereas the encapsulation rates were significantly lower in Old Harry (Fig. 3.7, Fig. 3.8). The same phenomenon was observed between the untreated and the induced Cyrus caterpillars in which encapsulation rates were significantly lower in the second case but average weights were almost the same (Fig. 3.7, Fig. 3.8). The above, considering also the described in the first experiment lack of correlation between larval weight and encapsulation rates, suggests that Brassicas chemistry apart from an indirect effect (reduced development- suitability of herbivore as host), had also a direct effect on the immune system of the herbivore by weakening its ability to support an encapsulation reaction.

Benrey and Denno (1997) reported that encapsulation rates of *C. glomerata* eggs by *P. rapae* larvae were lower in plant species where caterpillars were rapidly grown. They concluded that factors that induce in herbivores a stressful condition (e.g. starvation, sequestration of toxic compounds) reduce larval vigour and through this effect their encapsulation ability is degraded. In our research plant chemistry affected encapsulation also in a direct way. This seems to be connected to several ecological implications. Depressed larval growth due to plant defenses may facilitate on the one hand parasitoids' eggs to escape encapsulations due to weak immune responses of the hosts but on the other hand leads to reduced mass and nutrient value of herbivore's tissue available for the immature parasitoid. Moreover it has been suggested that low nutritional quality or presence of toxic allelochemicals in plant tissues may increase the vulnerability period (time that is exposed to natural enemies) of caterpillars by prolonging their development (Clancy and Price, 1987); of course this would also

apply for the parasitoid's offspring. So plants defending themselves from herbivores may harm their mutualists. If plant defenses could directly target their enemy without imposing risks for the parasitoids, than their defensive potential would be significantly enhanced. In the second experiment, 3rd instar caterpillars grown on the untreated Old Harry line gave compared to untreated Cyrus lower encapsulation rates without their development being considerably affected (results for untreated Kimmeridge were not available). In turn, in the first experiment, Kimmeridge inhibited significantly the encapsulation potential of 2nd instar caterpillars compared to Old Harry, whereas the weights of caterpillars fed on Kimmeridge were higher. It should be taken into consideration that wild populations embody a substantial level of genetic variation, so it would be risky to combine results of different experiments especially when they refer to different instars. However, what is important is that irrespectively of the complexity of the results and the several possible implications that could be hidden within them, wild *Brassica* lines seem to be of great ecological interest as they manage efficiently to suppress the encapsulation process without necessarily impeding herbivores' development.

Secondary metabolism in Brassicas refers mainly to glucosinolates and their breakdown products. As was already referred in the introductory part, glucosinolate levels vary considerably among wild and cultivated Brassicas (Rosa E.A.S., 1999). This could be attributed to a domestication process. Agronomic selection on characteristics related to higher growth and yields may affect the resistance of plants against pests and pathogens (Lindig-Cisneros et al. 1997). Specifically for *Brassica rapa*, Siemens and Mitchell-olds (1998) reported a negative correlation between genetic increase in myrosinase (enzymes that hydrolyze glucosinolates into active toxic compounds) production and estimated seed production. However, it would be expected for a specialist in Brassicas like *P. rapae* to have evolved a more adaptive immune system to its host armory. A possible explanation for the severe effect of plant chemistry on the immune potential of *P. rapae* is that as with plants defenses also their enemies' protection includes costs. Sequestering toxic metabolites by herbivores is a physiologically expensive procedure (Bowers and Collinge, 1992) that may reallocate energetic sources used for encapsulation. Evidence of this trade-off has been demonstrated for *P. brassicae* (Freitak et al., 2003).

In our study apart from a constitutive effect of plant defenses a significant effect of induced responses has also been verified both for domesticated and wild *Brassicas*. Plant defenses may be enhanced as a result of previous herbivore damage (Karban and Myers, 1989) and the ability of species to induce defenses is not necessarily negatively correlated with the levels of constitutive defenses (Siemens and Mitchell-olds, 1998). What is intriguing in our case is the magnitude of the induction effect on the encapsulation ability of herbivores. So, the "wild" Old Harry led to significantly lower encapsulation rates compared to its cultivated relative Cyrus; yet, this effect was even greater when defenses were induced by feeding of another herbivore. The above results indicate that herbivore community structure may considerably be affected by other forms of competition than the direct competition for resources. A small amount of herbivores that may never directly interfere (feed on the same plant parts) with the focal herbivore species (induced responses are systemic throughout the plant) can trigger plant defensive mechanisms strong enough to affect its physiology suggesting an indirect population regulatory mechanism (Agrawal, 2005).

5. Synthesis

A community within an ecosystem forms a complex web of interactions among species, influencing each other through competitive relationships (direct or indirect), parasitism and predation, behavioural interference and the subsequent changes of their interplay arena (Levins and Wilson, 1980). Understanding the factors that influence the dynamics of multitrophic interactions is a promising field both for fundamental research and agriculture. Natural processes and biological interactions can be enhanced through ecologically relevant knowledge by optimization of the synergies among organisms and by the obtainment of the proper balance between biotic and abiotic factors (Altieri and Nicholls, 2005).

Our research aimed on gaining knowledge on the processes that shape the defensive potential of plants. We chose to study two different attributes of ecological interactions: the behaviour of adult parasitic wasps and the development of wasps' immature offspring, occurring within different tritrophic contexts. Despite the constrained framework of our research (study of dissected relationships, greenhouse experiments, short term observations) the performance of the involved organisms was of great interest. We observed indirect effects to travel through cascading direct ones (induced defenses by feeding of a herbivore, through their effect on plant quality, to facilitate egg parasitoids to escape the immune traps of another herbivore, although these potential food competitors never met its other). Moreover, chemical interfering of different herbivore populations to alienate the behaviour of their non shared enemy (excited inexperienced parasitoids in the presence of host related cues were willing to parasitize non hosts whereas the intensity of this chemical effect was strong enough to trigger intense searching even in the physical absence of hosts). And if we could have a dynamic look in the past, we would observe man endeavor through ages to evolve Brassicas into their modern cultivated form, which in turn reduced the changes of plants mutualists to escape the defense of herbivores as was recorded in our experiment.

As noted by Callicott (1988) organisms are the product of their dialectic relationships with their surroundings; "they are what they are because of the complex concentration of relationships to which they are adapted". This is a very important statement that should be seriously taken into account when an efficient and an ecologically sound control of our food competitors is the case. Studies in multitrophic interactions embody the potential to unravel ecological mechanisms and in turn to translate them into principles that will make possible the appropriate in a space (local) and time scale technologies to be applied (Altieri, 2004). Coming back to the results of our research, it is possible to have an example. Brassicas have been the result of complex evolutionary processes, involving both environmental and socio-economical factors. Agronomic selection for high productivity affected plant defenses, as expressed by the concentrations of glucosinolates. Selecting and testing locally adapted resistant cultivars is possible to reduce the efficiency of herbivores. Metabolites such as glucosinolates had a negative effect on the immune ability of caterpillars (as recorded in the experiment) whereas it is known to act as attractants for natural enemies and feeding deterrents for not specialist herbivores (Raybould and Moyes, 2001). In our case wild *Brassica* lines, had interesting combinations of advantages as caterpillars' growth wasn't considerably reduced in some of the treatments, whereas encapsulation rates were almost always substantially lower. Moreover, cultivating multiple varieties or intercropping can delay the onset of pests and inhibit epidemics by establishing unfavorable conditions mainly for the specialist herbivores and have been reported to

enhance the natural enemy (mainly generalist) activities (Altieri, 2004; Vandermeer, 1989). In our experiment *C. glomerata* was quite efficient to find its hosts in heterogeneous environments in terms of distributions and densities of their enemies. Clear evidence that this efficiency is maintained in diverse agroecosystems does not exist; however it is speculated that multiple experiences will help the parasitoid to overcome the foraging constraints that polycultures impose (Perfecto and Vet, 2003). Going one step further maybe is time to re-assess our offensive behaviour against our potential food competitors. We observed that even in limited artificial tritrophic contexts stabilizing forces existed. Constitutive defenses attracted parasitoids and strongly influenced the development of their offspring, induced defenses affected by multitrophic interactions functioned quite well whereas the adult parasitoids had their own view on exploitation of their resources that didn't necessarily fit in any mechanistic human perception. If we could imagine our presence (as managers) in a more complex system as an agroecosystem and the dramatic changes that our direct actions against herbivores (pesticides, augmentative releases of enemies) may impose to its balance, than at least it would be better to wisely organize our steps before we move forward. Interestingly, there are farmers in our days that do not consider every living herbivorous arthropod in their cultivation as a pest and by applying sophisticated primarily preventative agricultural practices have managed to sustain themselves and the wealth of their ecosystem (Altieri, 2004). These people are the best examples of how important is to have an understanding of ecological interrelationships and to be able to find your role within them.

References

- Agrawal, A.A. (2005). Future directions in the study of induced plant responses to herbivory. *Entomologia Experimentalis et Applicata* 115: 97-105.
- Altieri M.A. (2004). Linking ecologists and traditional farmers in the search for sustainable agriculture. *Frontiers in Ecology and Environment* 2: 35-42.
- Altieri M.A. and C. I. Nicholls (2005). *Agroecology and the Search for a Truly Sustainable Agriculture*. Mexico: United Nations Environment Programme. <http://www.agroeco.org/doc/agroecology-engl-PNUMA.pdf>
- Altieri, M.A., P.B.Martin, Lewis, W.J., 1983. A quest for ecologically based pest management systems. *Environmental Management* 7: 91-100.
- Benrey, B. and R.F. Denno (1997). The slow growth-high mortality hypothesis: A test using the Cabbage Butterfly. *Ecology*, 78: 987-999.
- Bowers, D.M. and S.K. Collinge (1992). Fate of iridoid glycosides in different life stages of the Buckeye, *Junonia coenia* (Lepidoptera: Nymphalidae). *Journal of chemical ecology*. 18: 817-831
- Brodeur, J., and L. E. M. Vet (1995). Relationships between parasitoid and host range and host defense: A comparative study of egg encapsulation in two related parasitoid species. *Physiological Entomology* 20:7-12.
- Brodeur J. and J.B.F. Geervliet (1992). Host species affecting the performance of the larval parasitoids *Cotesia Glomerata* and *C. rubecula* (Hymenoptera: Braconidae). I. Preference for host developmental stage of *Pieris* (Lepidoptera: Pieridae). *Medelingen Faculteit Landbouwwet Rijksuniversiteit (Gent)* 57:543-545.
- Callicott J.B. (1988). Agroecology in Context. *Journal of Agricultural Ethics* 1: 3-9.
- Cheng, L. 1970. Timing of attack of *Lypha dubia* Fall (Diptera:Tachinidae) on the winter moth, *Operophtera brumata* (L.) (Lepidoptera:Geometridae) as a factor affecting parasite success. In: Barbosa P., J.A. Saunders and M. Waldvogel (1982). Plant mediated variation in herbivore suitability and parasitoid fitness. *Proceedings of 5th International Symposium on Insect-Plant Relationships*. Pudoc, Wageningen, The Netherlands, pp. 63-71.
- Chew F.S. Biological effects of glucosinolates. In: *Biologically active natural products: potential use in agriculture*. Cutler H.G. (ed), American Chemical Society, Washington, pp. 155-181.
- Clancy K. M., P. W. Price (1987). Rapid herbivore growth enhances enemy attack: sublethal plant defenses remain a paradox. *Ecology*. 68:736-738.
- Costamagna, A.C., F.D. Menalled and D.A. Landis. 2004. Host density influences parasitism of the armyworm *Pseudaletia unipuncta* in agricultural landscapes. *Basic and Applied Ecology*. 5: 337-355.
- De Moraes M., W.J. Lewis and J.H. Tumilson (2000). Examining plant-parasitoid interactions in tritrophic systems. *Anais da Sociedade Entomológica do Brasil* 29:189-203.
- Denno R.F., L. Danny and C. Gratton (2005). Spatial variation in the relative strength of top-down and bottom-up forces: causes and consequences for phytophagous insect populations. *Annual Zoology Fennici* 42: 295-311.
- Dicke M. and M.W. Sabelis (1988). How plants obtain predatory mites as bodyguards. *Netherlands Journals of Zoology* 38: 148-165.
- Driessen G., C. Bernstein, J.J.M. Van Alphen and A. Kacelnik (1995). A count down mechanism for host search in the parasitoid *Venturia canescens*. *Journal of Animal Ecology* 64: 117-125

- Eggleton, P., K.J.Gaston (1990). 'Parasitoid' species and assemblages: convenient definitions or misleading compromises? *Oikos* 59: 417-421.
- Elkinton J.S. and R.T. Cardé (1984). Odor dispersion. In: Wiskerke J.S.C., L.E.M. Vet (1994). Foraging for solitarily and gregariously feeding caterpillars: a comparison of two related parasitoid species (Hymenoptera: Braconidae). *Journal of Insect Behaviour* 7:585-603.
- Finch S.and A.R.Thompson (1992). Pests of cruciferous crops.In: Vegetable crops pests. McKinlay R.G (ed), MacMillan Academic and Professional Ltd, London, pp. 87-138.
- Freitak D., I. Ots, A. Vanatoa and P. Hõrak (2003) Immune response is energetically costly in white cabbage butterfly pupae. *Proceedings of the Royal Society of London, Series B Biological Sciences (Supplement)* 270: 220–222.
- Godfray, H.C.J. (1994). *Parasitoids: Behavioural and evolutionary ecology*. Princeton university press, Princeton, 475 p.
- Godfray H.C.J, M.Shimada (1999). Parasitoids: a model system to answer questions in behavioural, evolutionary and population ecology. *Researches on Population Ecology* 41: 3-10.
- Gómez-Campo C. and S. Prakash (1999).Origin and domestication. In: *In Biology of Brassica Coenospecies* . C. Gómez- Campo (ed). Elsevier Science B.V., Amsterdam, The Netherlands, pp. 33-58.
- Gripenberg, S and T. Roslin (2006). Up or down in space? Uniting the bottom-up versus top-down paradigm and spatial ecology. *Oikos*, 116:181–188.
- Harvey JA, N.M. van Dam and R. Gols 2003. Interactions over four trophic levels: foodplant quality affects development of a hyperparasitoid as mediated through a herbivore and its primary parasitoid. *Journal of Animal Ecology* 72: 520–530.
- Hunter M.D.(2003). Effects of plant quality on the population ecology of parasitoids. *Agricultural and Forest Entomology* 5:1-8.
- Hunter, M. D., and P. W. Price (1992). Playing chutes and ladders: heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. *Ecology* 73: 724-732.
- Karban, R., and J. H. Myers 1989. Induced plant responses to herbivory. *Annual Review of Ecology and Systematics* 20:331-348.
- Kessler A. and I.T. Baldwin (2001). Defensive Function of Herbivore-Induced Plant Volatile Emissions in Nature. *Science* 16:2141-2144.
- Kester, KM and P Barbosa. 1991. Behavioral and ecological constraints imposed by plants on insect parasitoids: Implications for biological control. *Biological Control: Theory and Applications* 1:94-106.
- Kitano H. (1968). The fate of a braconid parasitoid, *Apanteles glomeratus* L. in ten species of unusual hosts. In: Sato Y. and N. Ohsaki (2003). Response of the wasp (*Cotesia glomerata*) to larvae of the large white butterfly (*Pieris brassicae*). *Ecological Research* 19: 455-449
- Laing J.E. and D.B. Levin (1982). A review of the biology and a bibliography of *Apanteles glomeratus* (L.) (Hymenoptera: Braconidae). *Biocontrol News and Information* 3:7-23.
- Larsen P. O. 1981. Glucosinolates. In: Seimens, D.H. and T. Mitchell-olds (1998). Evolution of pest induced defenses in *Brassica* plants: Tests of theory. *Ecology* 79: 632-646.
- Levins R.and M. Wilson (1980). Ecological theory and pest management. *Annual Review of Entomology*. 25: 287-308.

- Lindig-Cisneros R., B. Benrey and F.J. Espinosa-Garcia (1997). Phytoalexins, resistance traits and domestication status in *Phaseolus coccineus* and *Phaseolus lunatus*. *Journal of Chemical Ecology* 23:1997-2011
- Loon, J.J.A. van and L.M. Schoonhoven (1999). Specialist deterrent chemoreceptors enable *Pieris* caterpillars to discriminate between chemically different deterrents. *Entomologia experimentalis et applicata* 91: 29-35.
- Matsumoto T., T. Itioka, T. Nishida and T. Inoue (2004). A test of temporal and spatial density dependence in the parasitism rates of introduced parasitoids on host, the arrowhead scale (*Unaspis yanonensis*) in stable host-parasitoids system. *Journal of Applied Entomology* 128: 267-272
- Ohara Y., A. Takafuji and J. Takabayashi (2003). Factors affecting the patch-leaving decision of the parasitic wasp *Diadegma semiclausum* (Hymenoptera: Ichneumonidae). *Applied Entomology and Zoology* 38:211-214.
- Paoletti M.G.P. and D. Pimentel (2000). Environmental Risks of Pesticides versus Genetic Engineering for Agricultural Pest Control. *Journal of Agricultural and Environmental Ethics* 12: 279-303.
- Perfecto I. and L.E.M. Vet (2003). Effect of a Nonhost Plant on the Location Behavior of Two Parasitoids: The Tritrophic System of *Cotesia* spp. (Hymenoptera: Braconidae), *Pieris rapae* (Lepidoptera: Pieridae), and *Brassica oleraceae*. *Environmental Entomology* 32:163-174.
- Phipps P.M. (1990). Control of *Cylindrocladium* Black Rot of peanut with soil fumigants having isothiocyanate as the active ingredient. *Plant Disease* 74:438-441.
- Porter J., 1997. Description of species (I). In: *The colour identification guide to caterpillars of the British isles (Macrolepidoptera)*. Viking Press, London, England, pp. 1-156
- Potter M., V. Vanstone, K. Davies, J. Kirkegaard and A. Rathjen (1999). Reduced susceptibility of *Brassica napus* to *Pratylenchus neglectus* in plants with elevated root concentrations of 2-phenylethyl glucosinolate. *Journal of Nematology* 31:291-298
- Price P.W., C.E. Bouton, P. Gross, B.A. McPherson, J. N. Thompson, A.E. Weis (1980). Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annual Review of Ecological Systems* 11: 41-65.
- Raybould A.F. and C.L. Moyes (2001). The ecological genetics of aliphatic glucosinolates. *Heredity* 87:383-391.
- Renwick, J.A.A., C.D. Radke, K. Sachdev-Gupta and E. Stadler (1992). Leaf surface chemicals stimulating oviposition by *Pieris rapae* (Lepidoptera: Pieridae) on cabbage. *Chemoecology* 3:33-38.
- Rosa E.A.S (1999). Chemical composition. In *Biology of Brassica Coenospecies*. C. Gómez-Campo (ed). Elsevier Science B.V., Amsterdam, The Netherlands, pp. 315-357.
- Rosenheim, J. A. 1999. The relative contributions of time and eggs to the cost of reproduction. *Evolution* 53: 376-385.
- Rosenthal J.P. and R. Dirzo (1997). Effects of life history, domestication and agronomic selection on plant defence against insects: Evidence from maize and wild relatives. *Evolutionary Ecology* 11:337-355.
- Seimens, D.H. and T. Mitchell-Olds (1998). Evolution of pest induced defenses in *Brassica* plants: Tests of theory. *Ecology* 79: 632-646.

- Steinberg S., M. Dicke, L.E.M. Vet, Wanninger R. (1992). Response of the braconid parasitoid *Cotesia* (= *Apanteles*) *glomerata* to volatile infochemicals effects of bioassay set-up, parasitoid age and experience and barometric flux. *Entomologia Experimentalis et Applicata* 63:163–175.
- Steinberg S., M. Dicke and L.E.M. Vet (1993). Relative importance of infochemicals from first and second trophic level in long-range hosts location by the larval parasitoid *Cotesia glomerata*. *Journal of Chemical Ecology* 19: 47-59.
- Strong D. R. Density independence in space and inconsistent temporal relationships for host mortality caused by a fairyfly parasitoid. *Journal of Animal Ecology* 1989. 58:1065–1076.
- Turlings T. and B. Benrey (1998). Effects of plant metabolites on the behaviour and development of parasitic wasps. *Ecoscience* 5:321–333.
- UNEP, *Global Environmental Outlook* (United Nation Environmental Programme, Nairobi, Kenya, 1997). In Paoletti M.G.P. and D.Pimentel (2000). Environmental Risks of Pesticides versus Genetic Engineering for Agricultural Pest Control. *Journal of Agricultural and Environmental Ethics* 12: 279-303.
- Vandermeer J. 1989. The ecology of intercropping. Cambridge, UK: Cambridge University Press.
- van Veen F.J.F., P.D. van Holland and H.C.J. Godfray (2005). Stable coexistence in insect communities due to density and trait mediated indirect effects *Ecology* 86:3182- 3189.
- van Alphen JJM, C. Bernstein, G.Driessen (2003). Information acquisition and time allocation in insect parasitoids. *Trends in Ecology and Evolution* 18:81–87.
- Van Dam N.M., L. Witjes, A. Svatos (2004). Interactions between aboveground and belowground induction of glucosinolates in two wild *Brassica* species. *New Phytologist* 161:801-810.
- Vet, L.E.M. and M. Dicke (1992). Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology* 37: 141-172.
- Vinson, S. B. and G. F. Iwantsch (1980). Host regulation by insect parasitoids. *Quarterly Review of Biology* 55: 143-165.
- Visser M.E., T.H. Jones and J. Driessen (1999). Interference among insect parasitoids: a multi patch experiment. *Journal of Animal Ecology* 68: 108-120.
- Vos M., L. Hemerik and L.E.M. Vet (1998). Patch exploitation by the parasitoids *Cotesia rubecula* and *Cotesia glomerata* in multi-patch environments with different host distributions. *Journal of Animal Ecology* 67:774-783.
- Vos M., S. Moreno Berrocal, F. Karamaouna, L. Hemerik, L.E.M. Vet (2001). Plant-mediated indirect effects and the persistence of parasitoid-herbivore communities. *Ecology Letters* 4:38-45.
- Waage J. (1979). Foraging for patchily distributed hosts by the parasitoid, *Nemeritis canescens* *Journal of Animal Ecology* 48:353–371.
- Wang X.G. and M.A. Keller (2002). A comparison of the host-searching efficiency of two larval parasitoids of *Plutella xylostella*. *Ecological Entomology* 27: 105-114.
- Walde S. J. and W.W. Murdoch (1988). Spatial density dependence in parasitoids. *Annual Review of Entomology* 1988. 33:441–466.
- Weisser W.W., A.I. Houston and W. Volkl (1994). Foraging strategies in solitary parasitoids: The trade-off between female and offspring mortality risks. *Evolutionary Ecology* 8:587-597.

Wiskerke J.S.C., L.E.M. Vet (1994). Foraging for solitarily and gregariously feeding caterpillars: a comparison of two related parasitoid species (Hymenoptera: Braconidae). *Journal of Insect Behaviour* 7:585-603.

Appendix: Statistical Results

A) Behavioural study

10h5hrem: 10 hosts of which 5 of them were removed before the observation

10h: 10 hosts

10nh: 10 non hosts

5h5nh: 5 hosts along with 5 non hosts

5h5nhrem: 5 hosts and 5 non hosts; the latter ones removed before observation

5nh5hrem: 5 non hosts and 5 hosts; the latter ones removed before observation

8h2nh: 8 hosts along with 2 non hosts

1) Percentage of parasitism

The SAS System- Genmod Procedure

LR Statistics For Type 3 Analysis

Source	NumDF	Den DF	Fvalue	Pr > F	Chi-Square	Pr > ChiSq
Treatment	5	153	2.24	0.0527	11.22	0.0472

SAS- Parasitism

Differences of Least Square Means

	1)10h5hrem	2)10h	3) 5h5nh	4) 5h5nhrem
2) 10h	0.0063*			
3) 5h5nh	0.4293	0,0273*		
4) 5h5nhrem	0.0404*	0.9631	2.35	
5) 8h2nh	0.1971	0.41	0.59	0.26

2) Frequency of non host oviposition.

The SPSS statistical programme

Oviposition non host

Chi-Square	11.507
df	3
Asymp. Sig.	0.009

a Kruskal Wallis Test

b Grouping Variable: treatment

Mann-Whitney test: Frequency of non host oviposition

	10nh	5h5nh	5nh5hrem
5h5nh	0.029*		
5nh5hrem	0.004*	0.0239	
8nh2h	0.097	0.139	0.016*

3) Time spent in patches

The SPSS statistical programme

Time spent in patch	
Chi-Square	37.564
df	6
Asymp. Sig.	0.00

a Kruskal Wallis Test
 b Grouping Variable: treatment

Mann –Whitney Test :Time spent in Patch

	1 10h5hrem	2 10h	3 10nh	4 5h5nh	5 5h5nhrem	6 5nh5hrem
2 10h	0.483					
3 10nh	0.000*	0.000*				
4 5h5nh	0.328	0.862	0.000*			
5 5h5nhrem	0.047*	0.104	0.000*	0.084		
6 5nh5hrem	0.356	0.878	0.000*	0.949	0.088	
7 8h2nh	0.026*	0.088	0.000*	0.124	0.88	0.112

4) Time allocation per behaviour in patches

	Fly	Search	Groom	Stop	Walk
Chi-Square	16.471	44.933	40.541	12.718	66.135
df	6	6	6	6	6
Asymp.Sig.	0.011	0.000	0.000	0.048	0.000

a Kruskal Wallis Test
 b Grouping Variable: treatment

Mann –Whitney Test : Fly

	1 10h5hrem	2 10h	3 10nh	4 5h5nh	5 5h5nhrem	6 5nh5hrem
2 10h	0.465					
3 10nh	0.001*	0.021*				
4 5h5nh	0.443	0.829	0.006*			
5 5h5nhrem	0.047*	0.383	0.200	0.103		
6 5nh5hrem	0.595	0.213	0.000*	0.199	0.014*	
7 8h2nh	0.788	0.492	0.006*	0.735	0.180	0.480

Mann –Whitney Test: Search

	1 10h5hrem	2 10h	3 10nh	4 5h5nh	5 5h5nhrem	6 5nh5hrem
2 10h	0.941					
3 10nh	0.000*	0.000*				
4 5h5nh	0.911	0.837	0.000*			
5 5h5nhrem	0.023*	0.030*	0.000*	0.140		
6 5nh5hrem	0.094*	0.118	0.000*	0.103	0.001*	
7 8h2nh	0.194	0.125	0.000*	0.930	0.675	0.004*

Mann –Whitney Test: Groom

	1 10h5hrem	2 10h	3 10nh	4 5h5nh	5 5h5nhrem	6 5nh5hrem
2 10h	0.602					
3 10nh	0.000*	0.000*				
4 5h5nh	0.304	0.622	0.000*			
5 5h5nhrem	0.192	0.135	0.000*	0.270		
6 5nh5hrem	0.091	0.270	0.000*	0.357	0.470	
7 8h2nh	0.007*	0.013*	0.000*	0.039*	0.513	0.151

Mann –Whitney Test: Stop

	1 10h5hrem	2 10h	3 10nh	4 5h5nh	5 5h5nhrem	6 5nh5hrem
2 10h	0.414					
3 10nh	0.018*	0.050*				
4 5h5nh	0.056*	0.302	0.,242			
5 5h5nhrem	0.077	0.321	0.432	0.932		
6 5nh5hrem	0.030*	0.106	0.290	0.505	0.,760	
7 8h2nh	0.007*	0.035*	0.507	0.237	0.354	0.533

Mann –Whitney Test: Walk

	1 10h5hrem	2 10h	3 10nh	4 5h5nh	5 5h5nhrem	6 5nh5hrem
2 10h	0,75					
3 10nh	0.000*	0.001*				
4 5h5nh	0,091	0.243	0.001*			
5 5h5nhrem	0.207	0.110	0.000*	0.003*		
6 5nh5hrem	0.000*	0.000*	0.440	0.000*	0.000*	
7 8h2nh	0.000*	0.000*	0.695	0.000*	0.000*	0.535

B) Developmental study

2nd Experiment

CtrCyr: Control Cyrus
 CtrOH: Control Old Harry
 IndCyr: Induced Cyrus
 IndKim: Induced Kimmeridge
 IndOH: Induced Old Harry

The SAS System: The Genmod Procedure

1) Encapsulation %

-Differences in treatments without including weights as a covariate

LR Statistics For Type 3 Analysis

Source	DF	Chi-Square	Pr> ChiSq
Treatment	4	32.61	< 0.0001

Contrasts

	CtrCyr	CtrOH	IndCyr	IndKim
CtrOH	0.0173*			
IndCyr	0.0204*	0.9621		
IndKim	<0.0001*	0.0112*	0.0106*	
IndOH	<0.0001*	0.0137*	0.0147*	0.8762

- Differences in treatments including weights as a covariate

LR Statistics For Type 3 Analysis

Source	DF	Chi-Square	Pr> ChiSq
Treatment	4	17.31	0.0017
Weight	1	34.27	<0.0001
Weight*Treatment	4	13.12	0.0107

Contrasts				
	CtrCyr	CtrOH	IndCyr	IndKim
CtrOH	0.0074			
IndCyr	0.0030	0.634		
IndKim	0.1553	0.4185	0.2717	
IndOH	0.0043	0.5145	0.7496	0.2137

2) Weight

The SPSS System: One Way Anova

ANOVA TABLE

	Sum of Squares	df	Mean Square	F	Significance
Between Squares	66.857	4	16.714	27.364	0.000
Within Groups	158.204	259	0.611		
Total	225.061	263			

	Treatment	N	Homogenous Subsets	
			α	b
Tukey HSD (α, b)	IndKim	54	1,8032	
	IndOH	35	2,1881	
	CtrOH	58		2,8223
	IndCyr	56		3,0287
	CtrCyr	61		3,0747
	Sig.		0.098	0.0482

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 50,719.

b The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

1st Experiment

The SAS System: The Genmod Procedure

1) Encapsulation %

-Differences in treatments without including weights as a covariate

LR Statistics For Type 3 Analysis

Source	DF	Chi-Square	Pr>ChiSq
Plant	2	15.98	0.003
Instar	1	82.15	<0.0001
Plant*instar	2	7	0.0302

Contrasts	Cyr1	Cyr2	Kim1	Kim2	OH1
Cyr2	<0.0001*				
Kim1	0.3189	<0.0001*			
Kim2	0.0505	<0.0001*	0.0045*		
OH1	0.0476*	<0.0001*	0.2828	0.0004*	
OH2	<0.0001*	<0.0372*	<0.0001*	0.0093*	<0.0001*

- Differences in treatments including weights as a covariate

LR Statistics For Type 3 Analysis

Source	DF	Chi-Square	Pr>ChiSq
Plant	2	9	0.011
Instar	1	3.32	0.0685
Weight	1	27.64	<0.0001
Plant*instar	2	8.29	0.0159

Contrasts	Cyr1	Cyr2	Kim1	Kim2	OH1
Cyr2	0.4789				
Kim1	0.1528	0.0654			
Kim2	0.1536	0.061	0.8829		
OH1	0.0530	0.0302*	0.5018	0.63	
OH2	0.0818	0.4260	0.0019*	0.0002*	0.0010*

2) Weights

The SPSS System: One Way Anova

ANOVA TABLE

	Sum of Squares	df	Mean Square	F	Significance
Between Squares	81.680	5	16.336	104.720	0.000
Within Groups	40.403	259	0.156		
Total	122.084	264			

	Treatment	N	Homogenous Subsets			
			a	b	c	d
Tukey	Cyr1	48	0.7842			
HSD	OH1	46	0.7844			
(a,b)	Kim1	46	0.9223			
	OH2	42		1.4735		
	Kim2	44			1,7260	
	Cyr2	39				2,3237
	Sig.		0.573	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 50,719.

b The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

