Plant mediated effects on the outcome of competition between two parasitoid wasps *Cotesia rubecula* and *Cotesia glomerata* (Hymenoptera: Braconidae)

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ABSTRACT

Parasitoid biology is tightly linked with their herbivore hosts, as their offspring develop in herbivores. Several parasitoid species exploit the same herbivore and as a result parasitoids may compete over herbivore hosts. In nature herbivore-parasitoid interactions occur within a complex insect community, which implies presence of several non-host species in the vicinity. Plants under herbivore attack emit specific volatiles, which selectively attracts parasitoids. Volatile information emitted by the plants, infested with non-host herbivore can distort searching environment of parasitoids. Thus, parasitoids involved in competitive interactions under complex environment have to rely on adaptive mechanisms, which would modify their host searching behavior.

We studied competition for the host *Pieris rapae* between two congeneric parasitoids, *Cotesia rubecula* and *C. glomerata* under different micro-environments: when non-host feed on neighboring plants and when non-host feed on the same plant with the host. Moreover, we studied which adaptive mechanisms of wasps were responsible for the outcome of competitive interactions; here we focused on learning capabilities of wasps. Hypothesis that *C. glomerata* wasps, being an inferior competitor to *C. rubecula* inside a host body, would suffer less from competition with *C. rubecula* was confirmed. Although *C. rubecula* parasitized more caterpillars in control situation, *C. glomerata* succeeded parasitization rates in complex environments. Further research showed that learning was not the key regulator of competitive interactions, due to the fact, that experienced *C. glomerata* wasps was not able to distinguish between host and non-host in wind tunnel assays, while *C. rubecula* showed significant preference to the host. This result suggested that *C. rubecula* has some plasticity in host searching behavior. Regarding to this finding, *C. rubecula* should succeed in parasitism in all environments. However, it was not the case. Analysis and comparison of superparasitism and spatial distribution of parasitism, caused by two parasitoids revealed difference in colonization ability of the study organisms, which leaded to the conclusion that *C. rubecula*, being a superior competitor, was inferior colonizer and *C. glomerata*, being an inferior competitor turned to be superior colonizer. Thus, advantage in colonization ability allowed *C. glomerata* wasps to succeed in parasitism under complex micro-environments. Further research is needed to obtain broader view on regulating factors of competitive interactions.

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INTRODUCTION

COMPETITION FOR SHARED RESOURCES

Competition of organisms for resources such as food, territory or mating opportunities is the main ecological phenomenon determining the direction of evolution (Jones et al., 2009). The majority of the traits, that organisms have, are likely to be adaptations that allow to succeed in proliferation (Pexton & Mayhew, 2004).

Competition encompasses many types of interactions, from symmetric to asymmetric forms in dependence on the outcome, whereas competitive mechanisms can be divided broadly as either exploitation or interference. In exploitative competition, individuals of one species get resources to a greater extend than individuals of another species. Interference competition implies that members of one species preclude another species access to resources (Reitz & Trumble, 2002).

On the level of individuals, interactions can have a form of intraspecific (between individuals of the same species) competition or an interspecific one (between individuals of different species). Interspecific competition is a widespread phenomenon among species of all taxa and is important in shaping communities (Pedata et al, 2002).

COMPETITION BETWEEN PARASITOIDS

There is a strong empirical support for the presence of competition in arthropod communities among phytophages, parasitoids, omnivores and predators (Reitz & Trumble, 2002). Among all of them parasitoids are excellent examples for studies of competition, as they are tightly connected to their hosts all life period (Hawkins, 2000). Such factors as development of parasitoids offsprings in herbivore, multiparasitism leading to interspecific competition within host body and parasitoids' distinct foraging behavior made these insects a favorite model system for ecologists and evolutionary biologists (Godfray & Shimada, 1999).

Parasitoids occur in several orders of insects (Diptera, Coleoptera, Lepidoptera, Trichoptera, Neuroptera, Strepsiptera), but they are especially common in Hemynoptera. It is estimated that parasitoids comprise 10 to 20% of all insect species (Pennachino & Strand, 2006).

Parasitoids lay their eggs on or in the body of its host-insect and the parasitoid larvae develop by feeding on the host leading it to death. Most parasitoids can be classified as either solitary or gregarious. Solitary parasitoids lay a single egg, whereas gregarious ones lay several eggs in one host. Due to specific larval trait, namely cannibalism only one solitary parasitoid is able to develop per host. Thus, all the larvae present within the host will perish except for one. For gregarious parasitoids several larvae develop in one host (Godfray, 1994). In order to detect the host, some parasitoids use every type of chemical trace left by a host, from feces and oral secretions to pheromones and chemicals linked with host symbionts. However, the array of chemicals produced by host and host plant upon herbivore damage often strongly attracts parasitoids (Godfray & Shimada, 1999).

Parasitoids are most likely to compete for hosts. It involves related adult behavior patters as well as interactions among offsprings. Thus, competition between parasitoids can happen during different life stages. Females compete for the most suitable host to oviposit, whereas immature parasitoids compete for resources essential for their development and survival.

During the adult stage the crucial function of the female parasitoids is search for the hosts in order to oviposit. In the situation of the exploitative competition all individuals have potential access to resources. But, while individuals of one species obtain sufficient resources, members of another species cannot get the resources. This difference in obtaining the resources is driven by the intrinsic (not agonistic) abilities of the species to gain access to the resources via host searching activities and survival strategies (Reitz & Trumble, 2002).

Competitive interactions between larval stages of parasitoids take place within the host body. When the host is attacked by more than one parasitoid (of the same species or different

one), competition occurs between them. Not all the larvae are able to complete the development in the host and thus some of them die. The elimination of the larvae can be as a result of physical attack, physiological suppression or starvation and introduction of a toxic compound by one of the parasitoids (Laing & Corrigan, 1987). When the super numerous larvae inside the host body are of the same parasitoid species this phenomenon is known as superparasitism, and when they are of different species it is called multiparasitism (Fisher, 1961). The main difference between superparasitism and multiparasitism is that competition is likely to be asymmetric in the case of multiparasitism, where the different development patterns of the two species may lead one species to be advantageous over another (Godfray, 1994).

A clear example of competition, caused by multiparasitism, is interaction between larvae of the solitary parasitoid *Cotesia rubecula* (Marshall) and gregarious *Cotesia glomerata* (L.) within a host. The larvae of *C. rubecula* readily attack conspecific as well as larvae of *C. glomerata*. The first instar larvae of *C. rubecula* are equipped with mandibles. When the larvae moult to the second instar and begin their rapid growth, the mandibles usually disappear (Laing & Corrigan, 1987). Moreover, parasitoid larvae actively move around the host hemocoel, searching for other larvae to eliminate (Godfray, 1994).

In some cases when females perceive multiparasitism occurrence they can make a decision to superparasitize, so these two phases of parasitism are closely connected to each other within competitive interactions (Godfray, 1994).

IMPACT OF ENVIRONMENT COMPLEXITY ON INSECT INTERACTIONS

Complexity of environment can be considered as habitat heterogeneity (plants polyculture) or insect community complexity (plants in policulture as well as in monoculture). However, insect interactions within either type of complexity are mediated by plants.

Composition and structure of the vegetation were proven to influence on the host-finding efficiency of parasitoids. It was shown that increase in habitat complexity reduces parasitoid foraging efficiency due to physical and chemical characteristics of the plants located close to the host-infested pant (Gols et al., 2005).

In crop monocultures parasitoids often overexploit host populations, resulting sometimes in extinction. It happens because in simple conditions it is much easier for parasitoids to detect its host (Gols et al., 2005). Habitat homogeneity can be intricated by the presence of variety of insects of different trophic levels, creating insect community complexity. In nature parasitoids often have to choose between plants infested with a complex of host and non-host herbivores versus plants containing only non-host species (Vos et al., 2001). Chemical analysis showed that plants damaged by multiple attackers emit more volatiles than plants attacked by one herbivore. It is possible, that plants damaged by several herbivores will emit volatiles which will enhance the attraction of parasitoids or impair it in comparison to attraction to the plants attacked by one pest (Dicke et al., 2009). For example, it was shown that plants simultaneously infested with two herbivores – *Plutella xylostella* and *Pieris rapae* were more attractive for *C. glomerata* (parasitizing *P. rapae),* than to *C. plutellae* (parasitizing *P. xylostella*) (Shiojiri et al., 2001). When presence of several herbivores on plants makes volatile information less perceptible for parasitoids, each of herbivores in such conditions might be relatively protected from specialist parasitoid and less from generalists (Vos et al., 2001). Thus, indirect interactions have impact on community persistence and stability of herbivores and parasitoids.

With relevance to this example it is presumable that in a situation, when one of the two herbivores attacking the plant is a shared host for two parasitoids, infochemicals emitted from the attacked plant will mediate the outcome of competitive interactions between parasitoids. Once a species experience niche overlap in complex environment, it will evolve adaptive behavior in order not to extinct. Variation in feeding, spatial or temporal niches, together with the possibility of trade-off between inferior competitor and superior dispenser to unexploited habitats are potential strategies of competitive exclusion prevention (van Veen et al., 2006).

AVOIDANCE OF COMPETITION

In natural conditions it is not rare when two species share the same host resources. The inferior species under the asymmetric competition will tend to avoid oviposition in hosts, in which the other species develops. When the inferior species is a generalist, it could easily switch to another host. For example, *C. glomerata* may actively avoid parasitism on *Pieris rapae* (L.) (Lepidoptera: Pieridae), which is the target for the specialist *C. rubecula* (Geervliet et al, 2000).

It is known that upon damage caused by the herbivore some plants show specific reaction, emitting volatiles, which affect performance of natural enemies of herbivorous insects positively (Poelman et al., 2009). Damaged plant derived volatiles are considered to be highly detectable, due to its larger biomass, but less reliable predictors of the herbivore presence (Schoonhoven et. al., 2005). However, plant volatiles play an important role in avoiding parasitoid competition. It is shown that species *C. rubecula* and *C. glomerata* are able to use herbivore-induced plant volatiles to discriminate in flight between the plants containing parasitized and unparasitized hosts. This ability enhances their reproductive success and helps in saving time and energy to find a suitable host (Fatouros et al, 2005).

In the environment with the restricted amount of unparasitised hosts, parasitoids may decide to oviposit onto the host that has been already parasitized by conspecific or individual of another species. Some specific adaptive strategies different for solitary and gregarious parsitoids can evolve upon such circumstances (van Alphen & Visser, 1990).

There is more complex adaptation to the environment, which is helpful for parasitoids in competition avoidance, known like learning.

LEARNING AS AN ADAPTATION

By learning parasitoids can solve the reliability-detectability problem. Linking highly detectable cues to highly reliable cues through associative learning, parasitoids can temporarily increase the reliability of detectable indirect cues (Vet et al., 1995). Parasitoids can learn all the range of characteristics of the host's environment like odor of the host's food plant, the color and form of the host's environment, the part of the plant where the host resides, etc. While searching for the host, especially from a long distance, parasitoids are used to rely on the volatiles emitted upon herbivore damage. Probably on this step plant volatiles are the most important cues. Parasitoid once gained a reward after being attracted to some plant volatile will be able to learn it and use it as an easy-to-detect stimulus which leads to hard to detect host. Attracted to the plant volatile and received non reward parasitoid will learn to avoid the experienced cue. It is important to note that learned responses disappear over time in the absence of continued experience (Papaj et al., 1994).

The host-foraging behavior of female parasitoids is mostly modified by the positive associative learning, which indicates that responses to stimuli are newly acquired or enhanced by linking them to a reinforcing stimulus. The reinforcing stimulus in associative learning is always host derived (reward) (Vet & Dicke, 1992). Successful oviposition in a host (rewarding experience) increases a parasitoid's searching effort in a host microhabitat of the type associated with an experience. It was determined that females after successful oviposition in either of two microhabitat types strongly preferred to forage subsequently on that microhabitat type. However, an unrewarding experience (failure to find a host in the previously rewarding microhabitat) caused females to change their preference in favor of a new microhabitat type. Thus, an unrewarding experience is also turned to be important in shaping parasitoid's behavior (Papaj et al., 1994).

It was shown that learning can already take place during the preadult stage (early adult learning) as well as during the adult stage. Preadult learning, as a consequence of development in a particular host and its food, can influence responses to odors by the adult, although it is less common compared to adult stage learning (Vet & Dicke, 1992). The fact that Brussels sprouts, the plants used in cultures of parasitoids, elicits the strongest responses in both *C. glomerata* and *C. rubecula* species may be attributed preadult learning or responses shaped by selection (Geervliet et al., 1996). Thus, wasps that newly emerged from their cocoons are able to find their first hosts

due to the fact that they have genetic inclinations for the odors of plants that were utilized by hosts as a food and that some elements and substances in their cocoons can bring a sort of experience to the wasps (Tumlinson et al., 1993). Although, wasps have some innate ability to respond to odors of host-infested plants, they cannot discriminate between the plants infested by host and non-host species without being experienced before (Geervliet, 1996).

Preference of parasitoids to particular host is an ecological factor setting specific values for learning. Learning is expected to play an insignificant role in species that experience little variation in host and plant species and strong innate responses to kairomones, and stimuli from the host's food are expected in this case. For species that experience a variation in host, information from the host's food is supposed to be relatively important in foraging and therefore strong innate responses to plant volatiles and other plant characteristics can be predicted. But, these plant specialists still have to deal with the intrinsic variability in these first trophic level cues which may dictate a need for some learning (Vet et al., 1995).

The strength of the learning potential in the naïve insects is set by natural selection, therefore learning evolved as an adaptation to the environment (Vet & Dicke, 1992).

THE IMPORTANCE OF COMPETITIVE INTERACTIONS INVESTIGATION

Although the degree of competition in parasitoid communities remains to be figure out, it is possible to state that competition is widespread due to the range of evolved physiological and behavioral attributes of parasitoids. For example, the host specificity or host preference of some species of parasitoids predicts that they will occur together in a particular host individual (Hawkins, 2000).

From the applied perspective, parasitoids play an important role in biological control of insect pests. To make the employment of parasitoids more effective, it is necessary to understand their behavior, and its consequences for the dynamics of parasitoid and host populations. Thus, fundamental studies on parasitoids behavior can contribute to the successful application of biological control.

The study of interactions which are involved in competition could be useful in predicting the outcome of multiple introductions of natural enemies against agricultural pests (Pedata et al, 2002). From the one side natural enemies which are superior in within-host competition may somehow displace a natural enemy that is better able to suppress pests (Pedata et al, 2002), from the other side the total effect of several competing species may be greater than the action of any species alone. Thus, to estimate more accurately the impact of competition on the result of biological control the investigations in this field are needed.

Also attention should be paid to the parasitoids' interactions with other components of the ecosystem before introducing them into a new habitat (Tylianakis et al., 2005).

Ecological impact by introduced species can be revealed at different levels of biological organization like genetic effects, effects on individuals, communities or populations of species, and finally effects on ecosystem processes (Kenis et al, 2009).

The majority of competitive displacements cause negative impacts. The most obvious impact is the loss of biodiversity when the invasive species displaces a native one (Rietz & Trumble, 2002). In extreme cases competition may lead to species extinction. The outcome depends subtly on the strength of local superiority of one species versus the dispersal rate of the other (Killingback et al, 2006).

Moreover as plants, herbivores and natural enemies are involved in multitrophic interactions, it is necessary to account environment where the interaction takes place. That is why it is important to investigate competition outcome within more complex situation as close to natural conditions as possible.

Testing the competition under relatively simple environments and then under more and more complex ones may give the impression of the mechanisms of interactions and predict the direction of the ecosystem processes. In other words, simple systems are that fundamental blocks

from which an understanding of more complex mutli-species interactions can be constructed (Hassell & Waage, 1984).

MODEL ORGANISMS AND THEIR BIOLOGICAL AND ECOLOGICAL CHARACTERISTICS

Considering the importance of the environment where interactions take place, I studied the outcome of competition between two parasitoid wasps under habitats that differ in level of complexity. It was done by involvement of 2 parasitoid species: *C. rubecula* and *C. glomerata*, 2 herbivores: *P. rapae* and *M. brassicae* and a host plant: *Brassica oleracea* var. *gemmifera* L. (Brassicaceae) cultivar Cyrus, which allowed to set research in a tritrophic context (Figure 1).
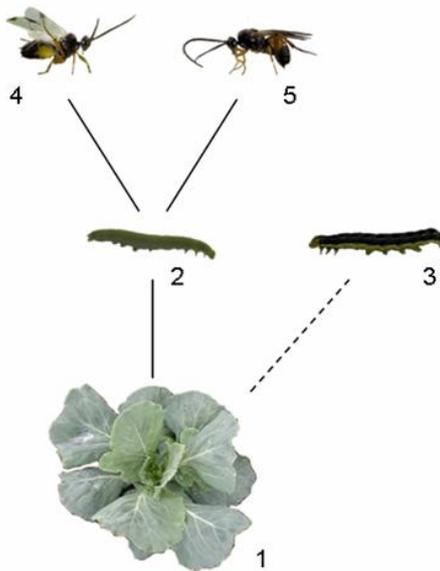
Figure 1. Schematic representation of the organisms involved in a tritrophic interactions investigated in current study: 1 - *Brassica oleracea* var. *gemmifera* L. (Brassicaceae) cultivar Cyrus; 2 – *P. rapae* caterpillar; 3 – *M. brassicae* caterpillar; 4 – *C. rubecula* adult wasp; 5 – *C. glomerata* adult wasp (created by Erik Poelman).

Each of the organisms, involved has their particular biological traits. We mostly were interested in those of parasitoid wasps, as their behavior was the main subject of the research.

C. rubecula lays single eggs in the body of *P. rapae* larvae and is considered to be a solitary parasitoid. Female wasps upon emergence have around 30 eggs and produce 40-80 more during their lifetime (Vos et al., 2001). In the laboratory individuals live in average 17 days, in the field conditions – 14 days (Geervliet et al, 1997).

C. glomerata on the contrary lays clutches of eggs in the caterpillars of several *Pieris* species as it is gregarious parasitoid. The average clutch size on *P. brassicae* in Europe is 27-30 eggs (Tanaka et al., 2007). For the life duration it only known that in the laboratory conditions females can live up to 5 weeks if feed with the honeywater. The fecundity of females fluctuates between 500-2000 eggs upon emergence. Moreover they can produce some hundred more during adult stage (Vos, 2001).

Both parasitoids parasitize caterpillars of the small cabbage white, *Pieris rapae*. *Pieris rapae* lays eggs singly usually on the lower surface of the outer leaves. The larva requires about 15 days (range 11 to 33 days) to complete its development (Capinera, 2001). Feeding behavior of the *P. rapae* larvae is described by frequent interruptions with the subsequent movement to another feeding place. This behavior pattern may help the larvae to circumvent the parasitoid which is attracted to the feeding damage site of its host. However, parasitoid avoidance strategy of *P. rapae* lacks of feeding at night and spending most of the time on the underside of the leaves, because the caterpillars are cryptic (Mauricio & Bowers, 1990).

C. glomerata is considered to be a generalist at both the host level and the food plant level and attack several Pieridae species, whereas *C. rubecula* is more specialized at the host level and

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the plant level and parasitize a small cabbage white fly *Pieris rapae*. These species overlap in niche, as they share host resources (Geervliet et. al., 1998) and, therefore, the competition exists between these two species. It was found that *C. rubecula* was superior to *C. glomerata* in *P. rapae* host species as the larvae of *C. rubecula* eliminated *C. glomerata* inside a host after multiparasitism (Laing & Corrigan, 1987).

Furthermore, it was estimated that only *C. glomerata* and not *C. rubecula* characterized by shift in preference after experience (Geervliet et al., 1998). These findings turned to be in agreement with the hypothesis that in specialists (*C. rubecula)* the behavior is less modified by experience than in generalists (*C. glomerata*) (Geervliet et. al., 1998; van Lenteren, 1986). Differences in learning of the parasitoids can be explained by the differences in spatial distribution of their hosts. *C. rubecula* is a specialist on the small cabbage white *P. rapae*, whereas *C. glomerata* prefers *P. bassicae* as a host in The Netherlands (Poelman et al., 2009; Brodeur et. al., 1996; Geervliet et al., 2000). *P. rapae* lays its eggs singly on the plants, so the presence of the caterpillar does not indicates the presents of more of them in the vicinity in the same plant odour. *P. brassicae* lays its eggs in clutches, so the presence of the host caterpillar indicated for *C. glomerata* that there are many more of the suitable hosts around (Bleeker et al., 2006).

It is useful to mention the factors, which particularly contribute to the increased response of the experienced wasps. The study was done in this direction. Four groups of female wasps were tested: naïve wasps, wasps experienced only with odor of the host infested plant, wasps, which has only oviposition experience and finally wasps with a complete experience (they were allowed to parasitize a host on the infested leaf). Testing revealed that *C. glomerata* and *C. rubecula* females that received a complete experience showed the highest response level in comparison to other 3 groups of treated wasps (Bleeker et al., 2006).

Besides two parasitoid wasps and their shared host, a non-host herbivore *M. brassicae* was included into the model system of the current study. This species commonly occur on cabbage plants. Volatiles emitted upon its damage can affect host searching behavior of parasitoid wasps (see Appendix I). Cabbage moth *M. brassicae* lays eggs in batches on the lower side of the leaves. Caterpillars develop within 24-50 days, depending on the environmental conditions (Sukhareva, 1999).

Thus, described model allowed to investigate the outcome of competition between two parasitoid wasps for shared host considering the effect of non-host presence in the foraging arena. Moreover, the role of adaptive strategies of wasps under given conditions was studied. Designed model system helped to answer following research questions.

RESEARCH QUESTIONS AND HYPOTHESIS

With references to previous research experience and considering biological differences between *C. rubecula* and *C. glomerata* we tested:

1. The outcome of competition between congeneric parasitoid wasp species *C. rubecula* and *C. glomerata* for the host *P. rapae* in the presence of non-host herbivore *M. brassicae* in two situations
- when the non-host was on the neighboring plant;
- when non-host fed on the same plant with the host;
2. The role of long term memory of *C. glomerata* in modifying host finding behavior of the parasitoid;
3. The effects of variation in herbivore-induced plant volatiles on attraction of experienced *C. rubecula* and *C. glomerata.*

From this point several research questions aroused:

1. Does the presence of the non-host herbivore affect the outcome of the competition between the two parasitoid wasp species?
2. Does learning play a role in the adaptation to competitive interactions?
3. How does experience influence the foraging behavior and efficiency of the parasitoid wasps of different host specialization?

Hypothesis:

1. *Cotesia glomerata* suffers less from competition with *C. rubecula* when the plant mediated non-host herbivore cues are present in the foraging arena. This hypothesis is based on the finding that *C. glomerata* showed more plasticity in host searching behavior compared to *C. rubecula*. Moreover, the fact that parasitoids differ in host specificity may play a role in the development of adaptive mechanisms to the environment (Geervliet et al., 1998).
2. We expect, that *C. glomerata* with inhibited memory formation suffers more from competition when the plant mediated non-host herbivore cues are present in the foraging arena, because only *C. glomerata* and not *C. rubecula* characterized by shift in preference after experience (Geervliet et al., 1998). Thus, treated with memory inhibitor, *C. glomerata* wasps will not be able to store information about the suitability of experienced contact with host or non-host and eventually will not manage to modify their behavior.
3. Experience is supposed to increase a parasitoid's foraging effort in a host microhabitat, i. e. more "choices" would be recorded on the plants infested with the host. In other words experienced wasps would be able to discriminate the cues elicited upon damage caused by non-host and host herbivore species. This expectation also aroused from findings that parasitoid wasp are able to modify their host searching behavior after series of ovipositions into host (Vet & Dicke, 1992)

MATERIALS AND METHODS

INSECTS

To test the outcome of competitive interactions under different micro-environments we used the two congeneric parasitoids *Cotesia glomerata* and *C. rubecula* that occur together in the same habitats and share host resources. Variety of treatments was obtained by combinations of microenvironments with the presence of host and non-host herbivores.

Parasitoids and herbivore species originated from individuals collected on Brussels sprouts fields near Wageningen, the Netherlands.

Two Lepidoptera species *Pieris rapae* L. (Pieridae) and *Mamestra brassicae* L. (Noctuidae) originated from respective cultures maintained in the Laboratory of Entomology, Wageningen University. Brussels sprouts (*Brassica oleracea* var. *gemmifera* L. cultivar Cyrus) were used as a host plant for herbivore rearing. *Mamestra brassicae* moths were offered filter paper to oviposit on, without contact to cabbage plants. Larvae of *P. rapae* and *M. brassicae* were obtained from colonies maintained separately in a climatic room at 20-22ºC, 50-70% RH and a L16:D8 photoregime. Larvae of late first instar were used in the experiments.

Cotesia glomerata and *C. rubecula* colonies were maintained on *P. brassicae* and *P. rapae* larvae respectively. Brussels sprouts leaves with first instar host larvae were offered to caged parasitoid females to parasitize on. After that the leaves were transferred to cages with Brussels sprouts plants in greenhouse compartments, at 20-22ºC, 50-70% RH and a 16L:8D photoregime. *Pieris brassicae* caterpillars were parasitized by *C. glomerata* in the first to third instar and *C. glomerata* larvae left the host body to form a cocoon when host caterpillar reached its last (fifth) instar. *Cotesia rubecula* in its turn parasitized early larval instars of *P. rapae* and then developed larvae also left the host body to spin a cocoon. Cocoons of parasitoid wasps were kept separately in Petri dishes (9 cm in diameter) in a climate controlled rooms, at 20-22ºC, 50-70% RH. and a L16:D8 photoperiod. After emergence of wasps, males and females were caged together to allow mating. Cocoons were transferred periodically to new cages to obtain parasitoid groups of uniform age. Cages (40X30X30 cm) were supplied with water and honey.

PLANTS

In field and laboratory experiments we used *Brassica oleracea* var. *gemmifera* L. (Brassicaceae) cultivar Cyrus plants that were reared in the greenhouse in plastic pots ($11 \times 11 \times 11$ cm), at 20-25ºC, 50-70% RH and a L16:D8 photoperiod.

To investigate plant - mediated competition between parasitoid wasps under the field conditions we created tents that contained different microenvironments for foraging parasitoids. We planted plants one week prior to the start of an experiment in order to adapt plants to field conditions. After that, larvae of *P. rapae* and *M. brassicae* were placed on the plants to initiate damaged-plant volatiles emission. To mimic natural distribution patterns, *P. rapae* and *M. brassicae* larvae were placed singly on the leaves of the plants (1-2 L1 per leaf). The number of caterpillars per plant varied with the treatment (see Experimental set-up for *Competition between C. rubecula and C. glomerata under field conditions*).

Later, in the second field experiment, testing the hypotheses regarding to the reasons of resulted outcome of competition, the same methodology as described above was applied in order to obtain plant volatiles. For the numbers of caterpillars per plant according to the treatment see Experimental set-up for *Competition between healthy C. rubecula and ANI-treated C. glomerata females under field conditions*.

To generate odors for wind tunnel assays, Brussels sprouts plants were randomly infested with the late first instar larvae of *P. rapae* and *M. brassicae* in different combinations (see Experimental set-up for *Adult learning: preference for damaged plant volatiles*). Larvae were allowed to feed on the plants for 24 hours prior to the experiments. Larvae were removed from the

plants just before placing plants into the wind tunnel in order to allow wasps to rely on the odors only while searching the host.

EXPERIMENTAL SETUP

Competition between *C. rubecula* and *C. glomerata* under field conditions

In agricultural field in the vicinity of Wageningen University we conducted an experiment testing plant-mediated competition between congeneric parasitoid wasps *C. rubecula* and *C. glomerata*. To study the influence of the environmental complexity on the parasitism success of either parasitoid species, we carried out an experiment in tents, which allowed to create and maintain relatively stable conditions within a particular treatment. In each of 12 tents (3mx4mx2m) (Fig. 2A), 16 cabbage plants were planted into the soil in a 4x4 grid (Fig. 2B).
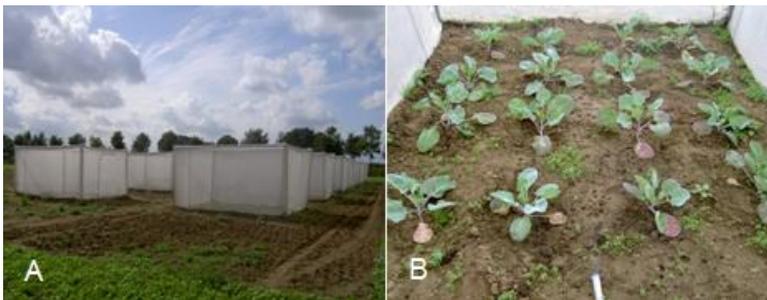

Figure 2. Experiment set-up: A – 12 tents located in two rows in the field; B – 16 plants planted in each of 12 tents.

Within an experiment three treatments with different levels of environmental complexity were designed (Fig. 3). A control treatment of hosts only was needed to compare the competitive interactions between parasitoids with effects caused by more complex microenvironments that included non-hosts. The two more complex microenvironments were established to provide a clue on how far the volatile information upon the influence of environment can mediate searching behavior of parasitoids.

To create an appropriate environment within each treatment, plants were infested with different combinations of host and non-host larvae:

1. Control – 10 *P. rapae* plants were introduced on a single leaf of each second plant;

2. Host and non-host situation – eight plants were infested with the 10 *P. rapae* larvae and another eight plants with 10 *M. brassicae* larvae;

3. Host and host + non-host situation – eight plants were infested with 5 *M. brassicae* larvae and the remaining eight plants contained 10 *P. rapae* + 5 *M. brassicae*.
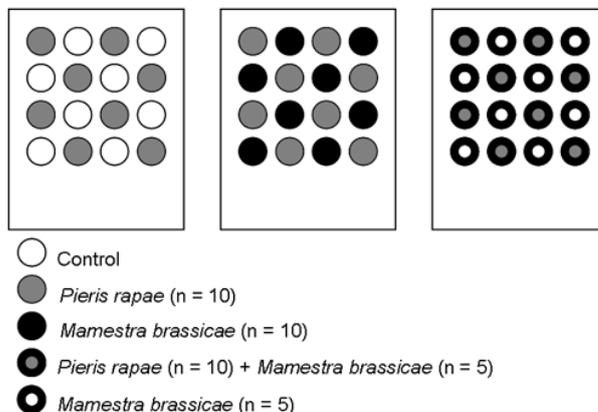
Figure 3. Scheme of plants' infestation with *P. rapae* and *M. brassicae* within treatments of experiment, testing outcome of competition between *C. rubecula* and *C. glomerata* under field conditions.

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To examine foraging behavior of *C. rubecula* and *C. glomerata* under competition, sixteen hours after infestation of plants with larvae, three females and three males of both parasitoid wasp species were released from the vials in each tent. Parasitoids were provided with honey as a source of food, placed in droplets on the inner side of the vials' covers. Upon release of the wasps, the cover with honey was placed in front of the opened vial.

Plants, infested with *P. rapae* caterpillars were recollected 48 hours after releasing the wasps and further caterpillars were dissected to detect the number of eggs oviposited by each of the parasitoid species.

The eggs of parasitoids differ in morphology (see Appendix II). Eggs of *C. rubecula* are much bigger in size then those of *C. glomerata,* and they are whitish in color and oblong in shape. Eggs of *C. glomerata* are more transparent and less oblong (Brodeur & Vet, 1995).

Further, data obtained upon dissection of caterpillars was analyzed for:

1. Number of caterpillars parasitized by each of the species of parasitoids.

2. Incidence of superparasitism and multiparasitism per tent; it is important to note, that for *C. glomerata* more than 35 eggs laid in a host were assumed as superparasitism incidence, for *C. rubecula* more than 1 egg laid in a host indicated occurrence of superparasitism.

3. Spatial pattern of parasitism for both *Cotesia* species.

During the season we managed to replicate the experiment 3 times and got 12 replicates per treatment, which gave enough data to run statistical analysis.

Performance of healthy and ANI-treated *C. glomerata*, competing with *C. rubecula* under field conditions

Previous research elucidated adaptive difference between *C. rubecula* and *C. glomerata,* suggesting that the latter species was more capable of learning (Geervliet et al., 1998; Bleeker et al., 2006). In other words, *C. rubecula* takes more time to consolidate memory than *C. glomerata.*

Memory is classified into temporally distinct forms:

1. Short-term memory or anaesthesia-sensetive memory;

2. Consolidated memory: long-term memory and anaesthesia-resistant memory.

It was estimated that *C. glomerata* formed long-term memory after only a single conditioning, whereas *C. rubecula* needed more experiences (Smid et al., 2007).

From our results of the previous experiment we observed that females of *C. glomerata* managed to parasitize higher number of *P. rapae* larvae in both complex environment treatments. Hence, to test whether learning was the reason of such outcome we inhibited long-term memory formation of a group of *C. glomerata* female wasps with a translator-inhibitor anisomycin (ANI).

The experiment was also conducted in the agricultural field in vicinity of Wageningen University. To maintain definite environment of the treatments, the experiment was carried out in tents. In each of 12 tents, 16 plants were planted one week before the experiment (Fig. 2). To create damaged-plant volatiles, which mediate foraging behavior of wasps, plants were infested with *P. rapae* and *M. brassicae* caterpillars, according to treatments' design (Fig. 4). Six tents represented a control situation, which means that 10 *P. rapae* larvae were introduced on leaves of each second plant in the tent, other six tents were used to create more complex environment: eight plants in each tent were infested with 10 *M. brassicae* (non-host) larvae and the remaining eight plants contained 10 *P. rapae* (host) larvae. Such distribution of caterpillars was similar to that of control and treatment with host and non-host on separate plants in the previous experiment.
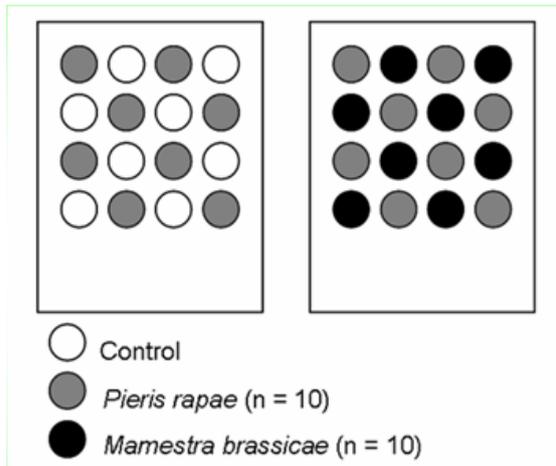
Figure 4. Scheme of infestation of plants with either host species *P. rapae* or non-host *M. brassicae* within treatments of experiment, testing outcome of competition between healthy *C. rubecula* and ANI-treated *C. glomerata* under field conditions.

For long-term memory inhibition 3-5 days old female wasps of *C. glomerata* were deprived of honey and water for 4 hours before the treatment. This measure was compulsory to make wasps starved and therefore stimulate them to consume ANI-solution. Wasps were placed in Eppendorf tubes and offered 0.5 μl of a solution containing 5mM of anisomycin in 2 % sucrose. Droplets of solution were spread on the walls of the vials. Wasps were kept in the vials for 1 h for consumption of the solution. Wasps, which refused to consume the solution, were discarded from the experiment. After this selection wasps were transferred to the glass cages with water and honey supply for four hours in order to reach stable memory inhibition (Smid et al., 2007).

The control group of *C. glomerata* wasps used in the experiment was offered to consume 0.5 μl of a solution containing 5mM of tap water in 2% sucrose. They also were starved for 4 hours prior to the experiment; they were kept for 1 hour to consume sucrose solution provided; and after consumption wasps were placed in glass cages with honey and water supply in it. It was done to make sure the effect of ANI was the only difference in treatment of wasps.

As we wanted to compare the performance of *C. glomerata* wasps with inhibited long term memory formation with healthy *C. rubecula* wasps, the latter ones were not treated anyhow.

Before release, wasps were placed into 50 ml vials with the help of an exhauster – 3 females of *C. rubecula* and 3 females of *C. rubecula* in each, in a combinations regarding to the designed treatments. Since we had two treatments (control and complex one) and two groups of *C. glomerata* wasps (healthy and ANI-treated), we used four treatments:

1. Control 1 - 10 *P. rapae* introduced on a single leaf of each second plant + 3 untreated healthy wasps of *C. rubecula* and 3 untreated *C. glomerata*;

2. Control 2 - 10 *P. rapae* introduced on a single leaf of each second plant + 3 anisomycin treated *C. glomerata* wasps and 3 healthy *C. rubecula* wasps;

3 Host and non-host situation 1 – eight plants infested with 10 *M. brassicae* larvae and the remaining eight plants contain 10 *P. rapae* +3 untreated healthy wasps of *C. rubecula* and 3 untreated *C. glomerata*;

4. Host and non-host situation 2 – eight plants infested with 10 *M. brassicae* larvae and the remaining eight plants contain 10 *P. rapae* + 3 anisomycin treated *C. glomerata* wasps and 3 healthy *C. rubecula* wasps;

Wasps were released 16 hours after plant's infestation. It was important to prepare anisomycin treated wasps in time, as they were fed with ANI in the morning and therefore released in the afternoon to have half a day for foraging. It is known that parasitoids are more active during the day hours (Juillet, 1964).

When released in the tents, parasitoids were provided with honey: droplets were placed on the covers of the vials.

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Plants with *P. rapae* larvae were recollected 24 hours after releasing the wasps, as within this time period ANI-treated wasps had stable and high memory retention (Smid et al., 2007).

In laboratory larvae of *P. rapae* were dissected to assess the rate of parasitism from the numbers of eggs oviposited by each of parasitoid species.

Adult learning: preference for damaged plant volatiles

To test the effects of variation in herbivore-induced plant volatiles on attraction of experienced wasps of *C. rubecula* and *C. glomerata*, two choice wind tunnel assays were conducted. As we assumed that naïve wasps released in the tents with herbivore infested plants after a sequence of contacts and ovipositions spaced in time became experienced, hence we decided to recreate this close to natural situation by the means of spaced training.

Spaced training
To create odor sources needed for spaced training, Brussels sprouts plants were infested each next day: 2 plants were infested with 40 late first instar of *P. rapae* caterpillars and another 2 plants were infested with 40 *M. brassicae* caterpillars of the same age, in the way that each plant contained 20 caterpillars of a particular herbivore species. Plants were left for at least 24 h in the cages in the laboratory.

Four to nine-days old females of *C. glomerata* and *C. rubecula* were collected from the cages and used in the wind tunnel as they were shown to perform better than younger ones (1-2 days old) (Steinberg et al., 1992). Wasps were placed in glass vials and released to an infested leaf of cabbage plant in a close vicinity of the caterpillars to ensure that parasitoids could perceive host stimuli. In most of the cases, especially with *C. rubecula* females, it resulted in immediate host-searching behavior. Female wasps were given a reward followed by unreward experience: first they were offered to have contact (followed by oviposition) with host *P. rapae* than after 1-2 minutes with non-host *M. brassicae*. Experimental data showed that both reward and unreward experiences turned to be important in shaping the parasitoids' behavior (Vet & Dicke, 1992; Papaj et al., 1994). The treatment was done for each wasp three times with 10 minutes interval. The incidence of oviposition into hosts was assessed during the observation of wasps stinging the caterpillars. Wasps, which did not oviposit due to whatever reasons in any of three chances, were excluded from the experiment.

Experienced wasps were transferred to cages with honey and water and were kept there for 4 hours until they were released in the wind tunnel (H. M. Smid, personal communication).

Windtunnel assays
Two-choice tests were carried out in a wind tunnel (Fig. 5). The air which came from outside was filtered over glasswool. The humidity of the coming air was controlled by the air humidifier. On the next step the air was pressurized, filtered for the second time in the charcoal filter and passed through a temperature controlled heating system. Finally the air was directed to the windtunnel. The size of the polyacrylate flight compartment was 200 x 60 x 60 cm. Both ends of the compartment were covered with fine mesh copper gauze.

Inside the windtunnel, temperature varied between 21-23 ºC, wind speed was held around 20 cm/s and the relative humidity fluctuated 60 and 70%.

The parasitoid's release site was represented by the horizontal glass cylinder (the size was 30 cm long and 15 cm in diameter) with both ends open, placed on a socket of 10 cm height at a distance of 70 cm from the odor sources. The cylinder kept wasps in the air flow and prevented them to fly directly to the ceiling. The opened vial with an individual female was placed on the bottom of the release site and females were free to fly out and choose between the two odor sources at the upwind end of the windtunnel (Geervliet et al., 1994).
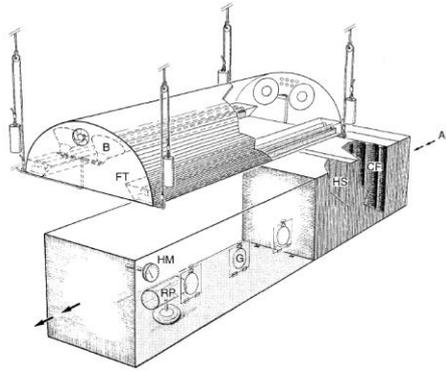
Figure 5. Windtunnel set-up: Al – air-inlet, CF – charcoal filter, HS – heating system, RP – parasitoid release point, FT – high frequency fluorescent tubes, B – bulbs, X – position of odor source, HM – hygrometer, G – gate (Geervliet et al., 1994).

According to field experiments trained parasitoid wasps were tested for host preference in two situations:

    1. *P. rapae* (10) infested plants versus *M. brassicae* (10) infested plants;

    2. *M. brassicae* (5) infested plants versus *P. rapae* (10) + *M. brassicae* (5) infested plants.

Infested cabbage plants were placed upwind in the wind tunnel with approximately 10 cm distance between them. Wasps were released inside the cylinder in the wind tunnel 70 cm downwind from the two cabbage plants. Each parasitoid species was tested for the preference separately (no competition conditions). From 8 to 15 wasps were released a day, depending on the responsiveness of the wasps during spaced training.

A "choice" was recorded as the female wasp landed on any of two offered plants within 10 minutes. If a wasp did not land on a plant during the given interval of time or if the wasp did not initiate flight within 5 minutes such behavioral patterns were assessed like "no response". Each female wasp had only one flight opportunity within a test and was offered one test treatment only and then discarded by freezing at -20ºC.

Results of preferences of naïve wasps under in the same treatments were assumed as control (see Appendix I).

STATISTICAL ANALYSIS

We used Generalized Linear Models (GLM) in the statistical program SPSS to analyze whether conditions of treatments had an effect on the difference between parasitism of *P. rapae*, superparasitism and multiparasitism in *P. rapae* and spatial distribution of parasitism of *P. rapae* caused by *C. rubecula* and *C. glomerata*. Since the field experiment was conducted in 3 different time sets, we compared the overall effect of treatments within replicates by modeling treatment as nested within time.

First we analyzed if difference in parasitism rate was affected by the treatment: the values of fraction of caterpillars parasitized by *C. glomerata* were subtracted from that by *C. rubecula*. This difference was included in the model as dependent variable with two factors: time and treatment.

Similarly we constructed separate models to test the effect of treatments within time for differences in superparasitism and spatial distribution of parasitism between *C. rubecula* and *C. glomerata*: differences were included in the model as dependent variables with time and treatment as factors.

Pair-wise comparison of treatments was done to figure out significant difference between the treatments.

Choices for odor sources in wind tunnel assays were analyzed using binomial probability functions.

RESULTS

COMPETITION BETWEEN *C. RUBECULA* AND *C. GLOMERATA* UNDER FIELD CONDITIONS

To evaluate parasitism rate of *C. rubecula* and *C. glomerata* 1953 larvae of *P. rapae,* collected with plants from the tents were dissected and the mean numbers of eggs oviposited by each of parasitoid species were calculated (Table 1). The mean number of eggs per parasitized caterpillar laid by *C. rubecula* was equal to 1.11; and by *C. glomerata* – 20.34. These data correlated with the solitary nature of *C. rubecula* and gregarious one of *C. glomerata.*

Table 1. Number of eggs per larva of *P. rapae* oviposited by *C. rubecula* and *C. glomerata*

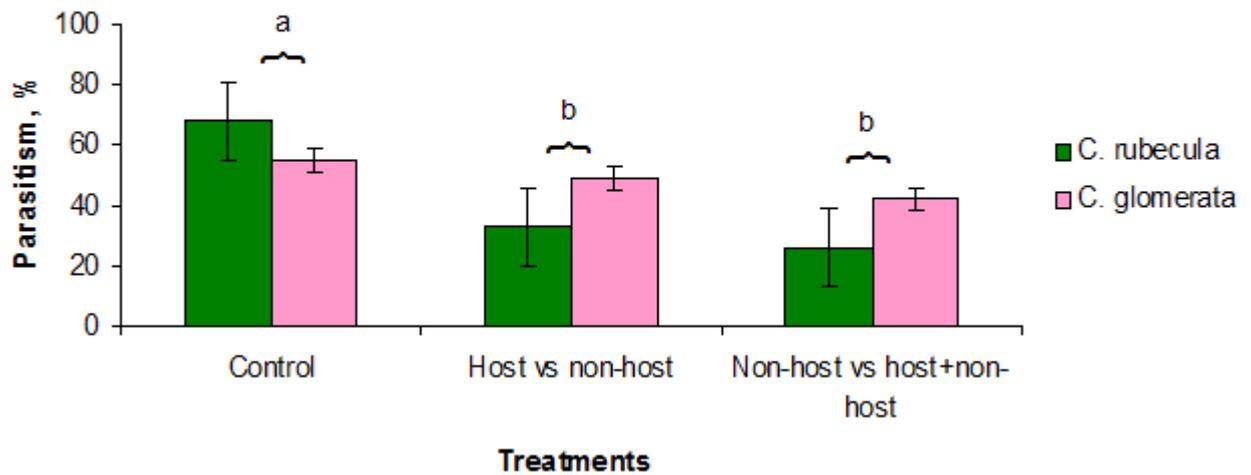| Treatment	*C. rubecula*	*C. glomerata*
Control	1.16	22.03
Host vs. non-host	1.08	19.89
Non-host vs. host+non-host	1.11	19.11
Mean	**1.11**	**20.34**

Estimation of parasitism rates of *C. rubecula* and *C. glomerata* allowed further to make a conclusion about the direction of competitive interactions between these parasitoid wasps upon different treatments (Table 2). In all series of experiment *C. glomerata* showed higher parasitism rates in comparison to *C. rubecula.* So, in three series *C. glomerata* parasitized 58, 49 and 39% of *P. rapae* larvae, whereas *C. rubecula* did 56, 44 and 28% respectively.

Table 2. Parasitism rates of *C. rubecula* and *C. glomerata* detected upon dissection of collected larvae of *P. rapae* from each of three series of filed experiment, testing outcome of competition between *C. rubecula* and *C. glomerata* parasitoid wasps.

Series no. and # tents analyzed	# larvae parasitized by C. rubecula	# larvae parasitized by C. glomerata	# larvae dissected	Parasitism rate, %	
				C. rubecula	C. glomerata
Series 1 (12 tents)	341	355	613	**56**	**58**
Series 2 (12 tents)	279	313	637	**44**	**49**
Series 3 (12 tents)	197	280	710	**28**	**39**

An overall outcome of competitive interactions from the values of parasitism rate of *C. rubecula* and *C. glomerata* revealed that there was a difference only between control treatment and each of the complex treatments. In control situation *C. rubecula* females parasitized more caterpillars of *P. rapae*, whereas in more complex situations *C. glomerata* females exceeded numbers of caterpillars parasitized by *C. rubecula*. Figure 6 summarizes the parasitism rates of two parasitoid wasps.

The rate of parasitism decreased with the increase in complexity of the environment.

Figure 6. Parasitism rates *C. rubecula* and *C. glomerata* in three treatments of experiment. Bars indicate mean values. SE calculated over 12 replicates. Wald Chi-Square = 74,950. df = 8. P < 0.0005
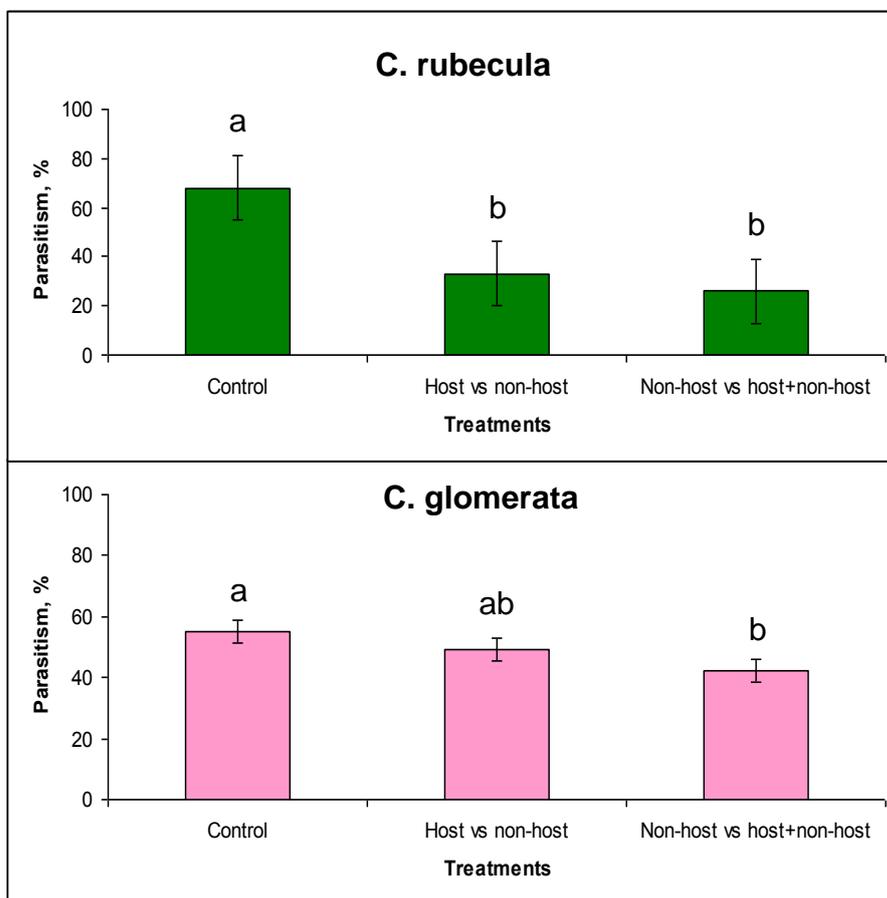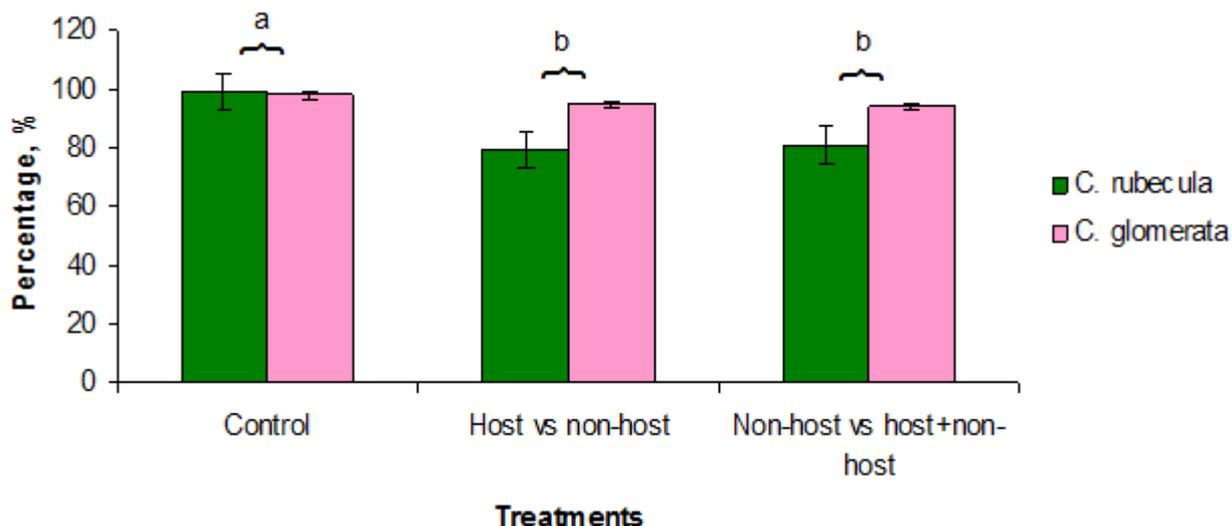

Figure 7. Parasitism rate of *C. rubecula* (Wald-chi square =181,692. df=2. P< 0.0005) and *C. glomerata* (Wald-Chi Square = 14,945. df = 2. P < 0,005) in three treatments. Bars indicate mean values. SE was calculated over 12 replicates.

Comparison of parasitism rates of each of the parasitoids separately, indicated that *C. rubecula* parasitized significantly more *P. rapae* larvae in the control situation than in the two other treatments where the non-host was present; *C. glomerata* also parasitized more caterpillars under control conditions, but this value was significantly different only from the treatment where non-host was present on the same plants with the host as well as on the neighboring plants.

Parasitism rate of *C. glomerata* in the treatment with the non-host on the neighbor plants was not different from that of in the control and second complex situation (Fig. 7).

Further, to analyze distribution of parasitism, caused by parasitoid wasps in the tents, we calculated the number of plants containing *P. rapae* larvae that were parasitized by either *C. rubecula* or *C. glomerata*. We found that in control treatment both species visited almost all plants in the tents (98.8% by *C. rubecula* and 98% by *C. glomerata*), but in complex treatments *C. glomerata* managed to parasitized caterpillars on 95% in host vs. non-host treatment and 94 % in non-host vs. host+non-host one; whereas *C. rubecula* visited 79 and 81% of plants respectively (Fig. 8).

Figure 8. Spatial distribution of parasitism of *C. rubecula* and *C. glomerata* . Bars indicate mean values. SE was calculated over 12 replicates. Wald Chi-Square = 30,056. df = 8. P < 0,0005.

Besides values of parasitism rate of *C. rubecula* and *C. glomerata*, dissection of *P. rapae* larvae followed by calculation of eggs oviposited by each of the wasp species revealed incidents of superparasitism and multiparasitism. *Cotesia rubecula* wasps caused more superparasitism in contrast to *C. glomerata*. Statistical analysis revealed significant difference between patterns of superparasitism behavior of wasps in control and both complex situations (Fig. 9).

Table 3. Numbers of *P. rapae* larvae superparasitized by either *C. rubecula* or *C. glomerata* and multiparasitized by both parasitoid wasp species from 1953 larvae collected.

| Treatment	# larvae superparasitized by:		# larvae multiparasitized
	C. rubecula	C. glomerata	
Control	115	30	197
Host vs. non-host	74	25	162
Non-host vs host+non-host	61	14	91

Analysis of the effect of treatments on the number of superparasitism incidents made by *C. rubecula* showed significant difference between control and the second complex treatment - non-host vs. host+non-host. In control it was higher. Superparasitism performance of *C. rubecula* in the first complex treatment, where the non-host was only present on the neighboring plants was the same as in control and in the second complex treatment (Fig. 9). In control situation the highest value of superparasitism by *C. rubecula* i. e. 115 incidents were fixed. In complex treatments wasps superparasitized 74 and 61 larvae of *P. rapae* respectively (Table 3).

In comparison to *C. rubecula*, *C. glomerata* showed low patterns of superparasitism, which even did not differ significantly among the treatments (Fig. 10). In control treatment *C.*

glomerata caused superparasitism to 30 caterpillars, in the host vs. non-host – 25 and in non-host vs. host + non-host treatment only 14 incidents of superparasitism were observed (Table 3).
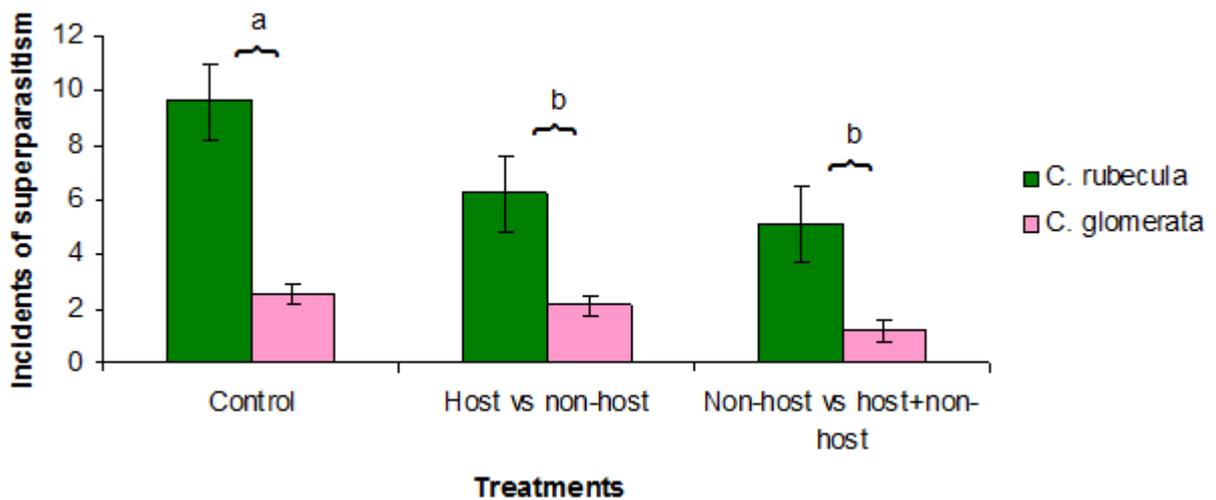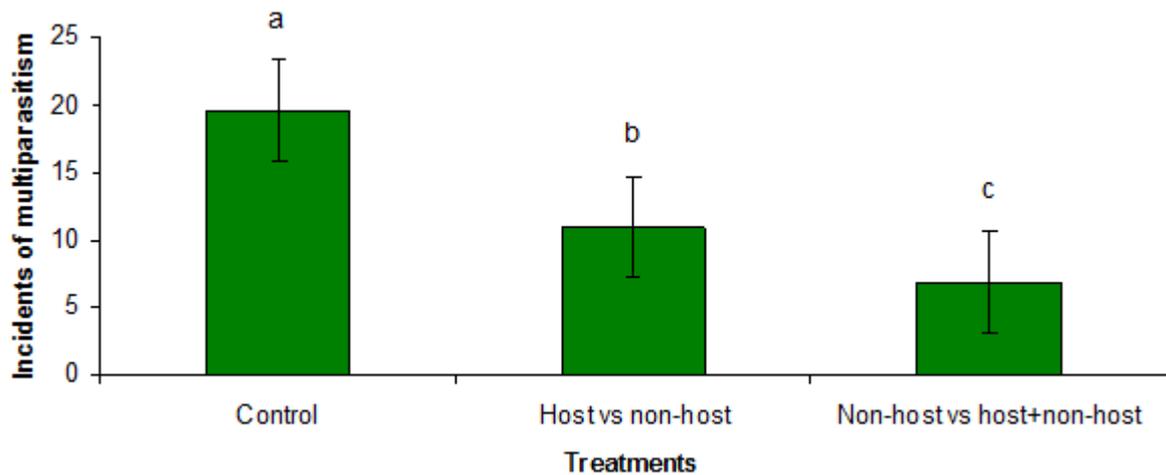

Figure 9. Incidents of superparasitism of *C. rubecula* and *C. glomerata*. Bars indicate mean values. SE was calculated over 12 replicates. Wald Chi-square =28,756. df = 8. P < 0,0005.
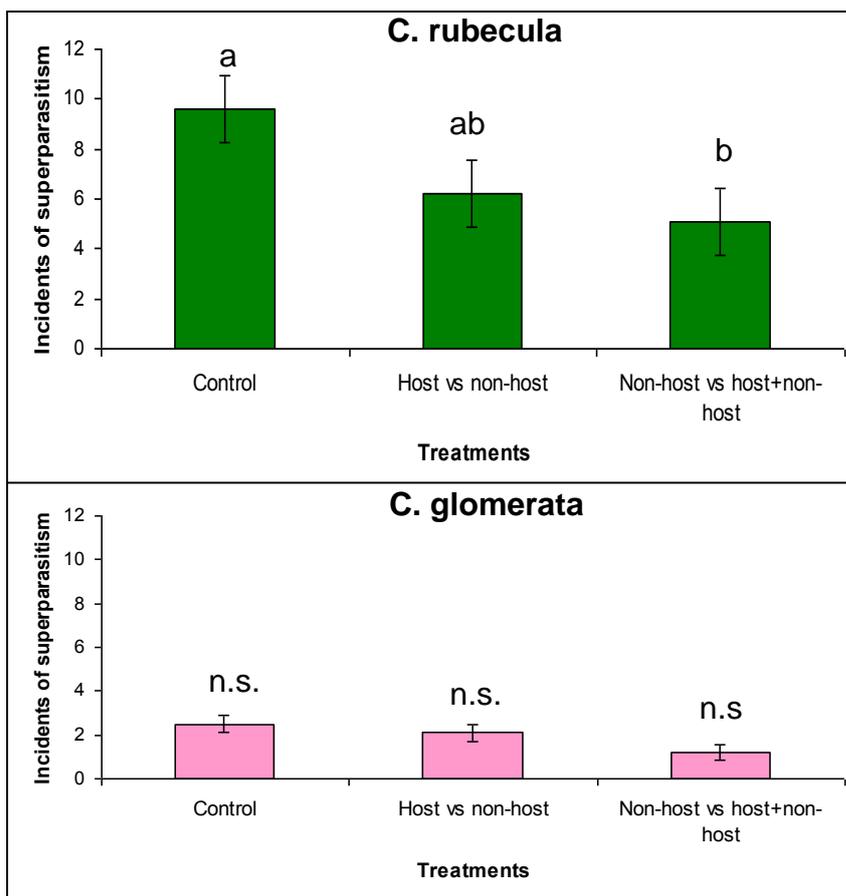
Figure 10. Incidents of superparasitism of *C. rubecula* (Overall test: Wald-Chi Square = 5,819. df = 2. P = 0,05) and *C. glomerata* (Overall test: Wald-Chi Square = 4,800. df = 2. P = 0.09). Bars indicate mean values. SE was calculated over 12 replicates.

Simultaneously with occurrence of superparasitism we observed multiparasitism in performance of parasitoid wasps. Multiple parasitism was indicated more often, comparing to

superparasitism. Under control conditions 197 hosts were parasitized by both *C. rubecula* and *C. glomerata*; whereas in the first complex situation 162 larvae and in the second complex treatment even less – 91 larvae were multiparasitized (Table 4). The mean values of multiple parasitism of parasitoids in three treatments turned to be significantly different from each other (Fig. 11).

Figure 11. Incidents of multiparasitism of *C. rubecula* and *C. glomerata.* Wald Chi-Square = 107,825. df = 8. P < 0,0005. Bars indicate mean values. SE was calculated over 12 replicates

PERFORMANCE OF HEALTHY AND ANI-TREATED *C. GLOMERATA*, COMPETING WITH *C. RUBECULA* UNDER FIELD CONDITIONS

Testing competition between healthy *C. rubecula* and ANI-treated *C. glomerata* wasps we aimed to find out if learning ability of wasps was responsible for the outcome of competitive interactions, which we observed in the previous experiment. For this, dissecting *P. rapae* larvae we counted number of eggs oviposited by each of parasitoids and parasitism rate of wasps was calculated (Table 4).

Table 4. Parasitism rates of *C. glomerata* and *C. rubecula* detected upon dissection of collected larvae of *P. rapae* from each of three series of filed experiment, testing competition between healthy *C. rubecula* and ANI-treated *C. glomerata* parasitoid wasps.

| Series no. and # tents analyzed	# larvae parasitized by C. rubecula	# larvae parasitized by C. glomerata		# larvae collected	Parasitism rate, %		
		healthy	ANI-treated		C. rubecula	C. glomerata	
						healthy	ANI-treated
Series 1 (4 tents)*	34	15	7	249	13	11	6
Series 2 (12 tents)	92	91	77	675	14	28	21
Series 3 (5 tents)**	4	0	0	240	2	0	0

* Series contained of 8 tents, each treatment was replicated twice
**Series contained 12 tents, eachtreatment was replicated three times. Caterpillars from 5 tents only were dissected, the rest of them was discarded because of extemely low parasitism rate.

The highest values of parasitism rate were revealed in series 2 of the experiment, caused by healthy (28%) and (21%) *C. glomerata,* followed by *C. rubecula* (14%). Parasitism rates in other two series of the experiment were very low, which did not allow full statistical analysis (Table 4).

The results of the second series gave some hint on the direction of the interactions within the arena where competition took place: *C. rubecula* parasitized fewer caterpillars in control

situation, as well as in more complex conditions. Parasitism rates of healthy and ANI-treated *C. glomerata* wasps were very similar, what indicates that ANI did not influence too much on the host searching behavior of wasps (Fig. 12). But still this interpretation of the results can be too preliminary due to lack of replicates.
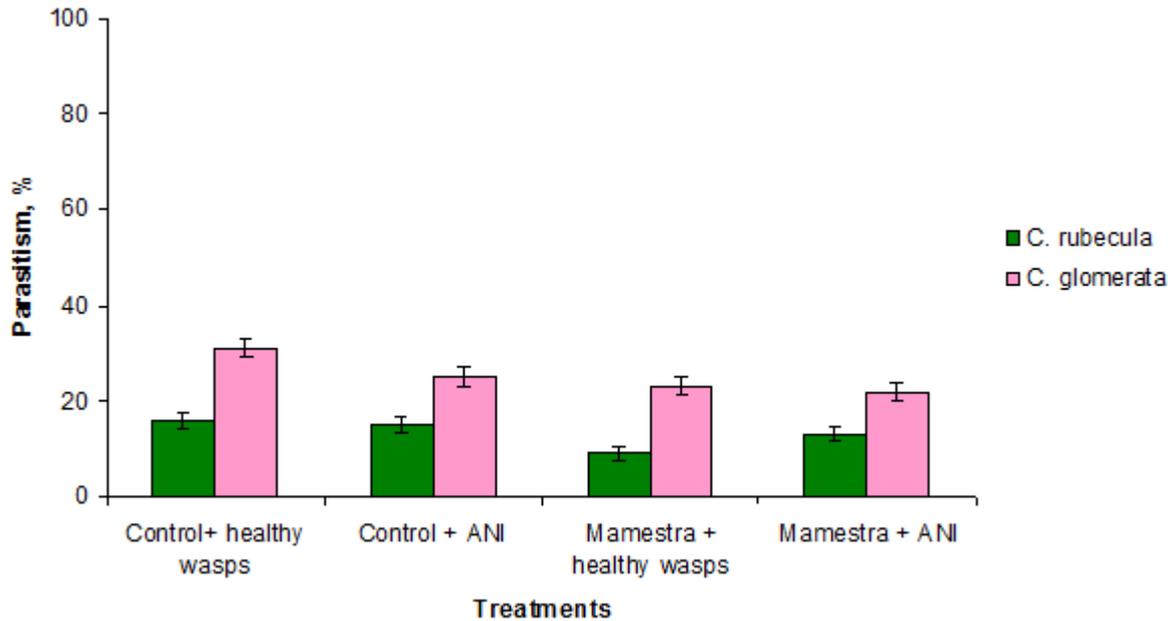

Figure 12. Fraction of caterpillars parasitized by *C. rubecula* and healthy or ANI-treated *C. glomerata* females under field conditions. Bars indicate mean values. SE was calculated over 12 replicates.


ADULT LEARNING: PREFERENCE FOR DAMAGED PLANT VOLATILES

In addition to the field experiment, the hypothesis that learning modifies searching behavior of wasps was tested in the laboratory conditions. Experienced *C. rubecula* female wasps preferred plants infested with *P. rapae* and *P. rapae + M. brassicae* over only *M. brassicae* – infested plants (Fig. 13).

Experienced females of *C. glomerata* did not show preference for *P. rapae* over *M. brassicae* – infested plants, whereas females strongly responded to *P. rapae + M. brassicae* – infested plants over *M. brassicae*-infested ones (Fig. 14).

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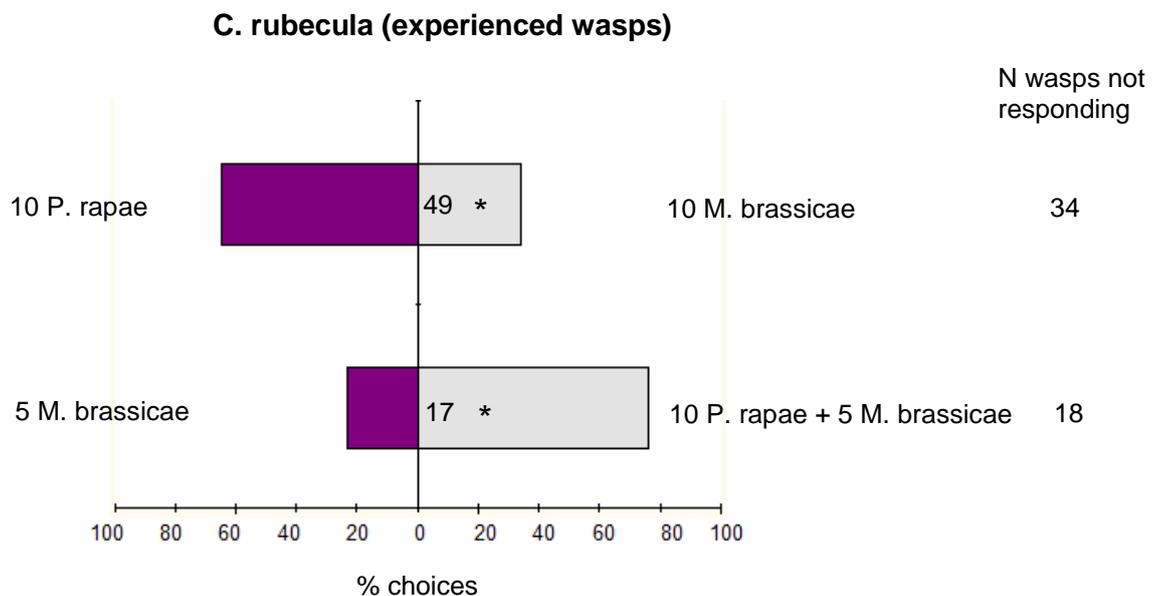
Figure 13. Choice distribution of responding experienced C. rubecula females over differently infested Brussels sprouts host plants. Numbers of responding females are indicated on the right bars. Asterisks show a significant difference within a choice test: * P < 0,05.

C. glomerata (experienced wasps)
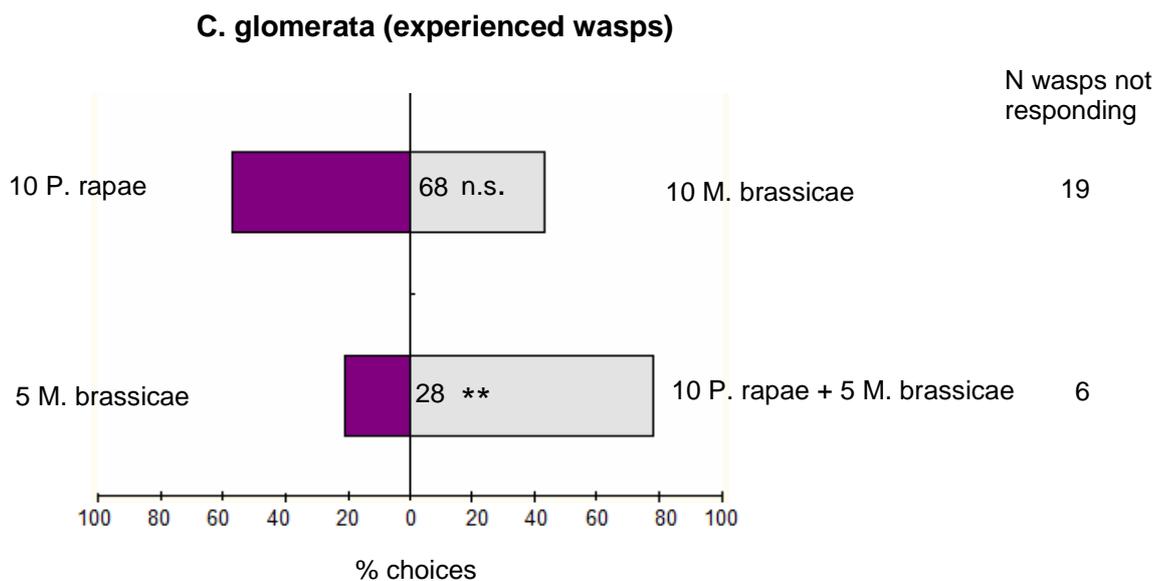
Figure 14. Choice distribution of experienced C. glomerata females over differently infested Brussels sprouts plants. Numbers of responding females are indicated on the right bars. Asterisks show a significant difference within a choice test: ** P < 0,005; n.s. not significant.

DISCUSSION

The purpose of this study was to figure out the direction of competitive interactions between *C. rubecula* and *C. glomerata* and the prerequisites of the outcome.

Assuming that parasitoid -host interactions take place within an insect community with a range of non-host insects present in the vicinity, the experiment was designed to investigate the impact of environment complexity on the performance of parasitoids. Particular level of complexity was reached through combinations of volatile information, emitted from the plants upon herbivore damage (caused by host or/and non-host); as it was elucidated that parasitoids use chemical cues to locate their host

We emphasized the role of damaged plant volatiles in the long range host searching behavior of parasitoid wasps from the estimations, that both *C. rubecula* and *C. glomerata* were mainly guided into the micro-habitat of the hosts by odors released from plants, whereas host-derived chemical cues were of secondary importance in this process (Geervliet et al., 1994).

Considering the strong dependence of wasps' host searching behavior on the presence of the chemical information in the environment, we first wanted to figure out if the volatiles emitted upon non-host damage influence on the performance of wasps.

The results of our experiment showed that, indeed presence of non-herbivore species in the foraging arena affects the outcome of competition. In a simple situation *C. rubecula* showed higher parasitism rate in comparison to that of *C. glomerata*. But under the conditions of complex treatments, recreating possible natural micro-habitats *C. glomerata* succeeded in parasitation of *P. rapae* larvae.

Thus, our hypothesis that *C. glomerata* suffered less from competition with *C. rubecula* in the presence of non-host herbivore cues within foraging area was confirmed. But still it left much to be discovered about the reasons of the obtained outcome of competitive interactions, which probably encompassed some adaptive mechanisms of organisms to a given environment.

Several studies revealed that *C. glomerata,* being a generalist larval parasitoid of several *Pieris* butterflies showed plasticity in the host-searching behavior modified by learning (Geervliet et al., 1998a; Geervliet et al., 1998b). Relying on this experience we expected that the outcome of the competition was mediated by the adaptive differences in parasitoid species characteristics, in other words *C. glomerata* was supposed to adapt to the environment through gaining experience.

In order to find out whether it was the case, we designed next experiment, which allowed to compare the performance of *C. glomerata* females treated with ANI, a substance inhibiting long term memory formation with the behavioral response of sucrose treated (healthy) wasps of the same species. The results of this experiment insinuated that long term memory did not influence the searching behavior of *C. glomerata,* as parasitism rates of ANI-treated and healthy wasps did not differ. However, we obtained data from fewer replicates as we expected.

In addition to this experiment, two-choice tests for host preference of experienced wasps were conducted in laboratory conditions. We hypothesized that spaced learning should modify behavior of wasps leading to higher responses to the plants infested with the host herbivore species.

Surprisingly, experienced *C. glomerata* wasps could not discriminate between the volatiles emitted from the plants infested by *P. rapae* and *M. brassicae.* This result was not different from that of the responses of naïve wasps (see Appendix I).

No preference for *P. rapae* infested plants can be explained by two main reasons: first, that volatiles emitted from the plants were too similar and, therefore, hardly distinguishable for the wasps (Hans Smit, personal communication); and the second reason was that *C. glomerata* preferred more *P. brassicae,* as a host in natural conditions (Poelman et al., 2009; Brodeur et. al., 1996; Geervliet et al., 2000).

The findings, related to *C. glomerata* host preference are in agreement with the results of some other studies. In one of them it was shown that *C. glomerata* was not able to discriminate between the volatiles emitted from artificially damaged leaves of Brussels sprouts treated with the

regurgitant from parasitized and unparasitized *P. rapae*, whereas they responded to the plants treated with the regurgitant of unparasitized *P. brassicae* (Fatouros et al., 2005).

Furthermore, after experience of 2 ovipositions in *P. rapae*, *C. glomerata* wasps still preferred *P. brassicae* infested plants but with less significance then after 2 ovipositions in *P. brassicae* (Geervliet et al., 1998).

Only after 3 consequent ovipositions in *P. rapae* on nasturtium, *C. glomerata* female wasps showed strong response to the volatiles of nasturtium in a wind tunnel 2-choice test (Majolein Kruidhof, personal communication).

Indeed, experience without a reward (oviposition) is not reliable enough to make a conclusion about preferences of wasps: *C. glomerata* wasps upon leaf-damage experience could not discriminate between the plants infested with *P. brassicae* and *P. rapae* (Blaakmeer et al., 1994). Only after experience with several ovipositions, *C. glomerata* was able to discriminate between correspondingly infested plants (Gervliet et al., 1998).

All together these facts suggest that the number and sequence of experience can influence on the preferences of wasps. In the current work wasps had contact with reward species and unreward, which was different from practices of mentioned experiments. Probably, by changing the sequence of the reward and unrewards experiences, other results can be obtained.

Moreover, the fact that *C. glomerata* was reared on *P. brassicae* caterpillars might enhance preference of the wasps, forming specific innate ability to respond to particular odors (Tumlinson et al., 1993).

Another explanation of no preference arises from the observation that during spaced training procedure *C. glomerata* was noticed to willingly parasitize larvae of *M. brassicae* although no offsprings could hatch from parasitized *M. brassicae*. Previously it was noticed that once *C. glomerata* females laid eggs into a host they continue doing it in the larvae offered of more or less suitable species and stage of herbivore (E. Poelman, personal communication).

Comparing results for naïve and experienced wasps, the difference was found for *C. rubecula* which after spaced training showed preference to *P. rapae.*

Previously it was estimated that only *C. glomerata* was able to shift its preference upon experience, but in fact, both *C. glomerata* and *C. rubecula* female wasps display increased flight response to the preferable host infested plant after an oviposition reward conditioning with the odor of this plant (Bleeker et al., 2006). Therefore, *C. rubecula* keeps some memory traces, caused by experience, showing plasticity in host selection (Kaiser & Cardé, 1992). This finding indicates that learning does play a role in specialized parasitoid species, but to a different extend in comparison to generalists.

In addition, being highly specialized on *P. rapae*, *C. rubecula* responded to the volatiles emitted from the artificially damaged Brussels sprouts plants treated with the regurgitant from unparasitized *P. rapae*, whereas *C. glomerata* did not show any preferences in the same treatment (Fatouros et al., 2005).

Thus, the results of performance of experienced wasps *C. rubecula* and *C. glomerata* disagreed with the hypothesis that learning could be an adaptation to the complexity of environment for *C. glomerata.*

Presumably, some other adaptive mechanisms were involved in modification of the outcome of competition between the wasps.

According to our results, in all three treatments of the experiment *C. rubecula* showed high numbers of superparasitism values, whereas *C. glomerata* showed quite low superparasitism.

Cotesia glomerata superparasitized more hosts when the probability to find unparasitised ones was low. Our results showed that the values of superparasitism by *C. glomerata* were not different one from another within the treatments, while these values were lower than that of *C. rubecula.* It suggests that *C. glomerata* did not experience a lot of difficulties in finding hosts to parasitize (van Alphen & Visser, 1990).

Probably, under the conditions of current study treatments, superparasitism was not advantageous for *C. glomerata*, even because this strategy leads to smaller offspring in gregarious parasitoids due to a trade-off between the number of offsprings, reared from a host and their size (Gu et al., 2003). Low superparasitism by *C. glomerata* could be also explained by the absence of preferable host *P. brassicae* in the foraging arena.

In contrast, for solitary parasitoid having more than one eggs in a host may provide a higher probability of gaining an offspring from that host than when there was only one egg to compete with eggs of other parasitoids (*C. glomerata* in our research). Hence, for *C. rubecula* superparasitism might be advantageous: there could be a situation when parasitoid leaving a patch where competitors were still present and searching for the host, might lose offspring because she left the hosts she parasitized vulnerable to further attack by competitor. Decision to prevent such outcome can be to stay longer and superparasitize (van Alphen & Visser, 1990). This data agree with the fact that *C. rubecula* had lowest leaving tendency on leaves containing *P. rapae,* in comparison to empty leaves and ones infested with unfavorable host (Vos et al., 1998).

Another explanation of high superparasitism caused by *C. rubecula* was based on the suggestion that it increased the probability of gaining an offspring from a host when it was able to encapsulate parasitoid eggs (van Alphen & Visser, 1990). Experimental data provided evidence that *C. rubecula* and *C. glomerata* had different strategies in overcoming encapsulation: *C. glomerata* had the capacity to overcome the immune responses of some Pieridae species, including *P. rapae,* whereas *C. rubecula,* which specialized on this herbivore, had not evolved an effective way to prevent egg encapsulation by its preferred host (Brodeur & Vet, 1995). That is why for *C. rubecula* superparasitism strategy may increase a probability to evade encapsulation.

Incidents of interspecific superparasitism, or multiparasitism were observed with the highest values in simple situation, where only host was present.

We cannot precisely estimate the sequence of interspecific parasitism into *P. rapae,* i. e. if it was caused by *C. rubecula* or *C. glomerata.* Behavioral studies showed that avoidance of multiparasitism by insects of different specialization was rare in nature and a possible reason of multiparasitism occurrence can be absence of interspecific mark recognition. But from the other side for superior competitor mark recognition was not beneficial. So, hypothetically multiparasitism was caused mostly by *C. rubecula,* which was a superior competitor over *C. glomerata* within host body (Liang & Corrigan, 1987). This estimation was supported by the fact that *C. rubecula* succeeded in parasitism of larger amount of *P. rapae* in simple environment, which could lead to high occurrence of multiparasitism.

Considering superparasitism as an adaptive strategy of *C. rubecula* we could state that this parasitoid spent more time on this strategy in complex environments and, therefore, *C. glomerata* got some advantage over *C. rubecula*, but the fact that superparasitism was the highest in control situation doubting this idea.

Together with investigations of superparasitism and multiparasitism, we detected spatial distribution of wasps, which showed that *C. glomerata* visited almost all plants in all three treatments, whereas *C. rubecula* succeeded only in control. This observation suggested that *C. glomerata* being an inferior competitor had superior colonization ability over *C. rubecula* in a complex environment (Amarasekare & Nisbet, 2001). This could be an example of interspecific trade-off between colonization and competitive abilities (Tilman et al., 1994). Therefore, if *C. glomerata* encountered more hosts, there was no advantage to superparasitize.

In addition to behavioral patterns which were directly investigated within current research, there could be other mechanisms, responsible for performance of wasps. Comparison of the patch exploitation behavior of two parasitoids revealed one of the possible reasons why *C. glomerata* did not show flexibility in host-searching behavior.

Two parasitoid species showed interspecific difference in the way they made patch leaving decisions. *C. rubecula* had a higher leaving tendency than *C. glomerata* in three environments of multiple patch set-ups: Brussels sprouts plants were infested by either *P. rapae* or *P. brassicae* or both herbivore species. *C. rubecula* used simple strategy – high leaving tendency on the empty

plants, low leaving tendency on *P. brassicae-* infested plants and the lowest one on preferable host *P. rapae* – infested plants. *C. glomerata* had a lower leaving tendency on plants with preferred host *P. brassicae* than on plants infested with *P. rapae.* Patch leaving decisions in *C. glomerata* were modified by experience. However, it was noticed that in environments, containing *P. rapae* information on previous patch did not affect the leaving tendencies of *C. glomerata,* hence, experience did not change patch leaving decisions and as a result *C. glomerata* followed the same simple strategy of patch exploitation as *C. rubecula* did (Vos et al., 1998).

This pattern of patch exploitation behavior added strength to the idea, that experienced on *P. brassicae, C. glomerata* would be able to show plasticity in host-searching behavior as it was more preferable host despite the fact that *C. glomerata* is a generalist on several *Pieris* species. However, we only can hypothesize the role of patch exploitation by wasps performance in our research.

Furthermore, if parasitoids gain access to the host through perception of damaged plant volatiles, they have to deal with qualitative and quantitative differences in infochemicals (Geervliet et al., 1998). In our experiment we introduced two herbivore species *P. rapae* and *M. brassicae.* Damage caused by each of herbivorous insect can be followed by emission of qualitatively different volatiles. Moreover, the specific content of volatiles produced by plant in response to herbivore can change when the same herbivore species infested one plant. Quantities of volatiles also play a role. Higher quantities are likely to be better detectable to the parasitoids (Geervliet et al., 1998). Experiments with naïve *C. rubecula* and *C. glomerata* wasps showed that it was true, however when naïve *C. glomerata* was offered to discriminate between plant infested with equal amounts of caterpillars (15 *P. brassicae* vs. 5 *P. brassicae* +10 *P. rapae*), they highly preferred plants, damaged by two herbivore species (Appendix I). This finding suggested that although wasps were attracted to the plants with higher quantity of volatiles, quality of emissions might play more important role.

As it was mentioned above, we experienced some difficulties in obtaining enough data from the field experiment, testing the role of learning in performance of *C. glomerata* wasps. Low parasitism rates turned to be the reason of lack of experimental data for sufficient statistical analysis. We summarized some factors which could interfere with the results of the work.

In fact, a range of abiotic and biotic conditions may influence on the responsiveness of parasitoids towards plant volatiles and therefore, on the rate of parasitism (Poelman et al., 2009).

The most important are the seasonal trend in emission of volatiles by plants and environmental conditions:

Headspace analysis of cabbage plants
Emission of volatiles by intact cabbage plants has a distinct seasonal trend. Production of volatiles is the highest during the summer, then it drastically declines in autumn (in September), very low in winter and then gradually increases in spring. This trend suggests carrying out experiments concerning plants headspace volatiles during summer months with intact plants as well as with herbivore-damaged ones (Blaakmeer et al., 1994).

The influence of environmental factors on the wasps' performance
Environmental conditions, namely temperature, relative humidity, wind velocity, light intensity and precipitation are known to play a great role in the tendency to take off and the ability to initiate an orientated flight by insects. It was shown that for braconids maximum flight activity can be expected when there is a mean temperature above 21ºC, a mean relative humidity of 30%, and a wind velocity of up to 0.7 m/s (Juillet, 1964).

Take off values were much higher under the conditions of the still air (10-30 cm/s) and high light intensity (1600 lux). Low light intensity mostly associated with high humidity (above 90%) and sometimes with low temperatures and consequently with reduced number of flights to the plants.

Moderate to heavy precipitation reduces braconid activity and can even destroy insect population (Juillet, 1964).

Thus, in general braconids prefer warm and dry habitats.

These results matches with our observations during the field experiment that on cloudy and windy days few wasps were seen flying around and therefore the level of parasitism of caterpillars did not give any fruitful results (Gu & Dorn, 2001).

Effect of barometric flux on the response of the wasps

Experimental data suggests the existence of dependence between the response of the wasps and direction of barometer change: with the increase of barometric pressure, the response of the tested *C. glomerata* wasps was higher in comparison to the days with decreasing or fluctuating barometric pressure. Increasing barometric pressure correlates with the stable weather conditions and therefore favors to better responses of the insects, while during the stormy days (decreasing or fluctuating barometric pressure) it may be more advantageous for the wasps to hide than to show flying responses (Steinberg et al., 1992).

In addition to seasonal trend of plant volatiles emissions and adverse weather conditions, one of the problems was presence of natural insect community on the plants used in the experiment. This fact can cause so called "volatile noise", which might have effect on the quality and quantity of damaged plant volatiles.

Negative effect of mentioned factors could be enhanced by the fact that the series if the experiment were held during September. Geervliet et al. pointed that the percentage of parasitism in September was relatively low in field experiments (Geervliet et al., 2000).

CONCLUSIONS

The outcome of competition between *C. rubecula* and *C. glomerata* was mediated by the complexity of environment: presence of non-host species was advantageous for generalist species – *C. glomerata.*

Overall data suggested that learning was not the key factor of *C. glomerata* wasps' successful performance in complex treatments.

Difference in superparasitism between wasps together with the data of spatial distribution of parasitoids suggests that for *C. rubecula* superparasitism was an adaptive strategy to compete with *C. glomerata* for host, whereas avoidance of superparasitism was adaptive for *C. glomerata* which managed to find sufficient number of unparasitized hosts through higher colonization ability in complex environments. Therefore, *C. rubecula,* being superior competitor turned to be inferior colonizer, whereas *C. glomerata,* being inferior competitor, performed as superior colonizer.

FUTURE PERSPECTIVES

To get more detailed insight into the mechanisms involved in preferences of experienced wasps, a GC analysis of headspace volatiles emitted upon the damage caused by *M. brassicae* would be useful. This data is already available for the plants infested by *P. brassicae* and *P. rapae* (Blaakmeer et al., 1994).

Furthermore, it would be useful to investigate the preferences of *C. glomerata* experienced on *P. brassicae* as a reward and *M. brassicae* as unreward in a situation where plants would be infested with mentioned herbivore species. Previously *C. glomerata* wasps, which had leaf-damage experience caused by either *P. brassicae* or *M. brassicae* experiments showed no preference for either *P. brassicae* or *M. brassicae* infested plants in two-choice experiment (Geervliet et al., 1998). Probably the results could be different if experienced by the meaning of spaced training *C. glomerata* would be tested for preference between *P. brassicae* or *M. brassicae* infested plants. Together with this responses of experienced wasps to the quality and quantity of damaged plants volatiles should be investigated to claim whether quality of volatiles play a great role in guiding parasitoid into host micro-habitat.

In addition, as it was mentioned above, the method of giving experience to the wasp seems to influence on the responses, so different sequences of reward and unreward experiences should be tested.

Moreover, it could be feasible to compare the values of minimal blend components which are necessary to trigger searching behavior of *C. glomerata* and *C. rubecula* for their hosts. But this data is difficult to discover (Joop J. A. van Loon, personal communication).

And finally, to clarify the extend of interspecific competition-colonization trade-off between *C. rubecula* and *C. glomerata*, field work is needed. This can be done by creating habitat destruction patches.

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APPENDIX I

PREFERENCES OF NAÏVE *C. glomerata* AND *C. rubecula* TO PARTICULAR HERBIVORE INDUCED PLANT VOLATILES
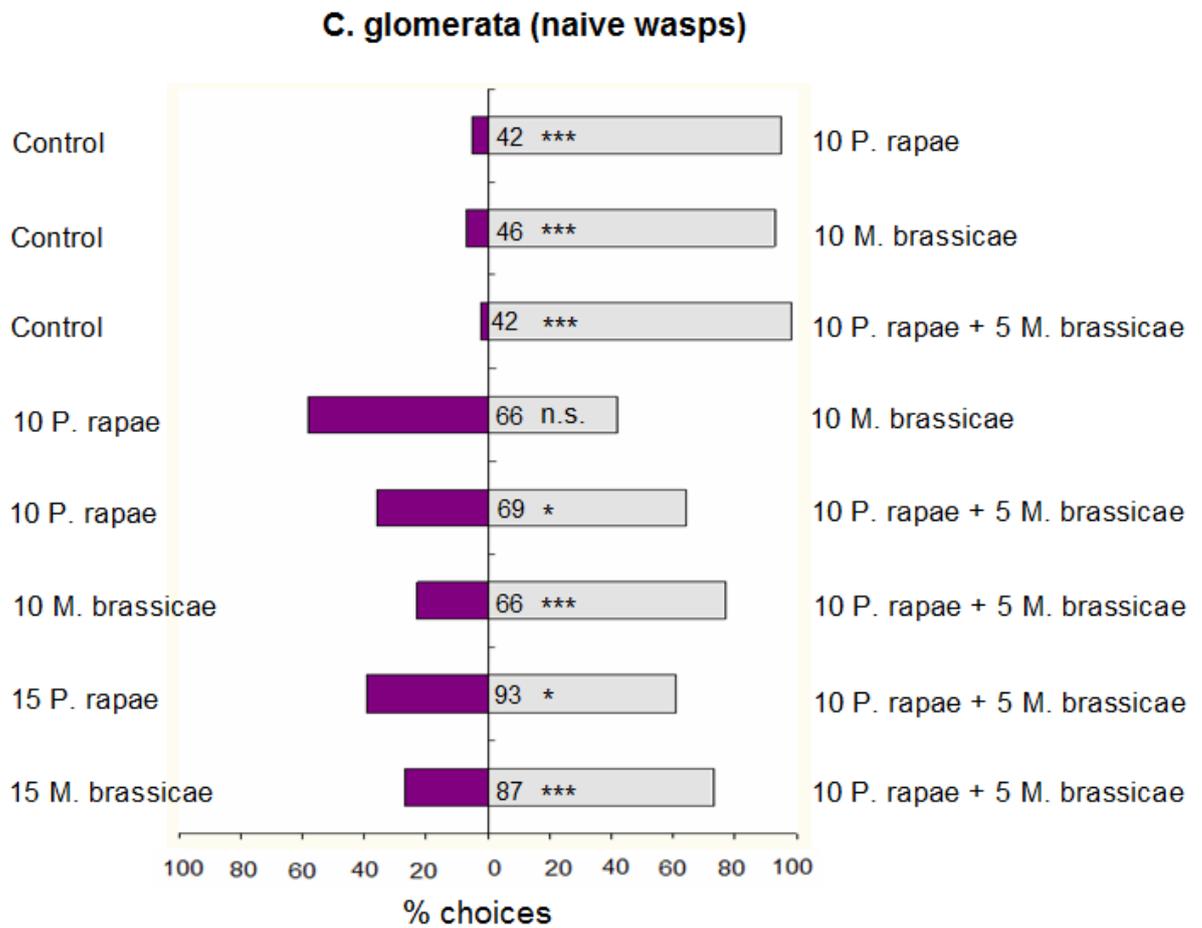

Figure 1. Distribution of choices of naïve *C. glomerata* females in windtunnel bioassays, where the plant were either undamaged, or induced by hosts (*P. rapae*) or non-hosts (*M. brassicae*). Herbivores were either introduced as pure or mixed patches in different densities. Asterisks indicate a significant difference within a choice test: * $P<0.05$, *** $P<0.0005$, n.s. not significant. Numbers of responding females are on the right bars (E. H. Poelman, unpublished data).
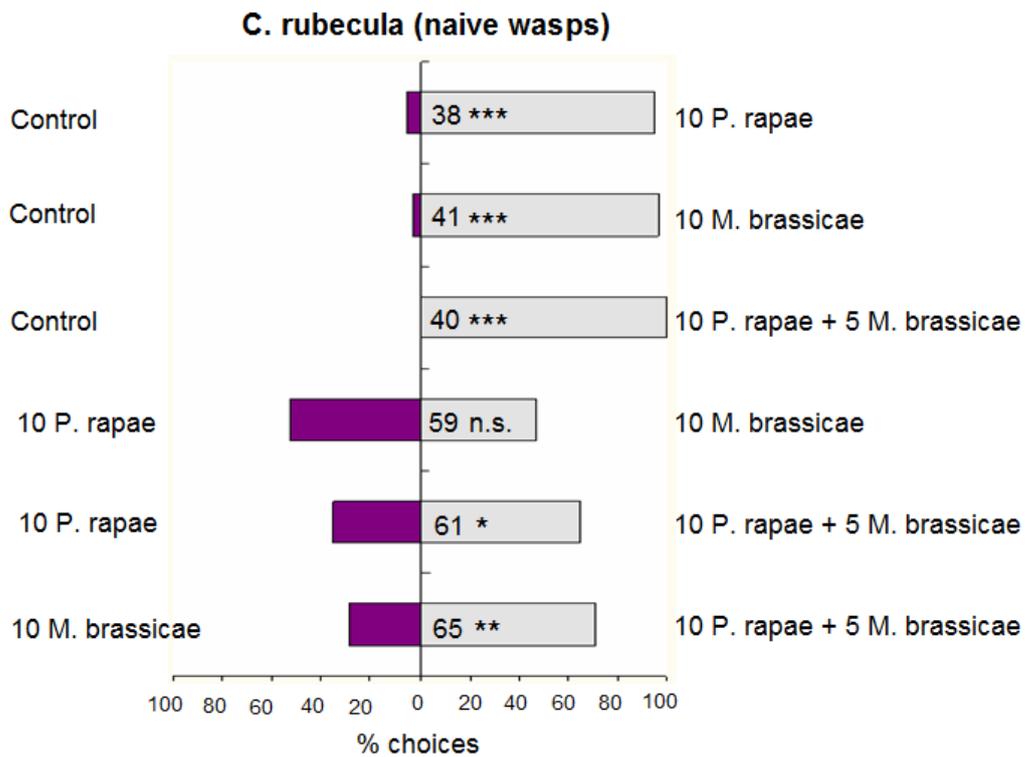
Figure 2. Distribution of choices of naïve *C. rubecula* females in wind tunnel bioassays, where the plant were either undamaged, or induced by hosts (*P. rapae*) or non-hosts (*M. brassicae*). Herbivores were either introduced as pure or mixed patches in different densities. Asterisks indicate a significant difference within a choice test: * P<0.05, *** P<0.0005, n.s. not significant. Numbers of responding females are on the right bars (E. H. Poelman, unpublished data).

APPENDIX II

DEVELOPMENTAL STAGES OF *C. rubecula* AND *C. glomerata* IN CATERPILLARS OF *P. rapae*
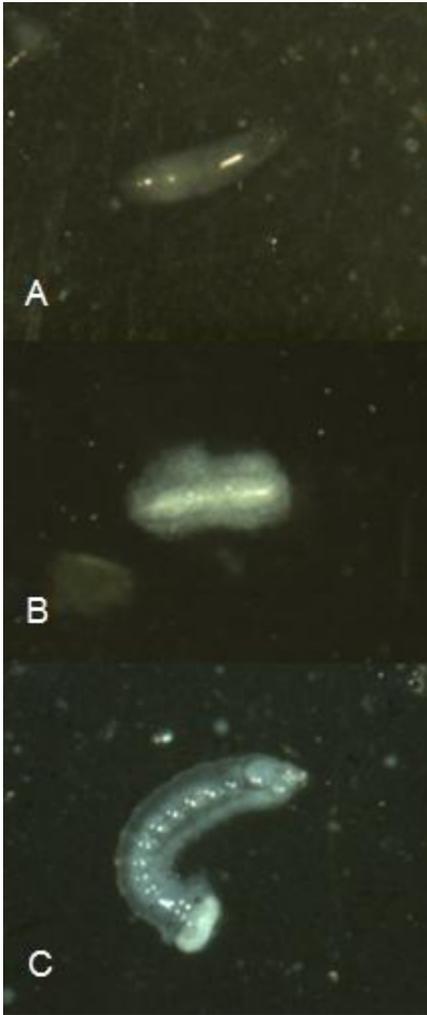
Figure 1. *C. rubecula* development stages within host *P. rapae*: A – freshly laid egg; B – encapsulated egg (third day after oviposition); C – newly hatched larva (fifth day after oviposition). Parasitized caterpillars of *P. rapae* were kept in climate chambers in 25°C.
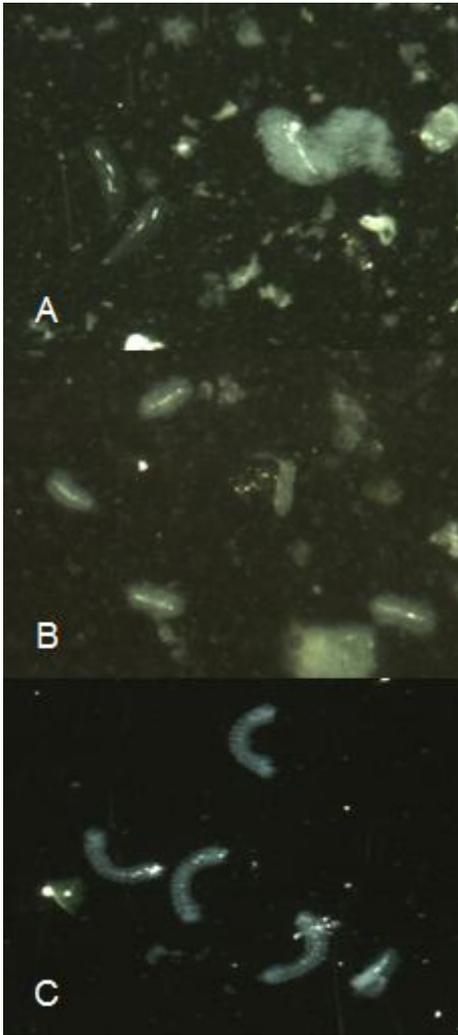
Figure 2. *C. glomerata* developmental stages in *P. rapae* caterpillar: A – encapsulated and not encapsulated eggs (next day after oviposition); B – encapsulated eggs (third day after oviposition); C – newly hatched larvae. Parasitized caterpillars of *P. rapae* were kept in climate chambers in 25ºC.