Foraging behaviour of parasitoids in multi-herbivore communities

2016

Marjolein de Rijk

Propositions

1. Field experiments are essential to understand insect behaviour.
(this thesis)

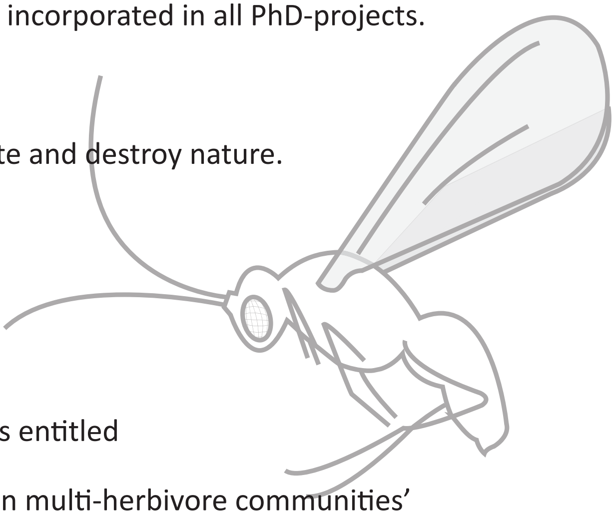
2. Parasitoids are not easily distracted from finding their herbivorous hosts.
(this thesis)

3. The expectation that interaction webs of ecosystems can be disentangled is not realistic.

4. Although science is objective in its content, its organisation is directed by subjectivity.

5. An industry internship should be incorporated in all PhD-projects.

6. Humankind has no right to pollute and destroy nature.



Propositions belonging to the thesis entitled

‘Foraging behaviour of parasitoids in multi-herbivore communities’

Marjolein de Rijk, 12 February 2016

Foraging behaviour of parasitoids in multi-herbivore communities

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Foraging behaviour of parasitoids in multi-herbivore communities

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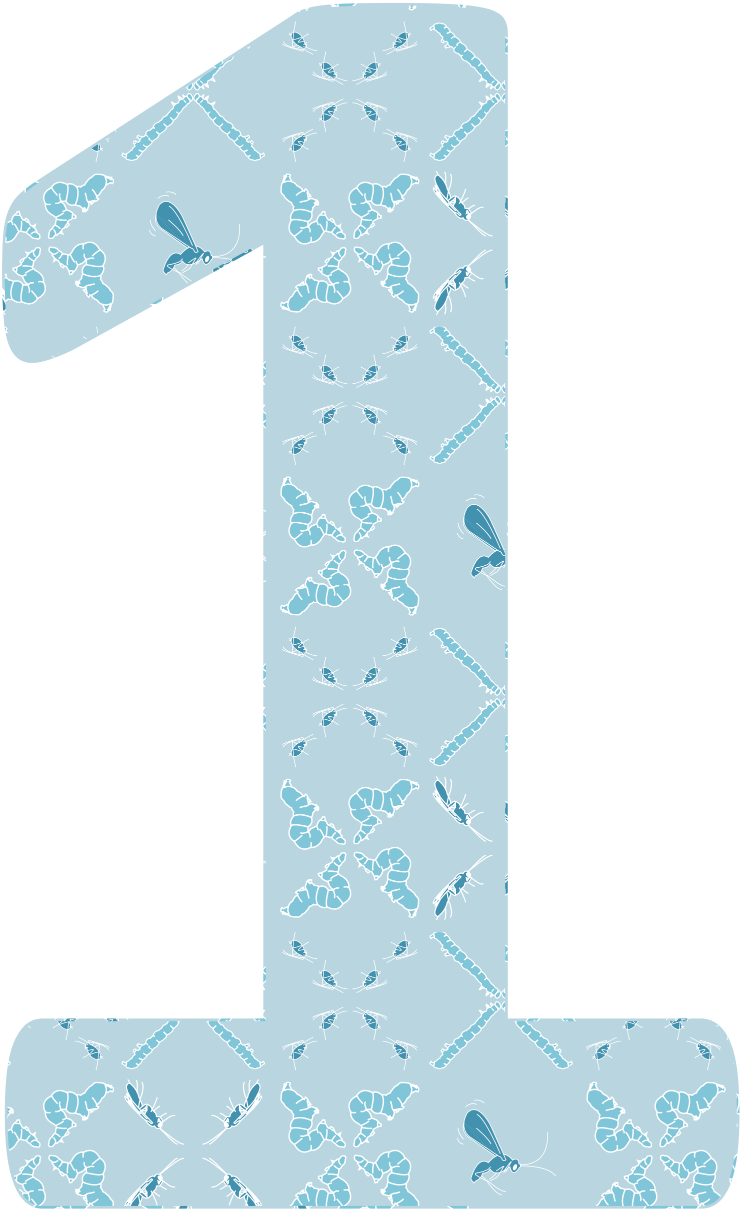

Chapter 1

General introduction

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Studies on animal foraging can elucidate how animals maximize their resource intake (Wajnberg 2006). Moreover, foraging behaviour has important effects on how animals affect population dynamics of their food organisms and, consequently, foraging behaviour may have community-wide effects (Lotka 1925, Volterra 1928, Utsumi et al. 2010). Finally, foraging theories are even applicable in robotics and computer science for designing optimal decision-making behaviours of task-processing agents (Pavlic and Passino 2011). An important part of animal foraging is making “decisions”, e.g. which patch to choose, which prey to accept, and when to leave the patch. These decisions have important consequences for an animal’s fitness (McArthur and Planka 1966, Van Alphen et al. 2003, Petchey et al. 2008). To maximize fitness, decision making and, thus, foraging strategies should be optimized. To achieve this, predators may use information from the environment to locate their prey and to assess the profitability of a food patch (Lima and Dill 1990, Vet and Dicke 1992, Schmidt et al. 2010, Kessler and Heil 2011), whereby the spatial structure of the environment can have a strong effect on decisions of foraging animals (Wajnberg 2006). The spatial structure may be determined by the presence of different environmental elements that are not of interest to the predator but make the environment more complex. An environment may, for instance, contain organisms that are not suitable as prey for the predator and these non-prey may have a negative or positive effect on predator foraging strategies (De Rijk et al. 2013). Parasitic wasps (parasitoids) are often used in studies of animal foraging. These carnivorous insects, comprising at least 50,000 species, are dependent on hosts for the development of their offspring. Parasitoids lay their eggs in or on the host, which is subsequently used by the parasitoid larvae as their food source and eventually dies (Godfray 1994). This strong link between foraging for hosts and the production of offspring, makes parasitoids very likely to be under strong selection to optimize their foraging behaviour (Thiel and Hoffmeister 2009).

The foraging process of parasitoids to find herbivorous hosts can be divided in 1) finding a host-infested plant and 2) finding, recognizing and accepting the host on the plant (Van Alphen et al. 2003, De Rijk et al. 2013). During the first phase, parasitoids use plant volatiles induced by herbivore feeding (HIPVs) (Vet and Dicke 1992, Hare 2011), whereas host-produced infochemicals can be used during the second foraging phase when the parasitoids search for hosts on the plant (Van Alphen et al. 2003, Colazza et al. 2014). During both phases of foraging, the presence of herbivores that are not suitable for larval development (non-host herbivores) can distract the parasitoid (De Rijk et al. 2013). Even though both phases of foraging can be influenced by non-host presence, previous studies often investigated only a single phase. Also, previous studies generally investigated the effect of only a single non-host species or trait (for overview see De Rijk et al. 2013). In the literature no examples were found of studies of a specific parasitoid-herbivore-plant complex in which one of the factors (plant, host, non-host, parasitoid) was comprehensively varied to elucidate its effect on that tritrophic system. As a result of this approach, the relatively large amount of available data on the impact of non-host presence on parasitoid foraging does not give a comprehensive overview from which generalizations could be deduced. In order to make general predictions, the influence of a high number of traits of plant, herbivore and parasitoid in several parasitoid-herbivore-plant complexes should be tested. Such an elaborate investigation would consist of several studies into individual parasitoid-host-food plant complexes from which generalizations for these specific plant-insects combinations could be deduced.

The aim of this thesis was to study the impact of non-host presence on the parasitoid-host-food plant complex of *Cotesia glomerata* with its host *Pieris brassicae* and a monoculture of the cultivated plant *Brassica oleracea*. Single non-host traits of several non-host species were systematically varied. As a result, a clear and comprehensive overview could be obtained of non-host effects on the behaviour of *C. glomerata* foraging in the chosen system. Additionally, I studied if the parasitoid could learn to associate non-host cues with the presence of hosts and if the parasitization preference was changed accordingly. Based on the results of this thesis, generalizations could be made that apply to the specific plant-insect community that was investigated.

Study system

Cotesia glomerata

Cotesia glomerata (Hymenoptera: Braconidae) is a gregarious endoparasitoid with a cosmopolitan distribution (Laing and Levin 1982). In The Netherlands it is a generalist on several species of Pieridae (Brodeur et al. 1996), with *Pieris brassicae* as its preferred host (Geervliet et al. 2000). First and second instar caterpillars are preferred for oviposition (Brodeur et al. 1996). The parasitoid utilizes plant volatiles to locate host-infested plants and close-range cues (e.g. leaf damage, frass and silk) to locate hosts on plants (Laing and Levin 1982). *Cotesia glomerata* is able to associate cues (e.g. herbivore-induced plant volatiles) with the presence of hosts and to adapt the foraging behaviour accordingly (Geervliet et al. 1998a, Geervliet et al. 1998b, Bleeker et al. 2006, Smid et al. 2007, Kruidhof et al. 2012a, Kruidhof et al. 2012b).

Pieris brassicae

Pieris brassicae (Lepidoptera: Pieridae) (large cabbage white) caterpillars are oligophagous, feeding on plants within the Brassicaceae family. The female butterfly lays egg clusters of 7-150 eggs in a patchy distribution over the plant population (Vos et al. 1998). Early-instar caterpillars feed gregariously from the food plant. Late-instar caterpillars spread over food plants in the environment.

Non-host herbivores

Brevicoryne brassicae (Hemiptera: Aphididae) and *Myzus persicae* (Hemiptera: Aphididae) were used as non-host aphids. Two strains of the asexually reproducing aphid *M. persicae* were used and considered different based on a clear difference in the stickiness and the amounts of honeydew produced. Five species of non-host caterpillars were used: *Mamestra brassicae* (Lepidoptera: Noctuidae), *Plutella xylostella* (Lepidoptera: Plutellidae), *Spodoptera exigua* (Lepidoptera: Noctuidae), *Autographa gamma* (Lepidoptera: Noctuidae), *Trichoplusia ni* (Lepidoptera: Noctuidae). All these non-hosts feed on *B. oleracea* plants.

Brassica oleracea

The cultivated *Brassica oleracea* (Brassicales: Brassicaceae) var gemmifera cv. Cyrus (Brussels sprout) was used as host plant.

Outline of thesis

Chapter 2 reviews the impact of non-host herbivore presence on the behavioural responses of parasitoids. Non-hosts can be present on neighbouring plants or share food plants with hosts and they can affect the plant-volatile-based as well as the host-infochemical-based searching behaviour of parasitoids. A literature-based overview of the effects of non-host herbivore presence on foraging by carnivorous arthropods is discussed as well as the possible effects of non-hosts in an ecological context.

Non-host herbivores can feed on different plant parts (e.g. shoots and roots) using different types of feeding (e.g. biting-chewing and piercing-sucking). In chapter 3, I studied the influence of leaf-chewing and phloem-feeding non-host herbivores on parasitoid foraging behaviour. Three species of leaf chewers and three types of phloem feeders were individually combined with *P. brassicae* hosts to form different insect communities. The plant-volatile-based and host-infochemical-based searching behaviour of C. *glomerata* in these communities was studied in the laboratory, whereas the foraging efficiency of the parasitoid was studied in a field setting.

The distribution of host and non-host herbivores over several food plants can vary, as well as the distribution over the leaves of an individual plant. In chapter 4, I studied how the position of herbivores on a single plant influenced the foraging behaviour of *C. glomerata*. In nature, the specialist host *P. brassicae* feeds on young, well defended leaves, whereas the generalist non-host *M. brassicae* feeds on old, less defended leaves. These herbivores were differently positioned on the plant to study how the feeding position of herbivores affected the behaviour of the parasitoid in laboratory and field settings.

In natural and agricultural environments, non-host herbivores occur in different densities. In chapter 5, I studied how the density of four non-hosts species (*Spodoptera exigua*, *Autographa gamma*, *Plutella xylostella* and *Mamestra brassicae*) influenced the foraging behaviour of *C. glomerata*. In this chapter, the plant-volatile-based and host-infochemical-based searching behaviour was studied in the laboratory and the resulting foraging efficiency of the parasitoid was studied in a field-tent set-up.

The influence of species diversity on ecosystem functions and services is extensively studied. In chapter 6, however, I studied how diversity and identity of non-host herbivores influenced a single community member, i.e. *C. glomerata*. In laboratory set-ups, five species of non-hosts were used to create environments with different levels of non-host diversity. Several combinations of non-host species were made to be able to identify both the effects of species diversity and species identity.

Cotesia glomerata parasitoids can learn to associate environmental cues with the presence of hosts. Based on this knowledge and the results described in chapter 3, in chapter 7 I studied if parasitoid foraging behaviour was adapted after acquiring oviposition experience on a plant infested with either hosts and non-host caterpillars or hosts and non-host aphids. The effect of learning on the landing preference of the parasitoid was studied in a laboratory setting, whereas the effect of learning on the parasitization preference was studied in a field-tent set-up.

Finally, in chapter 8, I discuss the impact of the presence of non-host herbivores on parasitoid individuals, parasitoid-host dynamics and parasitoids on a landscape level.

I address the importance of studying the foraging process as a whole and how knowledge of non-host interference could be used in biocontrol practices. The chapter ends with the main conclusions of the thesis and suggestions for future research.

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Chapter 2

Foraging behaviour of parasitoids in multi-herbivore communities

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Parasitoid foraging decisions are often affected by community characteristics such as community diversity and complexity. As part of a complex habitat, the presence of unsuitable hosts may affect foraging behaviour of parasitoids. First, unsuitable herbivores may affect the localization of patches where hosts are present. Second, encounters with unsuitable herbivores in the food-plant patch may affect parasitoid decisions during their searching behaviour in the patch. In this review, we outline the importance of the presence of unsuitable herbivores on the behavioural responses of parasitoids during both these foraging phases. Non-hosts feeding on a neighbouring plant or on the same plant individual the host is feeding from, may affect odour-based searching by parasitoids in a way specific for the species combination studied. Feeding by specific host and non-host-herbivore combinations may induce volatiles that are more, less or equally attractive compared to plants infested by the host only. Within the food patch, mixed presence of host and non-host may reduce the number of hosts parasitized per time unit and reduce parasitoid foraging efficiency. Importantly, we show that a single non-host species may have contrasting effects in terms of its effects on odour-based searching and patch-residence decisions. We conclude that studying host searching behaviour at both phases of foraging is essential for our understanding of parasitoid foraging behaviour in natural and agricultural settings. We further speculate on the ecological context in which unsuitable herbivores affect either of the two phases of parasitoid foraging.

Keywords
Foraging efficiency, herbivore induced plant volatiles, HIPV, multi-herbivory, non-host, parasitoid behaviour, patch residence time

Foraging in a complex habitat

To optimize their foraging strategies, predators may use information on where their prey is located and how profitable a certain food patch may be (Vet and Dicke 1992, Schmidt et al. 2010, Kessler and Heil 2011). However, prey are members of complex communities and share their environment with non-prey that may affect predator foraging decisions. Hence, predators foraging in species-rich communities are exposed to natural selection so as to deal with the presence of non-prey organisms and cues derived from these. Studies on parasitic wasps or parasitoids have played a major role in shaping and testing foraging theory (Godfray 1994). Parasitoids lay their eggs in or on other organisms that function as a host for the development of their offspring eventually resulting in the death of the host in which the larvae develop (Godfray 1994). The host-searching behaviour of various parasitoids has been studied extensively in tri-trophic systems consisting of a single food chain of plant, herbivore and parasitoid species (Vet and Dicke 1992, Heil 2008). In (agro-)ecosystems, however, parasitoids forage in a complex habitat of a diverse plant and herbivore community (Dicke et al. 2009). Only in the last decade, experimental studies have addressed parasitoid foraging behaviour in more natural, complex habitats. Results from these studies have shown that predictions on parasitoid foraging in simple tri-trophic communities should be nuanced for foraging behaviour of parasitoids in more complex habitats (e.g. Rodriguez-Saona et al. 2005; Bukovinszky et al. 2012b). One of the factors of a complex habitat is the presence of a community of other herbivores that may be unsuitable host species (here called: non-host herbivores). The presence of other herbivores in the habitat where parasitoids search for hosts has been shown to strongly affect parasitoid foraging behaviour (Rodriguez-Saona et al. 2005, Dicke et al. 2009). These non-host herbivores may either be present on neighbouring plants or share the same plant with host herbivores of a parasitoid. The shared food plant may be attacked simultaneously or sequentially (Vos et al. 2001, Poelman et al. 2010) and on the shared food plant the herbivores may feed on a single plant organ or may feed spatially separated on different plant organs above- as well as belowground (Van Dam and Heil 2011). As a result, the presence of non-host herbivores can affect parasitoid foraging behaviour on several levels, from finding the plant the host is feeding from to locating the host on the food plant and deciding whether or not to parasitize the host, each decision phase being important attributes to parasitoid fitness (McArthur and Planka 1966, Van Alphen et al. 2003). In host location, the presence of non-host herbivores may affect the parasitoid in two phases. First, like several other biotic and abiotic factors, non-hosts may influence the ability or efficiency of parasitoids to locate patches of host-infested plants from a distance (Gouinguené and Turlings 2002, Dicke et al. 2009). Parasitoids exploit so-called herbivore-induced plant volatiles (HIPVs) to locate their host (Vet and Dicke 1992, Heil 2008). HIPVs are blends of volatile compounds and are emitted by plants in response to attack by arthropod herbivores (Turlings et al. 1995, Arimura et al. 2005, Dicke and Baldwin 2010, Mumm and Dicke 2010). In more complex habitats, HIPV cues of host presence are surrounded by noise of volatiles induced by unsuitable hosts (Dicke et al. 2009). Second, non-hosts may affect foraging decisions in the patch: once a host-infested plant is located, encountering co-occurring non-hosts or their products on the plant could interfere with foraging decisions such as the time spent to search for hosts on the plant (Shiojiri et al. 2001, Bukovinszky et al. 2012b). Both of these phases together predict parasitoid host finding efficiency, but typically these two phases of host location are studied separately. Importantly, recent studies that did combine these two phases of parasitoid foraging have shown that effects of non-hosts on each phase may result in different predictions on foraging efficiency (Bukovinszky et al. 2012b). The effect of non-hosts on parasitoid foraging decisions may be determined by the host range specialization (generalist – specialist) of parasitoids. Moreover, there is a large potential of specificity of effects that non-hosts may have on either phase of parasitoid foraging that may be determined by the feeding guild (Van Poecke et al. 2003, Delphia et al. 2007, Dicke et al. 2009), species (Hare 2011), development stage (Yamamoto et al. 2011) and density (Zhang et al. 2009) of the non-host herbivore (Fig. 1). Here, we review the effects of unsuitable hosts on two phases in foraging, i.e. 1) responses of parasitoids to HIPVs and 2) decisions of parasitoids when foraging for their hosts on a plant. For each of these phases we scale down from effects of non-hosts when located on neighbouring plants to non-host presence on the same plant or leaf as the host herbivore is feeding on. We conclude that linking the responses of parasitoids to HIPVs and the responses to non-host encounters on the plant is crucial for our understanding of parasitoid foraging decisions under natural conditions where plants are attacked by multiple herbivore species.

Response to HIPVs induced by dual herbivory

In order to find their herbivorous hosts, parasitoids use information from their environment. Hosts are under strong selection to be inconspicuous, minimizing cues that directly give away their presence to their enemies. However, while feeding on their food plant, herbivores may give away their presence indirectly by inducing the emission of volatiles by their food plant. Although these HIPVs that plants produce are well detectable by parasitoids, the plant cues might not be as reliable as direct information received from the host (Vet and Dicke 1992). Parasitoids have to face this reliability-detectability

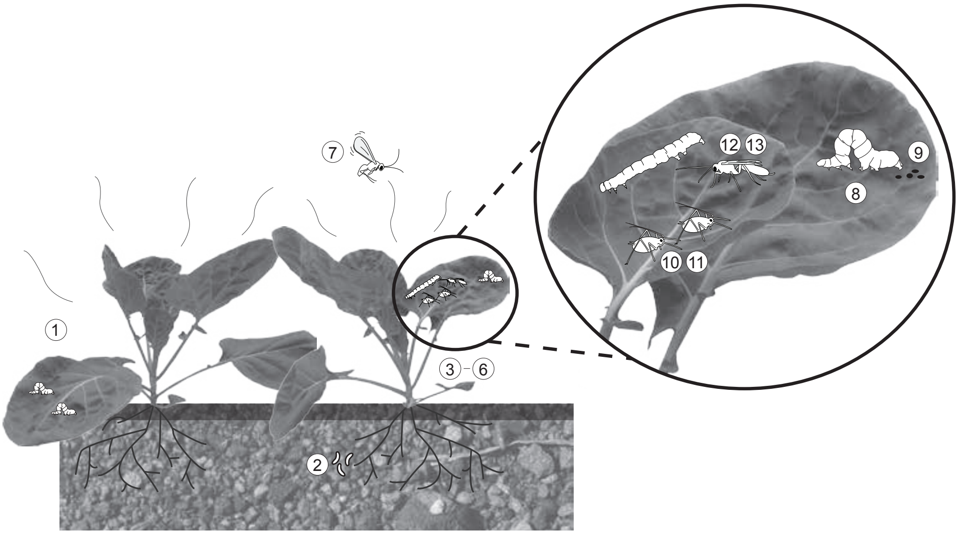

Figure 1. Factors potentially affecting the attractiveness of host-infested plants to foraging parasitoids: 1. non-host-infested neighbouring plants 2. feeding guild of non-host 3. development stage of non-host 4. density of non-host 5. order of host and non-host arrival 6. time period between infestation by host and non-host 7. parasitoid species. Potential factors affecting the plant residence time of parasitoids: 8. position of non-host on plant 9. handling of non-host (products) 10. density of non-host 11. developmental stage of non-host 12. parasitoid species 13. previous experience of parasitoid (associative learning). Figure created by Emma Tanis.

problem while foraging, and on top of that find themselves in an environment that is full of (volatile) cues that may distort information on host presence. For example, plants attacked by host herbivores may stand next to plants attacked by non-host herbivores, both plants releasing HIPVs indicative of the presence of some kind of herbivore (Mumm and Dicke 2010). In addition, a plant that is attacked by both host and non-host herbivores may release different cues compared to a plant under attack by the host only (Shiojiri et al. 2001, Schwartzberg et al. 2011, Zhang et al. 2013). Parasitoid species may respond differently to HIPVs of plants that harbour non-hosts. Parasitoids that have a narrow host range and are highly specialized in parasitizing one herbivore host species are hypothesized to have evolved to respond to a narrow range of HIPVs that reliably predict presence of their host (Vet and Dicke 1992). Generalists, on the other hand, are expected to use mainly general cues because specific information is less useful when searching for a wide variety of hosts (Vet and Dicke 1992, but see Gols et al. 2012). Therefore, specialist parasitoids may be more strongly affected by non-host presence in their response to HIPVs than generalists. However, also within such a group with a certain dietary breadth, differences occur. The specialist parasitoid *Cotesia vestalis,* for example, prefers volatiles from plants attacked by its host, *P. xylostella* caterpillars, over volatiles from plants attacked simultaneously by its host and the non-host caterpillar *P. rapae* (Shiojiri et al. 2000b). In contrast, the specialist parasitoid *Cotesia glomerata* prefers this dual infestation over infestations by its host *P. rapae* only (Shiojiri et al. 2000b). This shows that one plant-host-non-host complex can elicit different reactions by two distinct parasitoid species, despite the similarity in their host range specialization.

In addition to the specificity of parasitoid species, both when the non-host is present on the same or on a neighbouring plant, the characteristics of the herbivores may play an important role in determining the response of a parasitoid (Dicke et al. 2009).

Non-host herbivores on neighbouring plants

In habitats where non-host herbivores occur, parasitoids have to locate host-infested plants against a background of plants induced by non-host feeding. Plants respond to herbivore attack with the induction of plant defence signal-transduction pathways, e.g. the jasmonic acid (JA), salycilic acid (SA), ethylene (ET) and abscisic acid (ABA) pathways (Pieterse et al. 2009). These signal-transduction pathways elicit the biosynthesis and emission of HIPVs (Arimura et al. 2005), which are exploited by foraging parasitoids (Vet and Dicke 1992, Heil 2008). The role of HIPVs from a plant's perspective is still highly debated, as other functions of HIPVs are known in addition to the attraction of carnivorous arthropods (Kessler and Heil 2011). The different plant defence signal-transduction pathways are induced by different groups of herbivores, generally divided according to their feeding guild. Leaf chewers mainly induce the jasmonic acid pathway and phloem feeders mainly induce the salicylic acid pathway (Howe and Jander 2008). Within feeding guilds, however, herbivore species can induce more than one pathway simultaneously (Stout et al. 2006) and herbivores from different feeding guilds can induce the same pathways (De Vos et al. 2005). The plant's perception of the attacking herbivore is mediated by herbivore-associated elicitors such as oral secretions (Bonaventure 2012, Clavijo McCormick et al. 2012) which could even be affected by the physiological state of the host as influenced by parasitism (Poelman et al. 2011).

Because herbivore species may differ in the HIPVs being induced by their feeding activities, parasitoids may find information about the identity of the plant's attacker in HIPVs. This information may be so detailed as to reveal the developmental stage of the attacking herbivore (Takabayashi et al. 1995), herbivore densities (Girling et al. 2011) or duration since the last herbivore infestation (Ohara and Takabayashi 2012). Because of these differences in HIPVs, parasitoids may use HIPVs to discriminate between host-infested and neighbouring non-host-infested plants (Takabayashi et al. 2006, Clavijo McCormick et al. 2012). In half of the 26 published cases that we found to report on parasitoid preferences for volatiles from host versus non-host induced plants, volatiles from host-induced plants were discriminated from volatiles from non-host induced plants (fig. 2; table 1). In none of these studies were non-host induced plants more attractive to parasitoids than host-induced plants. Furthermore, by reducing the 'attractiveness' of neighbouring plants, non-host herbivores can enhance the preference in terms of number of parasitoids that fly to host-infested plants (Soler et al. 2007a). However, in the other half of the cases, parasitoids did not discriminate between plants infested with hosts or non-hosts, showing that non-host herbivores on neighbouring plants have strong potential to negatively affect the foraging efficiency of parasitoids (fig. 2) (references in table 1). This inability to discriminate between volatiles from host and non-host-infested plants, might be caused by the feeding guild of the non-host (Delphia et al. 2007, Dicke et al. 2009). To plants attacked by either of two herbivore species from the same feeding guild, parasitoids frequently show no preference for host or non-host infestations (references in table 1). In contrast, when host and non-host belong to different feeding guilds, for example JA-inducing leaf chewers versus SA-inducing phloem feeders, parasitoids do commonly discriminate (Van Poecke et al. 2003, Erb et al. 2010). This, however, is no general rule, as examples were found of parasitoids that do discriminate between plants infested by host and non-host from the same feeding guild (references in table 1). Most of the available literature presents effects of non-host-infested neighbouring plants on parasitoid foraging under controlled laboratory conditions. In natural situations, however, host-infested plants are components of plant communities. The structure of the surrounding, non-host-infested vegetation may affect existing volatile blends and, consequently, the perception by foraging parasitoids (Randlkofer et al. 2010).

Non-host herbivores on the same plant

Typically, in herbivore-rich arthropod communities several herbivores share the same host plant simultaneously or sequentially. Feeding by multiple herbivore species on the same plant may induce different HIPVs compared to when plants are exposed to feeding by host herbivores only. This may have important consequences for parasitoids to locate host-infested plants. In only six out of 20 cases of tested effects of non-host herbivores sharing the food plant with host herbivores, parasitoids, but also predatory arthropods, preferred volatiles from host-infested plants over those from dual-infested plants (i.e. plants infested with hosts and non-hosts). In almost half of the cases, parasitoids did not discriminate volatiles from host-infested from those of dual-infested plants and even more so in some cases dual-infested plants were more attractive than host-infested plants (fig. 2). When separating the cases where non-host herbivores were feeding from a different plant organ than the host herbivores, four cases of root feeding (non-)host herbivores are reported in the literature. Parasitoids in three of these cases did not show a preference for either host-infested plants or dual-infested plants. In only one of the cases, parasitoids were attracted more to host-infested plants than plants that had an additional non-host feeding on a different plant organ (table 1). The specificity of the effect of a non-host herbivore species on affecting the headspace of a plant while feeding together with a host herbivore, may be found in elicitation of (different) signal-transduction pathways by both herbivore species within the plant. Through cross-talk, signal-transduction pathways that are induced by either herbivore species can interact, which thereby may alter the volatile profiles emitted by the plant compared to plants induced by single herbivore species (Dicke et al. 2009). As herbivores from different feeding guilds generally induce different defence signalling pathways (Howe and Jander 2008), simultaneous feeding by non-hosts from other feeding guilds than the host could affect the biosynthesis and release of HIPVs (Schwartzberg et al. 2011, Zhang et al. 2013) and in this way the host-searching efficiency of parasitoids (Dicke et al. 2009). The leaf chewer-responsive JA and the phloem feeder-responsive SA signalling pathways are often found to act antagonistically (Dicke et al. 2009, Pieterse et al. 2009, Thaler et al. 2012), but an additive effect of inducing both pathways simultaneously has been observed in the plant *Arabidopsis thaliana* as well (Schenk et al. 2000, Van Wees et al. 2000). An example of the specificity of the effects on volatile emission caused by feeding by herbivores of different feeding guilds is the study of the endoparasitoid *Cotesia marginiventris* and its leaf-feeding host *Spodoptera littoralis* (African cotton leafworm). When the host is attacking the plant simultaneously with a root-feeding nonhost, *Diabrotica virgifera virgifera* (Western corn rootworm), the parasitoid prefers the host-infested plant over the dual-infested plant (Rasmann and Turlings 2007). However, when the root-feeding non-host is replaced by the phloem feeding non-host cicadellid *Euscelidius variegatus*, the parasitoid does not discriminate between plants infested by both herbivores simultaneously and plants infested by the host only (Erb et al. 2010). However, because only single members of different feeding guilds were compared, it remains to be identified whether the shift in preference is caused by feeding guild or other herbivore characteristics, for example herbivore developmental stage (Yamamoto et al. 2011) or density (Zhang et al. 2009). Also within feeding guilds, different species of non-hosts may differentially affect parasitoid responses to HIPVs. The parasitoid *Cotesia glomerata*, for example, does not discriminate HIPVs induced by its host *Pieris rapae* (small cabbage white), from HIPVs of plants induced by either of two non-host caterpillar species, *Plutella xylostella* (diamondback moth) or *Mamestra brassicae* (cabbage moth) (Shiojiri et al. 2000b, Vos et al. 2001, Bukovinszky et al. 2012b) and with both non-host herbivores the parasitoid prefers dual infestations over host-only infested plants (Shiojiri et al. 2000b, Bukovinszky et al. 2012b). The parasitoids did, however, respond differently to both non-host species when discriminating between plants infested with a non-host and plants infested with both host and non-host. With the non-host *M. brassicae*, the parasitoid prefers dual infestations over non-host infestations (Bukovinszky et al. 2012b), whereas with the non-host *P. xylostella* the parasitoid does not discriminate between dual and non-host infestations (Vos et al. 2001). This may imply that within the same feeding guild, some non-host species feeding simultaneously with the host make

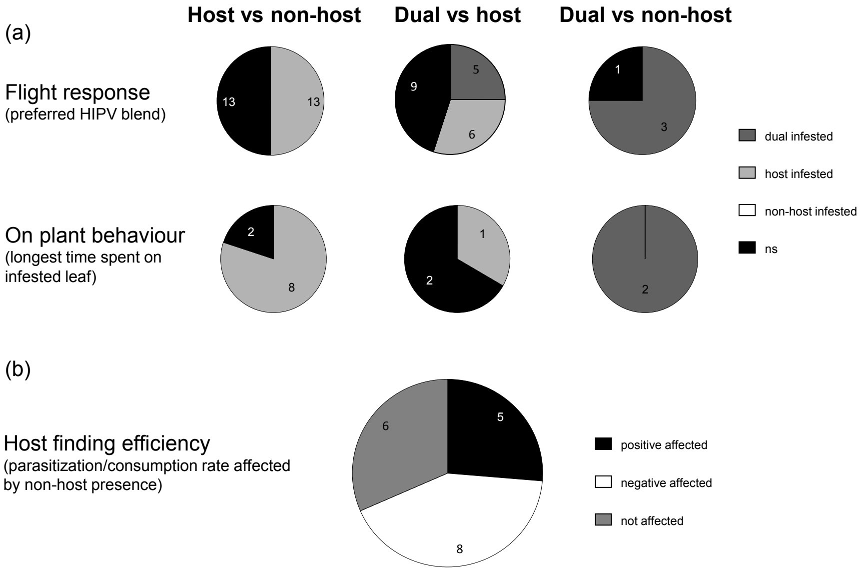

Figure 2. Overview of the effects of non-host herbivore presence on carnivorous arthropods foraging based on results obtained from literature, testing (a) discrimination of host versus non-host infestation, dual infestation versus host infestation and dual infestation versus non-host infestation at two levels of carnivore foraging behaviour and (b) the effect of non-host presence on host finding efficiency. Numbers in the pie sections indicate the number of cases that report a neutral, positive or negative effect. A detailed table of the studies and their results is provided in table 1. Note that some pie charts show understudied combinations.

Table 1. Overview of studies from literature testing the effects of non-host/non-prey presence on host/prey finding behaviour of naïve parasitoids/predators.

Parasitoid / predator	**Host / prey**	**Non-host / non-prey**	**Plant**	**Host / prey finding efficiency (parasitization/ consumption rate affected by non-host/non-prey presence)**	**Flight response (preferred HIPV blend)**			**On plant behaviour (longest time spent on infested leaf)**		
				positive / negative / neutral	**host vs non-host**	**dual vs host**	**dual vs non-host**	**host vs non-host**	**dual vs host**	**dual vs non-host**
Leaf chewing non-host / non-prey										
C. glomerata (g [1])	*P. rapae* (l.c.)	*M. brassicae* (l.c.)	*B. oleracea*	negative [2]	ns [2]	dual [2]	dual [2]	host [2]	ns [2]	dual [2]
C. glomerata (g [1])	*P. rapae* (l.c.)	*P. xylostella* (l.c.)	*B. oleracea* or *R. sativus* or *R. indica*	*B. oleracea*: positive [3]	*B. oleracea*: ns [4], [5]	*B. oleracea*: dual [5]	*B. oleracea*: ns [4]	*B. oleracea*: host [6] *R. sativus*: host [6] *R. indica*: host [7]	*B. oleracea*: ns [8]	*B. oleracea*: dual [4]

Parasitoid / predator	Host / prey	Non-host / non-prey	Plant	Host / prey finding efficiency (parasitization/ consumption rate affected by non-host/non-prey presence)	Flight response (preferred HIPV blend)			On plant behaviour (longest time spent on infested leaf)		
				positive / negative / neutral	host vs non-host	dual vs host	dual vs non-host	host vs non-host	dual vs host	dual vs non-host
C. glomerata (g [1])	*P. rapae* (l.c.)	*A. infumata* (l.c.) or *P. napi* (l.c.) or *P. melete* (l.c.)	*R. indica*					*A. infumata*: host [7] *P. napi*: ns [7] *P. melete*: ns [7]		
C. glomerata (g [1])	*P. brassicae* (l.c.)	*M. brassicae* (l.c.)	*B. oleracea*		ns [1]					

Parasitoid / predator	Host / prey	Non-host / non-prey	Plant	Host / prey finding efficiency (parasitization/ consumption rate affected by non-host/non-prey presence)	Flight response (preferred HIPV blend)			On plant behaviour (longest time spent on infested leaf)		
				positive / negative / neutral	host vs non-host	dual vs host	dual vs non-host	host vs non-host	dual vs host	dual vs non-host
D. fenestrale (g [9])	*P. xylostella* (l.c.)	*P. brassicae* (l.c.)	*B. oleracea* or *S. abla* or A feral *Brassica* strain		*B. oleracea*: host [9] *S. abla*: ns [9] A feral *Brassica* strain: ns [9]					
D. rapae (g* [10])	*M. persicae* (p.s.)	*P. xylostella* (l.c.)	*B. oleracea*		host [11]	ns [11]				
C. nigriceps (s [12])	*H. virescens* (l.c.)	*H. zea* (l.c.)	*N. tabacun* or *G. hirsutum*		*N. tabacun*: host [12] *G. hirsutum*: host [12]					

Parasitoid / predator	Host / prey	Non-host / non-prey	Plant	Host / prey finding efficiency (parasitization/ consumption rate affected by non-host/non-prey presence)	Flight response (preferred HIPV blend)			On plant behaviour (longest time spent on infested leaf)		
				positive / negative / neutral	**host vs non-host**	**dual vs host**	**dual vs non-host**	**host vs non-host**	**dual vs host**	**dual vs non-host**
C. rubecula (s [13])	*P. rapae* (l.c.)	*M. brassicae* (l.c.)	*B. oleracea*		ns [1]					
C. rubecula (s [13])	*P. rapae* (l.c.)	*P. xylostella* (l.c.)	*B. oleracea* or *A. thaliana*		*B. oleracea*: ns [14] *A. thaliana:* ns [13]					
C. rubecula (s [13])	*P. rapae* (l.c.)	*S. exigua* (l.c.)	*A. thaliana*		ns [13]					

Parasitoid / predator	Host / prey	Non-host / non-prey	Plant	Host / prey finding efficiency (parasitization/ consumption rate affected by non-host/non-prey presence)	Flight response (preferred HIPV blend)			On plant behaviour (longest time spent on infested leaf)		
				positive / negative / neutral	host vs non-host	dual vs host	dual vs non-host	host vs non-host	dual vs host	dual vs non-host
C. vestalis (s [8])	*P. xylostella* (l.c.)	*P. rapae* (l.c.)	*B. oleracea* or *R. sativus* or *R .indica*	*B. oleracea*: negative [3]	*R. sativus*: host [5]	*B. oleracea*: host [5] *B. oleracea* five young non-host: host [15] *B. oleracea* single young non-host: ns [15] *B. oleracea* single old non-host: host [15]		*B. oleracea*: host [6] *R. sativus*: host [6] *R. indica*: host [6]	*B. oleracea*: host [8]	

Parasitoid / predator	Host / prey	Non-host / non-prey	Plant	Host / prey finding efficiency (parasitization/ consumption rate affected by non-host/non-prey presence)	Flight response (preferred HIPV blend)			On plant behaviour (longest time spent on infested leaf)		
				positive / negative / neutral	**host vs non-host**	**dual vs host**	**dual vs non-host**	**host vs non-host**	**dual vs host**	**dual vs non-host**
D. semiclausum (s [9])	*P. xylostella* (l.c.)	*P. brassicae* (l.c.)	*B. oleracea* or *S. abla* or A feral *Brassica* strain		*B oleracea*: host [9] *S. abla*: ns [9] A feral *Brassica* strain: ns [9]					
P. persimilis (s [16])	*T. urticae* (p.s.)	*S. exigua* (l.c.)	*P. lunatus*, or *C. sativus*			*P. lunatus*: dual [16] *C. sativus*: dual [16]	*P. lunatus*: dual [16] *C. sativus*: dual [16]			
T. rapae (s [17])	*D. radicum* (r.h.)	*P. brassicae* (l.c.)	*B. rapa*	negative [17]		ns [17]				

Parasitoid / predator	Host / prey	Non-host / non-prey	Plant	Host / prey finding efficiency (parasitization/ consumption rate affected by non-host/non-prey presence)	Flight response (preferred HIPV blend)			On plant behaviour (longest time spent on infested leaf)		
				positive / negative / neutral	host vs non-host	dual vs host	dual vs non-host	host vs non-host	dual vs host	dual vs non-host
Unknown parasitoids	*B. brassicae* (p.s.) + *L. erysimi* (p.s.) + *Aphis sp*. (p.s.) + *M. persicae* (p.s.) + *M. euphorbiae* (p.s.)	*S. litoralis* (l.c.)	*S. arvensis*	neutral [18]						
Unknown parasitoids	*T. ekebladella* (l.m.)	*A. psi* (l.c.)	*Q. robur*	neutral [19]						

Parasitoid / predator	Host / prey	Non-host / non-prey	Plant	Host / prey finding efficiency (parasitization/ consumption rate affected by non-host/non-prey presence)	Flight response (preferred HIPV blend)			On plant behaviour (longest time spent on infested leaf)		
				positive / negative / neutral	host vs non-host	dual vs host	dual vs non-host	host vs non-host	dual vs host	dual vs non-host
Piercing-sucking non-host / non-prey										
A. ervi (g [20])	*A. pisum* (p.s.)	*A. fabae* (p.s.)	*V. faba*		host [21]					
C. glomerata (g [1])	*P. brassicae* (l.c.)	*B. brassicae* (p.s.)	*B. oleracea*			ns [24]				
C. marginiventris (g [25])	*S. littoralis* (l.c.)	*E. variegatus* (p.s.)	*Z. mays*		host [25]	ns [25]				
N. cucumeris (g* [27])	*F. occidentalis* (p.s.)	*T. urticae* (p.s.)	*C. sativa*	negative [27]						
C. rubecula(s [13])	*P. rapae* (l.c.)	*T. urticae* (p.s.)	*A. thaliana*		ns [13]					
C. rubecula (s [13])	*P. rapae* (l.c.)	*M. persicae* (p.s.)	*A. thaliana*		host [13]					
D. semiclausum (s [9])	*P. xylostella* (l.c.)	*B. rabaci* (p.s.)	*A. thaliana*			host [32]				

Parasitoid / predator	**Host / prey**	**Non-host / non-prey**	**Plant**	**Host / prey finding efficiency (parasitization/ consumption rate affected by non-host/non-prey presence)**	**Flight response (preferred HIPV blend)**			**On plant behaviour (longest time spent on infested leaf)**		
				positive / negative / neutral	**host vs non-host**	**dual vs host**	**dual vs non-host**	**host vs non-host**	**dual vs host**	**dual vs non-host**
P. persimilis (s [16])	*T. urticae* (p.s.)	*B. tabaci* (p.s.)	*P. lunatus*			host [35]				
				Root feeding non-host / non-prey						
C. glomerata (g [1])	*P. brassicae* (l.c.)	*D. radicum* (r.h.)	*B. nigra*	On same plant: negative [22] On neighbouring plant: positive for foraging efficiency [23]		On same plant: ns [22]				
C. marginiventris (g [25])	*S. littoralis* (l.c.)	*D. virgifera virgifera* (r.h.)	*Z. mays*		host [26]	host [26]				

Parasitoid / predator	Host / prey	Non-host / non-prey	Plant	Host / prey finding efficiency (parasitization/ consumption rate affected by non-host/non-prey presence)	Flight response (preferred HIPV blend)			On plant behaviour (longest time spent on infested leaf)		
				positive / negative / neutral	host vs non-host	dual vs host	dual vs non-host	host vs non-host	dual vs host	dual vs non-host
T. chloromerus (s) and *P. elevatus* (g* [28])	*T. ruficauda* (s.f.)	*P. horticula* (r.h.) + *O. sulcatus* (r.h.) + *T. oleracea* (r.h.)	*C. palustre*	neutral [29]						
C. kazak (s* [30])	*P. daplidice* (l.c.) + *E. crameri* (l.c.)	*C. gypsicola* (r.h.)	*M. moricandioides*	*C. gypsicola*: neutral [31]						
M. croceipes (s [33])	*H. zea* (l.c.)	*M. incognita* (r.h.)	*G. hirsutum*		host [33]	ns [33]				
M. grandii (s [34])	*O. nubilalis* (l.c.)	*Diabrotica spp.* (r.h.)	*Z. mays*	negative [34]						

Parasitoid / predator	Host / prey	Non-host / non-prey	Plant	Host / prey finding efficiency (parasitization/ consumption rate affected by non-host/non-prey presence)	Flight response (preferred HIPV blend)			On plant behaviour (longest time spent on infested leaf)		
				positive / negative / neutral	host vs non-host	dual vs host	dual vs non-host	host vs non-host	dual vs host	dual vs non-host
Unknown parasitoids	*B. brassicae* (p.s.) + *L. erysimi* (p.s.) + *Aphis sp*. (p.s.) + *M. persicae* (p.s.) + *M. euphorbiae* (p.s.)	*Agriotes sp.* (r.h.)	*S. arvensis*	neutral [18]						

Parasitoid / predator	Host / prey	Non-host / non-prey	Plant	Host / prey finding efficiency (parasitization/ consumption rate affected by non-host/non-prey presence)	Flight response (preferred HIPV blend)			On plant behaviour (longest time spent on infested leaf)		
				positive / negative / neutral	host vs non-host	dual vs host	dual vs non-host	host vs non-host	dual vs host	dual vs non-host
Arbuscular mycorrhizal fungi non-host / non-prey										
A. ervi (g [20])	*M. euphorbiae* (p.s.)	*G. mosseae* (amf)	*L. esculentum*		ns [20]					
D. isaea (g [36])	*C. syngenesiae* (l.m.)	*G. caledonium* (amf), or *G. fasciculatum* (amf), or *G. mosseae* (amf)	*L. vulgare*	*G.caledonium*: negative [36] *G.fasciculatum*: positive [36] *G. mosseae*: negative [36]						

Parasitoid / predator	Host / prey	Non-host / non-prey	Plant	Host / prey finding efficiency (parasitization/ consumption rate affected by non-host/non-prey presence)	Flight response (preferred HIPV blend)			On plant behaviour (longest time spent on infested leaf)		
				positive / negative / neutral	**host vs non-host**	**dual vs host**	**dual vs non-host**	**host vs non-host**	**dual vs host**	**dual vs non-host**
A. rhopalosiphi (s [37])	*R. padi* (p.s.)	*G. intraradices* (amf), or *G. mosseae* (amf)	*P. pratense*	*G. intraradices*: positive [38] *G. mosseae*: neutral [38]						
Phytopathogenic fungi non-host / non-prey										
C. marginiventris (g [25])	*S. exigua* (l.c.)	*S. rolfsii* (p.f.)	*A. hypogaea*			dual [39]				
C. marginiventris (g [25])	*S. littoralis* (l.c.)	*S. turcica* (p.f.)	*Z. mays*		host [40]	ns [40]				
M. rufiventris (g* [41])	*S. littoralis* (l.c.)	*S. turcica* (p.f.)	*Z. mays*		host [40]	ns [40]				
M. tristis (s [42])	*H. bicruris* (s.f.)	*M. violaceum* (p.f.)	*S. latifolia*	positive [42]						

Parasitoid / predator	**Host / prey**	**Non-host / non-prey**	**Plant**	**Host / prey finding efficiency (parasitization/ consumption rate affected by non-host/non-prey presence)**	**Flight response (preferred HIPV blend)**			**On plant behaviour (longest time spent on infested leaf)**		
				positive / negative / neutral	**host vs non-host**	**dual vs host**	**dual vs non-host**	**host vs non-host**	**dual vs host**	**dual vs non-host**
Unknown parasitoids	*T. ekebladella* (l.m.)	*E. alphitoides* (p.f.)	*Q. robur*	positive [19]						

Overview of studies from literature testing the effect of non-host/non-prey presence on host/prey finding efficiency of naïve parasitoids/predators and host infestation versus non-host infestation, dual infestation versus host infestation and dual infestation versus non-host infestation, at two levels of foraging behaviour of naïve parasitoid/predators: response to HIPV blends and behaviour on the plant. Literature was found using the database Scopus and combinations of the keywords: parasitoid/parasitic wasp, behaviour, nonhost/non-host, nonprey/non-prey, herbivore induced plant volatiles, multiple herbivory, plant-insect interactions. Amf: arbuscular mycorrhizal fungus, d: decomposer/detrivore, g: generalist, l.c.: leaf chewer, l.m.: leaf miner, p.f: phytopathogenic fungus, p.s.: piercing sucking, r.h.: root herbivore, s: specialist, s.f.: seed feeder, *: species are considered generalists if more than one host species is reported, +: species tested within one system, or: species tested as separate systems. (1) Geervliet et al. (1996), (2) Bukovinszky et al. (2012), (3) Shiojiri et al. (2002), (4) Vos et al. (2001), (5) Shiojiri et al. (2000a), (6) Shiojiri et al. (2000b), (7) Sato et al. (1999), (8) Shiojiri et al. (2001), (9) Gols et al. (2012), (10) Antolin et al. (2006), (11) Agbogba and Powell (2007), (12) DeMoraes et al. (1998), (13) Van Poecke et al. (2003), (14) Agelopoulos and Keller (1994), (15) Yamamoto et al. (2011), (16) De Boer et al. (2008), (17) Pierre et al. (2011), (18) Poveda et al. (2005), (19) Tack et al. (2012), (20) Guerrieri et al. (2004), (21) Du et al. (1996), (22) Soler et al. (2007b), (23) Soler et al. (2007a), (24) Soler et al. (2012), (25) Erb et al. (2010), (26) Rasmann and Turlings (2007), (27) Pallini et al. (1998), (28) Hoebeke and Wheeler (1996), (29) Masters et al. (2001), (30) Singh et al. (1982), (31) Megías and Müller (2010), (32) Zhang et al. (2013), (33) Olson et al. (2008), (34) White and Andow (2006), (35) Zhang et al. (2009), (36) Gange et al. (2003), (37) Gonzáles et al. (1999), (38) Hempel et al. (2009), (39) Cardoza et al. (2003), (40) Rostás et al. (2006), (41) Hegazi and Führer (1985), (42) Biere et al. (2002)

the plant more attractive to parasitoids (also observed by Rodriguez-Saona et al. 2005) while other non-host species do not.

In addition to variation in HIPV induction caused by herbivores from different feeding guilds, other factors may enhance the level of specificity in plant odours emitted in response to multiple herbivory. Effects of multiple herbivory on HIPV emissions may be plant-species or plant-genotype specific. For example, combined infestation of lima bean plants by one combination of herbivores, the spider mite *Tetranychus urticae* and the caterpillar *Spodoptera exigua*, resulted in the majority of compounds being more strongly induced than the emission rates induced by either herbivore alone, while in cucumber it resulted in two compounds being emitted in lower amounts from dual-infested plants than the sum of the amounts emitted by plants treated with single infestation of either herbivores. This suggests that the effects of dual infestation are driven by different mechanisms in lima bean and cucumber plants (De Boer et al. 2008). However, for both plants the effect of dual infestation versus single infestations on the response of, in this case, a predator were the same: the predator was more attracted to volatiles from dual-infested plants (De Boer et al. 2008). Observed effects of non-hosts may also be dependent on the density of host and non-host herbivores attacking the plant (Zhang et al. 2009). At least for certain species, non-host presence negatively interferes with host attractiveness only when non-host density is above a certain threshold (Zhang et al. 2009, Yamamoto et al. 2011, Zhang et al. 2013). Moreover, also the developmental stage of non-hosts may determine the attractiveness of a dual infestation. A single second instar non-host caterpillar, for example, did not affect the attractiveness of dual-infested plants to parasitic wasps, while a single fifth instar non-host caterpillar negatively affected the attractiveness of these plants. Yet, the exact factors that caused the change in attractiveness need to be identified (Yamamoto et al. 2011).

Hosts and non-hosts usually do not arrive simultaneously on the plant. The order of arrival could affect plant responses in terms of interfering signalling pathways (Dicke et al. 2009) and can affect plant-mediated interactions between herbivores (Kessler and Baldwin 2004, Erb et al. 2011). Interference among signalling pathways may affect HIPV emission and this may result in altered parasitoid searching behaviour (Dicke et al. 2009). In at least one system, however, non-prey infestation interferes with the attraction of a carnivore irrespective of the order of infestation (Zhang et al. 2009).

Based on the available literature, no general patterns on parasitoid response to HIPVs affected by several aspects of the plant-host-non-host-parasitoid complex can be found. However, we conclude that specificity of plant responses to herbivory is the main driver of specificity in parasitoid responses to situations of non-hosts inducing plant volatiles.

Encounters with non-host herbivores on the food plant

When a parasitoid arrives on a plant, it has to decide how long to search for its herbivore host on that plant. The decision when to give up searching and to leave the plant to fly to another plant can be mediated by an incremental or decremental mechanism. This means that each oviposition increases, or decreases respectively, the probability of staying on a plant (Van Alphen et al. 2003). These mechanisms are dynamic and individual parasitoids can show a switch between the two mechanisms (Outreman et al. 2005, Lucchetta et al. 2007). Many other cues could also affect the decision when to give up searching, e.g. the presence of competitors (Goubault et al. 2005), encounters with parasitized herbivores, quality of a previously visited plant, abiotic factors indicating upcoming changes in environmental conditions, (reviewed by Van Alphen et al. 2003) as well as the encounters with non-host herbivores.

Parasitoid behaviour on plants infested with single herbivore species

Host-infested leaves can increase residence time of parasitoids, when compared to uninfested leaves (Wiskerke and Vet 1994) and artificially damaged leaves (Shiojiri et al. 2000a). Infochemicals characteristic for the plant-herbivore combination (Shiojiri et al. 2000a), but also host-related kairomones were found to be important factors in altering searching behaviour (Bukovinszky et al. 2012b). In contrast, parasitoids often show short residence times on leaves infested with unsuitable hosts only (Sato et al. 1999). In the ten literature cases that we found on residence times of parasitoids on host-infested versus non-host-infested plants, we found that in the majority of these cases, parasitoids spent more time on host-infested leaves than on non-host-infested leaves (fig. 2). In 20% of the cases, however, parasitoids have also been observed to spend an equal amount of time on non-host-infested and host-infested leaves (fig. 2). Parasitoids could for example be arrested by cues related to the non-host that resemble host related cues, like the larval parasitoid *Apanteles kariyai* that is arrested by faecal pellets of non-host *Acantholoeucania loreyi* larvae (Takabayashi and Takahashi 1990).

Parasitoid behaviour on plants infested with two herbivore species

Three cases are reported of detailed analyses of the structure of behaviour of parasitoids when encountering non-hosts as compared to hosts. Two cases showed that parasitoids spent a similar amount of time on dual-infested leaves as on host-only infested leaves, whereas in a single case parasitoids spent more time on host-infested leaves than on dual-infested leaves (fig. 2). For example, the parasitoid *C. glomerata*, showed a high leaving tendency on non-host-infested leaves but an equal leaving tendency on host-infested leaves compared to dual infestations (Shiojiri et al. 2001, Bukovinszky et al. 2012b). When observed in more detail, *C. glomerata* showed no difference in time spent on several behaviours (Shiojiri et al. 2001) nor in the structure of the behavioural repertoire (Bukovinszky et al. 2012b) on host-infested compared to dual-infested leaves. Removing hosts, but not host-related cues, just before the experiment, or manipulating the host: non-host ratio, neither did affect residence time nor behavioural structure of the parasitoid *C. glomerata* (Bukovinszky et al. 2012b). In contrast, the parasitoid *Cotesia vestalis* spent more time on leaves infested with only hosts compared to leaves infested with hosts and non-hosts (Shiojiri et al. 2001).

As suggested by Bukovinszky et al. (2012b), the response of the parasitoid may depend on the position of the non-host relative to the position of the host. When the herbivores are positioned on different leaves, the leaf on which the parasitoid lands may in addition affect subsequent behaviour (Bukovinszky et al. 2012b). When herbivores are encountered on the plant, the density and developmental stage of non-hosts may affect searching behaviour of parasitoids, similar to effects on HIPV response (Yamamoto et al. 2011). Handling certain non-host species or their products after contact is a waste of time and may, therefore, affect host finding efficiency (Bukovinszky et al. 2012b). Just as for the response to HIPVs, different parasitoid species may also have different behaviours towards dual infestations of herbivores on the plant (Shiojiri et al. 2000b).

Because only three studies have reported detailed analysis of the behaviour of parasitoids in host, non-host and dual-infested patches, concluding on the generality of effect of non-host herbivores on patch residence times and foraging efficiency is pre-mature. However, even though only three studies report on parasitoid behaviour on the plant, these studies show that foraging decisions on the patch can be strongly affected by non-host presence and that responses are different within different systems.

Linking two phases of foraging behaviour

Parasitoid foraging behaviour can have important effects on species interactions and consequently population dynamics (Vet 2001, Vos and Hemerik 2003). Studies that link foraging strategies to population dynamics mostly address a single phase (but see Shiojiri et al. 2000b, 2001, 2002, Vos and Hemerik 2003, Bukovinszky et al. 2012b). In this review, we have shown that responses to HIPVs and foraging decisions of parasitoids on the plant may both be affected by several characteristics associated with dual infestations, leading to enhanced or attenuated effects on parasitoid behaviour (fig. 2). Importantly, the studies that did combine both phases of foraging show that studying a single phase only may lead to incorrect conclusions on parasitoid foraging efficiency (Shiojiri et al. 2000b, 2001, 2002, Bukovinszky et al. 2012b).

A study by Bukovinszky et al. (2012b) shows that the observed response of the parasitoid *C. glomerata* to volatiles induced by dual infestation is not in accordance with the parasitism rate found on dual-infested plants. The parasitoid prefers volatiles of plants infested by a mixture of hosts and non-hosts over volatiles of plants infested by only hosts, while the parasitism rate of this parasitoid is lower on dual-infested plants compared to plants infested by only hosts (Bukovinszky et al. 2012b). This reduced parasitism rate on dual-infested plants can be explained by the parasitoid wasting time on finding a host on a dual-infested plant (Vos et al. 2001, Bukovinszky et al. 2012b), because the parasitoid has an equal leaving tendency on dual-infested plants as on host-infested plants (Bukovinszky et al. 2012b) (fig. 3). The encounters with non-host herbivores result in a reduced number of parasitized hosts during the equal time spent on dual and host-only infested plants. The parasitoid *C. vestalis*, however, shows a consistency between both host searching based on HIPVs and foraging behaviour when searching for hosts on the plant, and parasitism rates on dual infestations (Shiojiri et al. 2000b, 2001, 2002) (fig. 3). The parasitoid prefers HIPVs induced by its host over HIPVs induced by dual infestations of hosts and non-hosts (Shiojiri et al. 2000b), which is in accordance with a longer time spent on plants infested by hosts only compared to host and non-host-infested plants (Shiojiri et al. 2001) and decreased parasitism rates on dual infestations (Shiojiri et al. 2002) (fig. 3).

Importantly, these studies by Shiojiri et al. (2000b, 2001, 2002) and Bukovinszky et al. (2012b) show that studying the response to either volatiles or the behaviour on the patch of a parasitoid alone could result in incorrect or poor predictions of the actual consequences for foraging efficiency caused by the presence of non-host herbivores in the

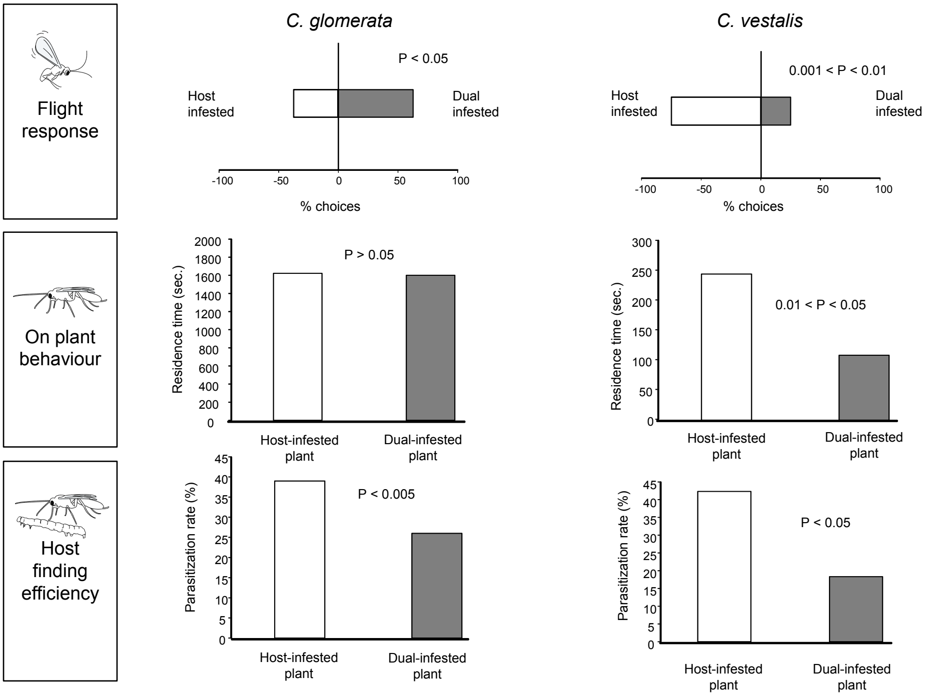

Figure 3. Response of *Cotesia glomerata* and *Cotesia vestalis* to host-infested versus dual-infested plants, residence time of *C. glomerata* and *C. vestalis* on host-infested versus dual-infested plants and parasitization rate of *C. glomerata* and *C. vestalis* on host-infested versus dual-infested plants. Graphs adjusted from Bukovinszky et al. (2012b) and Shiojiri et al. (2000b, 2001, 2002).

community. Studying only one phase of foraging behaviour could also disregard the fact that parasitoids can use information from previous experiences. After exploring a patch, parasitoids can combine the information gained with the experience they have from the previous patch they visited. Using this information, patch quality can be estimated and leaving decisions can be made (Louâpre et al. 2011, Froissart et al. 2012). In turn, the experience of patch quality can be associated with plant odours and affect the subsequent responses of parasitoids to HIPVs (Hoedjes et al. 2011). Extensive evidence shows that parasitoids can optimize their foraging efficiency by associative learning. Behavioural responses can be modified by associating odour cues (e.g. HIPVs) with rewarding experiences (e.g. hosts) (Hoedjes et al. 2011). Consequently, behaviour towards HIPVs can be affected by experiences on the previously visited plant. Moreover, behaviour on the subsequent patch might also be altered by learning. Thus, responses to HIPVs and foraging decisions on the food plant are linked in the dynamics of parasitoid behavioural plasticity that may be driven by experience and their memory formation.

Conclusion

We have identified that non-host herbivores may strongly affect the efficiency of host location by parasitoids, both during the response to HIPVs and during parasitoid behaviour on the food patch. Furthermore, comparisons among studies show large specificity in the effect of different types of non-host herbivores and responses by different parasitoids to the same mix of herbivore species.

We speculate that feeding habits of herbivores would be highly important in the effects of the presence of non-hosts on the host location by the parasitoid. The induction of different plant defence signal-transduction pathways by herbivores from different feeding guilds, and the cross-talk between these signal-transduction pathways, could have a substantial effect on the plant volatiles emitted (Dicke et al. 2009) and therefore on the efficiency of a parasitoid to locate host-infested plants.

On the level of parasitoid behaviour on the plant, herbivore feeding guild could also considerably affect parasitoid efficiency because of non-host herbivore cues that are (dis)similar to those of the host herbivore (Takabayashi and Takahashi 1990). Effects of herbivore specialization and herbivore phylogenetic relatedness are expected to be of marginal importance as these factors are shown to be less significant in the specificity of plant responses than herbivore feeding guild (Bidart-Bouzat and Kliebenstein 2011). A late-instar non-host individual, however, is expected to have a stronger effect compared to an early-instar non-host (Yamamoto et al. 2011) because of more damage to the plant and a higher production of herbivore cues on the plant. However, this would also depend on the preferred host developmental stage of the parasitoid. Similarly, a high non-host density is expected to have a strong effect on parasitoid behaviour (Zhang et al. 2009) during both phases of host location, because a high non-host density is expected to overshadow both the effects of the host on the HIPVs and the cues of the host present on the plant.

We further speculate that host distribution in relation to non-host distribution may play a major role in parasitoid decisions affecting encounters with non-hosts. Parasitoids that parasitize host herbivores that have a clumped distribution may experience lower fitness consequences of wasting time by handling non-host herbivores than parasitoids that forage for hosts that have a solitary distribution. Parasitoids foraging for clumped distributions of hosts may be able to parasitize a large number of the clumped hosts and may consequently be egg-limited and, therefore, not able to parasitize them all. Time wasted in handling non-hosts likely has stronger fitness consequences when parasitoids are not egg limited but time limited when having to spend a longer searching time to locate their solitary hosts (Vos et al. 1998, Vos and Hemerik 2003).

Furthermore, in a highly diverse plant-herbivore community, parasitoid foraging decisions are more strongly shaped by foraging decisions on the food plant than responses to variation in HIPV, because the abundance of HIPV cues might negatively affect the host-finding efficiency of parasitoids on the level of HIPV use (Sheehan 1986). In such cue-rich communities, parasitoids might rely more on their capacity to find hosts on the plant and use the herbivore-induced odour profile of the food plant their hosts are associated with to locate plants that potentially harbour hosts. In this situation, non-hosts would play a minor role in distorting the localization of a host-infested plant, but a major role in distorting finding hosts once the parasitoid is present on the plant. On the other hand, agro-ecosystems consisting of mono-cultures are more uniform with each plant potentially harbouring a mix of host and non-host herbivores. In such communities, parasitoids that use hosts with a clumped or heterogeneous distribution may be selected to specialize on HIPVs of plants infested by hosts in any background of non-host infestation. For parasitoids that locate host-infested plants, any encounter with a non-host on such plants should not result in a strong effect on the patch residence time. In this situation, non-hosts would have more impact on the parasitoid response to HIPVs of host-infested plants, compared to non-host effects on the parasitoid behaviour on the plant.

To understand parasitoid foraging behaviour in more realistic habitats, basic ecological questions such as the influence of feeding guild (Dicke et al. 2009), density (Zhang et al. 2009), diversity (Vos et al. 2006) and developmental stage (Yamamoto et al. 2011) of non-host herbivores, and influences of plant species and parasitoid host ranges, remain to be answered. Moreover, it will be interesting to study the influence of the order of, and time period between herbivore arrival on the searching behaviour of parasitoids (Dicke et al. 2009) and the role of unsuitable herbivores on competition between parasitoids (Meisner et al. 2007). Furthermore, most of our understanding of parasitoid responses to non-host herbivory comes from laboratory studies and we need to identify the importance of effects caused by non-host herbivory over other factors such as surrounding vegetation that may affect parasitoid foraging in the field. Answering these research questions requires a multidisciplinary approach to understand the consequences of non-host herbivory on plant responses and parasitoid behaviour.

To understand how various herbivore species affect plant responses when feeding simultaneously, we require understanding of the physiological machinery of plants that underlies volatile emission when responding to multiple attack. Although studies in the past decade have identified how molecular signal-transduction pathways may cross-talk during dual herbivore attack, we are only scratching the surface of understanding plant physiological rules for interactions among herbivore feeding guilds, timing of their arrival or consequences when herbivores are feeding on different leaves. Let alone how these responses at the molecular level translate into variation in volatile emission and the ability of parasitoids to discriminate between those volatile blends.

Studying the many layers of specificity in parasitoid responses to non-host herbivore presence simultaneously could be very complex with an experimental approach only. We therefore may be able to make the best progress by combining experimental work with a modelling approach. By studying one complex of food plant, herbivore host and parasitoid and experimentally changing species on one trophic level at the time, we may be able to predict with a modelling approach which of those factors most strongly affects parasitoid foraging efficiency. These predictions should then be empirically tested in both controlled and open field experiments to ultimately understand behaviour of parasitoids in natural environments and agro-ecosystems.

Ultimately, we need to scale up from dual herbivore infestations to species-rich dynamic communities, with both understanding of plant responses as well as parasitoid behaviour on the food patch to accurately predict how parasitoids affect their host's population in both natural and agricultural environments.

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Chapter 3

Feeding guild of non-host community members affects host-foraging efficiency of a parasitic wasp

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Interactions between predator and prey, or parasitoid and host, are shaped by trait- and density-mediated processes involving other community members. Parasitoids that lay their eggs in herbivorous insects locate their host through herbivore-induced plant volatiles (HIPVs) and host infochemicals. Hosts are frequently accompanied by non-host herbivores that are unsuitable for the parasitoid. These non-hosts may interfere with host location primarily through direct and indirect trait-mediated processes, respectively by their own infochemicals and their induction of the emission of plant volatiles. Specific non-host herbivore characteristics can play a role in the sign and strength of their trait-mediated effect on parasitoid host location. Here, we addressed whether the feeding guild of non-host herbivores differentially affects foraging of the parasitoid Cotesia glomerata for its common host, caterpillars of Pieris brassicae feeding on Brassica oleracea plants. We used different phloem-feeding and leaf-chewing non-hosts to study their effects on host location by the parasitoid when searching for host-infested plants based on HIPVs and when searching for hosts on the plant using infochemicals. To evaluate the ultimate effect of these two phases in host location, we studied parasitism efficiency of parasitoids in small plant communities under field-tent conditions. We show that leaf-chewing non-hosts primarily affected host location through indirect trait-mediated effects via plant volatiles, whereas phloem-feeding non-hosts exerted direct trait-mediated effects by affecting foraging efficiency of the parasitoid on the plant. These trait-mediated effects resulted in associational susceptibility of hosts in environments with phloem feeders and associational resistance in environments with non-host leaf chewers.

Keywords

Feeding guild, foraging efficiency, HIPV, leaf chewer, multi-herbivory, non-host, parasitoid behaviour, patch residence, phloem feeder, trait-mediated indirect interactions

Natural environments in which predators forage for prey are complex because of the presence of all kinds of organisms that are of no interest to the predator. Both the density and traits of these organisms may directly or indirectly affect the interaction between predator and prey and thereby influence major ecological processes such as food web interactions and community dynamics (Křivan and Schmitz 2004, Schmitz et al. 2004, Trussell et al. 2006, Vos et al. 2006). Indirect trait- or density-mediated interactions occur when one organism, through its traits or abundance, affects another via an intermediate species (Steinke et al. 2002, Werner and Peacor 2003). There is convincing evidence that predator-prey interactions, as well as community processes, are most strongly influenced by indirect trait-mediated interactions, rather than density-mediated processes (Luttbeg et al. 2003, Trussell et al. 2006). However, it often remains unknown which traits of community members may indirectly affect predator-prey interactions and whether these traits represent generalities in processes of predator-prey relationships.

Trophic relationships in aquatic and terrestrial environments are mediated by chemical cues (Lima and Dill 1990, Dicke and Sabelis 1992, Savoca and Nevitt 2014, Raguso et al. 2015). This involves both the cues emitted by prey that are exploited by predators or vice versa (Lima and Dill 1990, Ferrari et al. 2010) as well as indirect interactions where the herbivore induces the production of cues in its food with consequences for foraging decisions of the herbivore’s predator (Vet and Dicke 1992, Nevitt et al. 1995). For instance, antarctic procellariiform seabird species use dimethyl sulfide produced by phytoplankton in response to grazers as a foraging cue to selectively forage for phytoplankton grazers (Savoca and Nevitt 2014).

The trophic relationships between parasitic wasps (parasitoids) and their hosts are particularly shaped by both direct and indirect trait- and density-mediated processes (Vet and Dicke 1992, Utsumi et al. 2010). Parasitoids are dependent on herbivorous hosts for the development of their offspring and lay their eggs in or on the host. The parasitoid larvae subsequently use the host as their food source and eventually the host dies (Godfray 1994). During host-habitat location, parasitoids make use of herbivore-induced plant volatiles (HIPVs) that are emitted by the plant when their host herbivore is feeding on its tissues, which represents an indirect trait-mediated process (Vet and Dicke 1992, Ohgushi 2008, Hare 2011). At closer range, parasitoids use infochemicals derived from the hosts themselves to locate their exact positions on the plant, a direct trait-mediated interaction (Van Alphen et al. 2003, Colazza et al. 2014). After finding the host, the parasitoid examines its quality before accepting it for oviposition (Van Alphen et al. 2003). The presence of non-host herbivores, i.e. herbivores in which the parasitoid cannot develop, may interfere with these steps in host location through trait- and density-mediated processes (De Rijk et al. 2013). Previous studies identified that non-host feeding may alter the emission of plant volatiles that are induced by the host herbivore and consequently may hamper the use of HIPVs by parasitoids to locate host-infested plants (Shiojiri et al. 2001, Bukovinszky et al. 2012b, Chabaane et al. 2015). Encounters with these non-hosts or their infochemicals may require handling time and reduce host location efficiency in a direct trait-mediated fashion. Although the density of non-host herbivores may affect the strength of these processes, this has been found to play a minor role in determining the sign of the effect caused by non-host herbivore presence (Bukovinszky et al. 2012b). For example, the parasitoid *C. glomerata* displayed the same preference for HIPVs emitted by plants infested with hosts and non-hosts over volatiles emitted by plants infested by only hosts, regardless of the number of herbivores feeding on both plants being equal or different (Bukovinszky et al. 2012b). Through these direct and indirect trait-mediated processes, non-host herbivores may either provide associational resistance in the form of enemy-free space or associational susceptibility of the host to parasitism by enhancing attraction of parasitoids (Shiojiri et al. 2000b, Barbosa et al. 2009, De Rijk et al. 2013). It remains to be determined which non-host traits provide associational resistance or susceptibility.

The feeding guild, a classification of herbivores based on their feeding mode, of these non-hosts may be an important determinant in trait-mediated processes. Plants respond differently to herbivore attackers belonging to different feeding guilds. Generally, phloem feeders induce the salicylic acid (SA) signal-transduction pathway and leaf chewers induce the jasmonic acid (JA) pathway (Arimura et al. 2011). Whenever herbivores of different feeding guilds attack the same individual plant, this may lead to the induction of different plant defence signal-transduction pathways simultaneously which may interact through cross-talk, thereby generating an altered volatile blend emitted by the plant (Dicke et al. 2009, Arimura et al. 2011, Soler et al. 2012a, Zhang et al. 2013). The feeding guild of non-hosts might, therefore, affect the volatile-based behavioural response of parasitoids towards a plant infested with both hosts and non-hosts (Dicke et al. 2009, De Rijk et al. 2013). Moreover, the presence of non-host herbivores from different feeding guilds and their products could differentially affect the infochemical-based foraging decisions of parasitoids when searching for their host on the plant. The available evidence suggests that foraging efficiency is reduced when parasitoids encounter non-hosts that occupy a similar feeding guild as their intended host (Takabayashi and Takahashi 1990, Bukovinszky et al. 2012b, Chabaane et al. 2015). The effect of non-host feeding guild on volatile-based flight behaviour of parasitoids towards the attacked plant and searching behaviour after landing that is based on herbivore-produced infochemicals may lead to combined effects on host-finding efficiency of the parasitoid and hence associational resistance or susceptibility of the host to parasitism (De Rijk et al. 2013).

Here, we studied whether the feeding guild of non-hosts differentially affects the foraging behaviour of parasitoids through direct and indirect trait-mediated interactions. In a natural setting, all plants are commonly attacked by several herbivore species (Vos et al. 2001, Stam et al. 2014). Of these plants, only some are attacked by the specific species for which the parasitoid is searching. We therefore studied the behaviour of the gregarious parasitoid *Cotesia glomerata* (Hymenoptera: Braconidae) when foraging for its gregarious caterpillar host *Pieris brassicae* (Lepidoptera: Pieridae) in a background of non-host herbivore presence. The environment consisted of *Brassica oleracea* (Brassicales:Brassicaceae) plants that were all attacked by either one of three species of phloem feeders or one of three species of leaf chewers. In addition, half of these plants were simultaneously attacked by the host caterpillar that was thereby sharing the food plant with the non-host herbivore. We specifically addressed the question of whether the feeding guild of non-hosts affects 1) indirect trait-mediated location of an host-attacked plant based on HIPVs, 2) direct trait-mediated location of hosts on the plant based on herbivore-produced infochemicals, and 3) the resulting host location efficiency in field environments (Bukovinszky et al. 2012b, Chabaane et al. 2015). We hypothesized that through trait-mediated interactions non-host feeding guilds would differently affect parasitoid foraging and thereby either provide associational resistance or susceptibility of the host herbivore to parasitism.

Materials and methods

Plants and insects

Brassica oleracea var gemmifera cv. Cyrus plants were used in all experiments. Plants were grown in pots with 0.7 litre of potting soil (Lentse potgrond) and fertilized two to three times per week using a liquid fertilizer (greenhouse conditions: 20±2°C, 60±10% RH, 16:8 L:D photoperiod). Five-week-old plants were used in the wind tunnel and flight chamber experiment. Plants used in the outdoor tent experiment were transferred outside after four weeks to acclimatize. After five weeks, these plants were transplanted in the tents where they could acclimatize for another week and a half.

Native caterpillars (*P. brassicae*, *Mamestra brassicae* (Lepidoptera: Noctuidae) and *Plutella xylostella* (Lepidoptera: Plutellidae)) and aphids (*Brevicoryne brassicae* (Hemiptera: Aphididae), *Myzus persicae* (Hemiptera: Aphididae) strain 1 and *Myzus persicae* strain 2) were reared on *B. oleracea*, (one of) their host plants, and maintained at 20±2°C, 60±10% RH and 16:8 L:D photoperiod. *Spodoptera exigua* (Lepidoptera: Noctuidae) is an exotic species and was reared on an artificial diet mainly composed of polenta, beer yeast and wheat-germs and maintained at 25.5°C, 50%RH and 16:8 L:D photoperiod. Because different aphid species that have been reported to colonize *B. oleracea* in the field (e.g. *Macrosiphum euphorbiae* (Hemiptera: Aphididae)) reproduced poorly on our *B. oleracea* cultivar, we collected two different strains of *M. persicae* to ensure three aphid treatments. The two asexually reproducing *M. persicae* strains clearly differed in honeydew production; strain 2 produces larger amounts of stickier honeydew than strain 1. The parasitoid *C. glomerata* was reared on *P. brassicae* caterpillars. Parasitoid cocoons were kept in a climate cabinet (25°C, 12:12 L:D photoperiod) and emerged parasitoids were given honey and water *ad libitum*. Parasitoids were allowed to mate and one-to-six-day old naive parasitoids were used in the experiments.

Wind tunnel experiment

A wind tunnel, as described by Geervliet *et al.* (1994), was used in October 2012 (wind tunnel conditions: 0.1 m/s wind speed, 24-26°C, 59-64% RH) to test the hypotheses that non-host presence would reduce HIPV-based host location by *C. glomerata* and that feeding guild of the non-host would affect the magnitude of this effect. As a reference control for parasitoid responses to host-infested plants in the absence of non-hosts, we tested the preference of naive parasitoids for plants infested with ten hosts only in a choice test with an undamaged plant (fig. 1A). For the other treatments, both plants in a choice test pair were infested with the same non-host species by gently transferring either 40 wingless aphids or ten first-to-second instar caterpillars of one of the six non-host species to the youngest fully expanded leaf one day before testing. One plant per pair was infested with ten host (*P. brassicae*) caterpillars (fig. 1 B, C). These amounts of herbivorous individuals are known to induce plant defences (Bukovinszky et al. 2012b, Kroes et al. 2015). Caterpillars of all species were approximately the same size. The combination with *M. persicae* strain 2 was also tested with 72 hours between infestation of the plant and conduction of the wind tunnel experiment to test for an effect of infestation time of aphids. Infested plants were kept in a greenhouse (23±2°C, 60±10% RH, 16:8 L:D photoperiod). A piece of cotton wool was wrapped around the petiole of the infested leaf to prevent the herbivores from moving to other leaves. The cotton wool was removed just before the experiment. Per plant pair, ten female parasitoids were individually tested and allowed to make a choice during a maximum of five minutes. Parasitoids that did not land on one of the plants within five minutes were considered unresponsive. After testing five parasitoids, the position of the two plants in the wind tunnel was switched in order to limit any effect of plant position. Per treatment, six pairs of plants were tested on six different days and the specific combinations tested were randomly divided over the testing days.

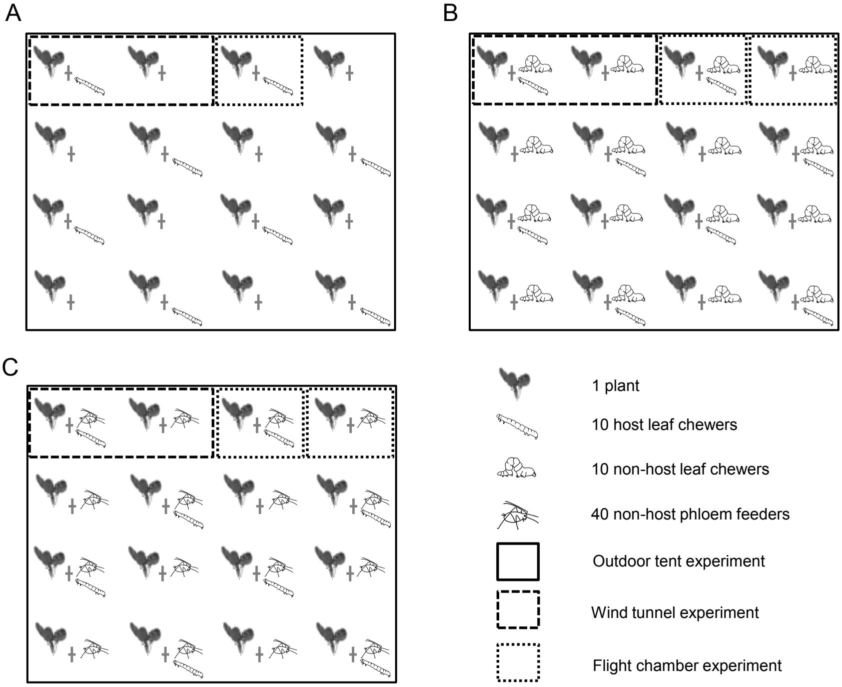

Figure 1. Set-up of the experiments. In the outdoor tent experiment and the wind tunnel experiment, environments were created with (A) clean plants and host-infested plants, (B) host + non-host leaf chewer infested plants and non-host leaf chewer-infested plants and (C) host + non-host phloem feeder infested plants and non-host phloem feeder-infested plants. In the flight chamber experiment, plants were infested with (A) hosts, with (B) hosts + non-host leaf chewers or only non-host leaf chewers, with (C) hosts + non-host phloem feeders or only non-host phloem feeders. Plant: *Brassica oleracea*, host: *Pieris brassicae*, non-host leaf chewers: *Mamestra brassicae*, *Plutella xylostella*, *Spodoptera exigua*, non-host phloem feeders: *Brevicoryne brassicae*, *Myzus persicae* strain 1 and *Myzus persicae* strain 2.

Flight chamber experiment in greenhouse

A flight chamber was used from November 2012 to January 2013 to test the hypotheses that the presence of non-hosts and their feeding guild would affect the on-plant host location behaviour of *C. glomerata*. The flight chamber was a tent (290 cm x 200 cm x 230 cm in height) made of insect screen (mesh size 0.6 mm) situated in a greenhouse compartment (25±2 °C, 60±10% RH) (see also Bukovinszky et al. 2012b). This was a no-choice experiment in which the effect of one non-host caterpillar species and one non-host aphid species was tested due to the time-consuming nature of the experiment. Plants were infested with a non-host only (either ten *M. brassicae* caterpillars or 40 *M. persicae*-strain-2 aphids) (fig. 1 B,C), with ten hosts only (*P. brassicae* caterpillars) (fig. 1A), or with ten hosts (*P. brassicae*) and either ten non-host caterpillars (*M. brassicae*) or 40 non-host aphids (*M. persicae2* strain 2) (fig. 1 B,C), using the same methods as described for the wind tunnel experiment. On a table inside the flight chamber, three infested plants of the same treatment were placed 50 cm apart from each other, forming a triangle. One female parasitoid at a time was released 50 cm from the nearest plant. The parasitoid was allowed to fly to one of the plants of her choice before starting the observation. Using a Psion Workabout PRO 3 device (Motorola Solutions, Inc., Schaumburg, Illinois, USA) and Observer XT 10 software (Noldus Information Technology, Wageningen, The Netherlands), the following behaviours of the parasitoid were recorded: excursions outside the vicinity of the plant (off-plant excursions), flying in the vicinity of the plant (flying), landing on the plant (landing), walking, preening, standing still, attacking a host, ovipositing in a host, ovipositing in a non-host. For all but two behavioural components, both the duration and the frequency were recorded. For the behavioural components ‘ovipositing in a host’ and ‘ovipositing in a non-host’, only the frequency was recorded because it was not possible to accurately record the duration of these sometimes rapid behaviours. Parasitoids were observed on the same plant for a maximum of one hour; the observation was stopped before the maximum time was reached when the parasitoid showed an off-plant excursion lasting more than one minute. Parasitoids staying on the walls or ceiling of the tent for five minutes immediately after being released were considered unresponsive. After each observation, plants visited by the parasitoid were replaced by a new plant before continuing with the next observation. Parasitized herbivores were dissected to count the number of parasitoid eggs that had been injected into the host by using a stereomicroscope. Because the behavioural structure in the ethogram of *C. glomerata* on host only or host and non-host infested plants has been found to be very similar (Bukovinszky et al. 2012b), here we focused on duration and frequency of each of the behaviours.

Outdoor tent experiment

We then hypothesized that non-host presence and their feeding guild would affect the overall parasitization efficiency of *C. glomerata* in a small plant community and tested this in an outdoor tent experiment from May to September 2012 in an agricultural field in Wageningen, The Netherlands (51°99 N, 5°66 E). Fourteen tents (l x w x h: 3m x 4m x 2m) made of insect screen (mesh size 0.6 mm) were used. Per series (seven in total), each of seven treatments was randomly assigned to two tents. Due to logistics, not all treatments could be tested in every series, but some treatments could be tested one extra time. In each tent, 16 plants were planted in a four by four grid. One and a half weeks later, plants were infested with herbivores. For the control treatment, ten first-instar host caterpillars (*P. brassicae*) were transferred to the youngest fully expanded leaf of eight plants (every other plant) (fig. 1A). For the other six treatments, next to the presence of the host caterpillars on every other plant, all 16 plants were infested with individuals of one of the six non-host species: either ten first-to-second instar non-host caterpillars (caterpillars of all species were approximately the same size) (fig. 1B) or 40 wingless non-host aphids (fig. 1C). One day after herbivore infestation, three female and three male naive *C. glomerata* parasitoids were released in each tent. At the same time, two plastic lids (diameter 3 cm) with drops of honey were left as food source in the tent. Three days after parasitoid release, host-infested plants were harvested, kept separately in labelled plastic bags and stored at 5°C. All *P. brassicae* caterpillars found back on the collected plants were dissected and parasitoid eggs were counted using a stereomicroscope.

Statistical analysis

For the wind tunnel experiment, a binomial test was conducted per treatment for equal preference of parasitoids for plants infested by hosts and non-hosts and plants infested by non-hosts only. Second, logistic regression analyses were conducted by feeding guild on the number of parasitoids choosing the host and non-host-infested plant out of the total number of responding parasitoids per plant pair. The model comprised effects for herbivore identity on the logit scale. Since no herbivore identity effects were found, results from these treatments were pooled per feeding guild. This was a step-up to a logistic regression analysis that included the host-only and the two feeding guild treatments. The model comprised an extra unknown multiplicative dispersion factor for the binomial variance, an explanatory variable for the age of the parasitoid and main effects for feeding guild on the logit scale. Since parasitoid age was not significant, it was subsequently dropped from the model. Statistical inference was by maximum quasi-likelihood (McCullagh and Nelder 1989). The quasi-likelihood ratio test (McCullagh and Nelder 1989) was preferred over the Wald test, because of an extremely high proportion for the control treatment (which was slightly lowered, to avoid a large number on the logit scale).

Duration of a behaviour in the flight chamber experiment was calculated per individual observation only when that behaviour was performed during this observation. Duration, expressed as a proportion of residence time, was analysed with logistic regression. The model comprised a multiplicative dispersion factor for the binomial variance and main effects for treatment on the logit scale. The 'binomial total' was fixed at value 1. Residence time per parasitoid was analysed similarly, with 60 minutes as the 'binomial total'. The number of hosts parasitized per parasitoid was also analysed in this way, with the number of parasitized hosts as the response variable and a 'binomial total' of ten hosts. Rates per minute of observed behaviours were analysed for all observations with a generalized linear model comprising a log link function. The model comprised an extra unknown multiplicative dispersion factor in combination with the Poisson variance and main effects for treatment on the log scale. The number of eggs per oviposition event and the number of eggs per host were analysed with the same model but with an extra offset: log of oviposition frequency or log of number of hosts parasitized. All models of the flight chamber experiment comprised an explanatory variable for the age of the parasitoid. Whenever parasitoid age was not significant, it was dropped from the model. In the analyses of the behaviours 'attack host', 'oviposition host', 'hosts parasitized', 'hosts parasitized per minute', 'eggs per host' and 'eggs per oviposition', five observations were left out in which the parasitoids did not parasitize any hosts.

The percentage of parasitized hosts per tent and the percentage of plants with parasitized hosts per tent in the outdoor tent experiment were analysed with a generalized linear mixed model (GLMM), comprising a logit link function and a multiplicative dispersion parameter for the binomial variance function. The number of parasitized hosts per plant on which parasitized hosts were found per tent and the number of eggs per parasitized host per tent was analysed with a GLMM, comprising a log link function and a multiplicative dispersion parameter for the Poisson variance function. The response was the number of parasitized hosts per tent or the number of eggs per tent, with the log of the number of plants with parasitized hosts per tent or the log of the number of parasitized hosts per tent as an offset. All four models of the tent experiment comprised fixed effects for series in time and either herbivore identity or feeding guild and random effects for tents, the latter effects allowing for dependence between observations from the same tent (being used in all series). Inference was based on penalized quasi-likelihood (PQL) (Breslow and Clayton 1993). Quasi-Wald tests and approximate F-tests (Kenward and Roger 1997) applied to the approximate linear mixed model from the last step of the iterative re-weighted REML algorithm, were used. Calculations were performed using GenStat® 17th edition.

Results

Wind tunnel experiment

To test the response to herbivore-induced plant volatiles, 278 responding parasitoids were observed. Parasitoids preferred plants infested with both hosts and non-hosts over neighbouring plants, infested with non-hosts only, for all treatments (herbivore identity and feeding guild) (fig. 2). These results were similar when phloem feeders fed on the plant for 72 hours instead of 18 hours (binomial test, $P < 0.001$). Within feeding guild treatments, no differences in the degree of preference were found (leaf chewers: GLM, $F = 0.21$, d.f. = 1, $P = 0.656$, phloem feeders: GLM, $F = 0.00$, d.f. = 1, $P = 0.963$), so herbivore identity groups within feeding guilds were pooled. In the presence of leaf-chewing non-hosts, parasitoids less frequently preferred the dual-infested plant over the non-host-only-infested neighbouring plant compared to the control situation without non-hosts. The presence of phloem-feeding non-hosts, on the other hand, did not affect the percentage of parasitoids attracted to the dual-infested plant. Comparing the two groups of non-host feeding guilds shows that the presence of non-host leaf chewers negatively affected the response of parasitoids towards the dual-infested plant compared to the presence of non-host phloem feeders (Quasi likelihood ratio test, $F = 24.28$, d.f. = 2, $P < 0.0001$ (fig. 2).

Flight chamber experiment in greenhouse

The behaviour of naive, mated female parasitoids on the plant was observed for 103 responding parasitoids. After arriving on a plant, the residence time of the parasitoids was affected by plant treatment (GLM, $F = 36.47$, d.f. = 4, $P < 0.001$). On plants with single infestation by the non-host leaf chewer (*M. brassicae*) or phloem feeder (*M. persicae* strain 2), the residence time was similar with 593.7 ± 37.3 s for the non-host leaf chewer and 512.5 ± 40.5 s for the non-host phloem feeder (fig. 3A). When hosts were present on the plant in single or in dual infestations, the residence time significantly increased up to

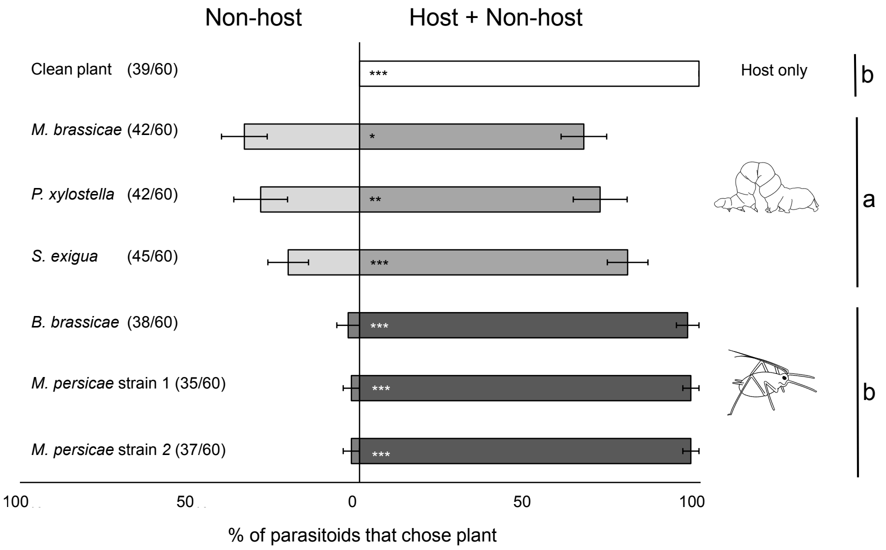

Figure 2. Effects of non-hosts sharing the food plant with host herbivores in an environment of non-host infestation, on location of host-infested plants by the parasitoid wasp *C. glomerata* in a wind tunnel. Response to volatiles from non-host only infested plants (left) versus host and non-host-infested plants (right) in the wind tunnel experiment for the different non-host herbivores. Error bars indicate standard errors of different plant pairs (n = 6 plant pairs per treatment), asterisks indicate significance level based on binomial tests *:P < 0.05, **:P < 0.01, ***:P < 0.001. Numbers between brackets indicate the number of parasitoids that responded/the number of parasitoids tested. Different letters for the feeding guild treatments indicate different means based on a quasi-likelihood ratio test (P < 0.0001) and least significant differences (α = 0.05).

six-fold to 3118 ± 115 s (P < 0.001) (fig. 3A). Parasitoids spent less time on dual-infested plants with hosts and phloem feeders compared to plants infested by hosts only (P = 0.021) (fig. 3A). The time spent on dual-infested plants with hosts and leaf chewers did not significantly differ from the time spent on host-only infested plants, nor from dual-infested plants with hosts and phloem feeders.

Parasitoids were less frequently involved in, and spent a smaller proportion of the residence time on, off-plant excursions when host herbivores were present on the plant than when only non-host herbivores were present (rate: GLM, F = 25.61, d.f = 4, P < 0.001, proportion of residence time: GLM, F = 20.79, d.f. = 4, P < 0.001) (fig. 3 B,C). Parasitoids spent a significantly higher proportion of the residence time on off-plant excursions when plants were infested with only non-host phloem feeders compared to only non-host leaf

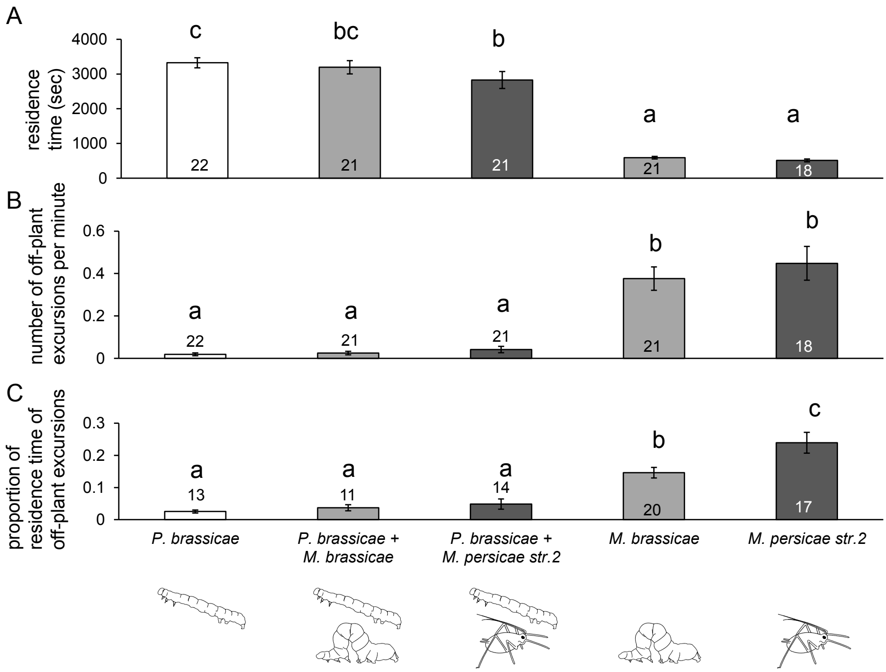

Figure 3. Residence time (A), number of off-plant excursions per minute (B) and proportion of residence time of off-plant excursions (C) ± SE of *C. glomerata* parasitoids on herbivore-infested plants in the flight chamber experiment. Numbers in/on top off bars indicate the number of observations analysed per treatment. Different letters indicate significantly different means based on generalized linear models (P < 0.001) and least significant differences (α = 0.05).

chewers (P = 0.002) (fig. 3C). In treatments where hosts were present, the number of hosts parasitized, the number of eggs per oviposition and the number of eggs per host was not significantly affected by the presence of non-host herbivores of either feeding guild (fig. 4 A,B,C). No differences in the remaining observed behaviours on the plant, such as flying, walking and preening, were observed when plants were infested with hosts, either alone or in combination with a non-host (see fig. S1, Appendices).

Outdoor tent experiment

In experimental habitats where parasitoids were exposed to different non-host herbivores in outdoor tents, the week in which experiments were conducted (series) strongly affected the percentage of parasitized host caterpillars, the percentage of plants with parasitized hosts and the number of eggs found in parasitized hosts (fig. S2, Appendices). There was no effect of the used tents in any of the models. In total 6348 (81.8%) host caterpillars

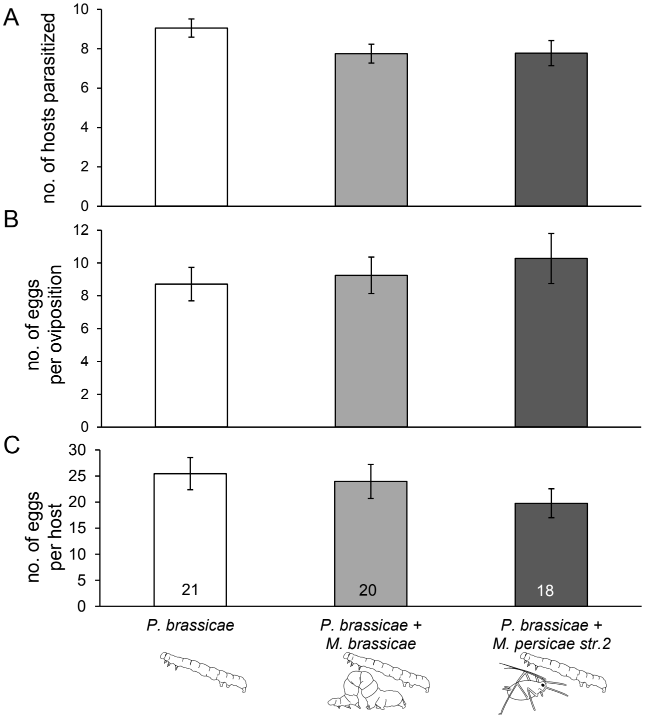

Figure 4. Number of hosts parasitized (A), number of eggs per oviposition (B) and number of eggs per host (C) ±SE by *C. glomerata* parasitoids that did parasitize at least one host on herbivore infested plants in the flight chamber experiment. Numbers in bars indicate the number of observations analysed per treatment, equal for all three analyses. No significant effects of treatment were found using generalized linear models.

were remaining on the plants of which 2342 (36.9%) were parasitized. Although there was a marginally non-significant effect of feeding guild on the overall parasitism level (GLMM, F = 2.77, n.d.f. = 2, d.d.f. = 88.0, P = 0.068) (fig. 5A), feeding guild did significantly affect the percentage of plants on which hosts were parasitized (GLMM, F = 3.54,n.d.f. = 2, d.d.f. = 88.0, P = 0.033) (fig. 5B). The presence of phloem-feeding or leaf-chewing non-hosts on all plants in the environment, i.e. on host-infested plants and on the neighbouring plants, did not affect the percentage of plants with parasitized hosts compared to a situation where non-hosts were absent. However, when phloem-feeding non-hosts were present, the percentage of plants with parasitized hosts was higher compared to treatments in which leaf-chewing non-hosts were present (P = 0.02). The total number of parasitized hosts on plants on which one or more hosts had been parasitized was not different for environments with non-host phloem feeders or with non-host leaf chewers (fig. 5C). The number of eggs found in parasitized hosts, in contrast, was significantly affected by the feeding guild of the non-hosts (GLMM, F = 6.36, n.d.f. = 2, d.d.f. = 86.3, P = 0.003) and by the species/strain of non-hosts (GLMM, F = 3.38, n.d.f. = 6, d.d.f. = 70.9, P = 0.005). When phloem feeders were present, the number of eggs per host was higher compared to the control situation where only hosts were present (P = 0.003) and compared to the situation where non-host leaf chewers were present (P = 0.008). The presence of non-host leaf-chewers did not affect the number of eggs found in parasitized hosts compared to the control situation without non-hosts (fig. 5D).

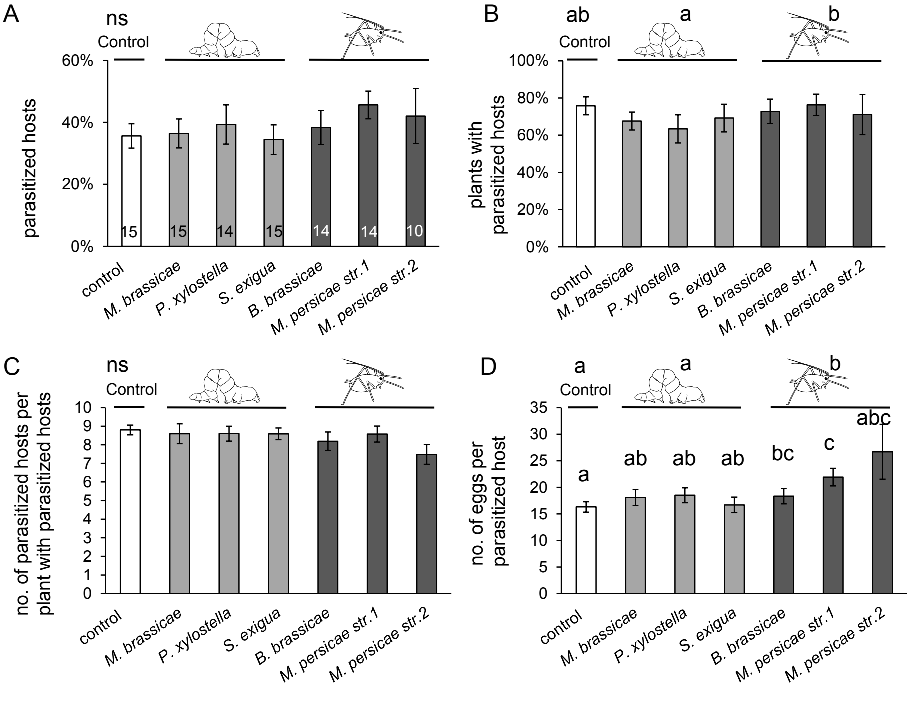

Figure 5. Percentage of hosts parasitized (A), percentage of plants with parasitized hosts (B), number of parasitized hosts per plant with parasitized hosts (C) and number of eggs per parasitized host (D) ±SE found in the outdoor tent experiment for the different non-host herbivores. In the treatment 'control' no non-hosts were present next to the host. Numbers in bars indicate the number of tents per treatment, equal for all four analyses. Different letters for the herbivore identity and feeding guild treatments indicate different means based on generalized linear mixed models ($P < 0.05$) and least significant differences ($\alpha = 0.05$).

Discussion

It is well known that trait-mediated direct and indirect interactions influence foraging animals and microorganisms in terrestrial and aquatic environments (Schmitz et al. 2004, Miki and Jacquet 2010, Utsumi et al. 2010). Several trait-mediated interactions could interfere with each other (Ohgushi 2005, Desurmont et al. 2014). In this study, this has been investigated for plant-herbivore-parasitoid interactions. Foraging parasitoids are faced with trait-mediated indirect interactions of which volatile-based searching is used by parasitoids to find host-infested plants (Vet and Dicke 1992, Ohgushi 2008, Hare 2011). Additionally, parasitoids face direct trait-mediated interactions of which host-derived infochemicals are used to locate hosts on a plant (De Rijk et al. 2013). These interaction units of (plant-)host-parasitoid are expanded or even linked to other interaction units when intervening herbivores (non-hosts) are present (Utsumi et al. 2010). Non-hosts, therefore, can alter interactions between host and parasitoid (Shiojiri et al. 2000b, 2001, 2002, Bukovinszky et al. 2012b). Here, we found that, in particular, the feeding guild of non-hosts can determine the sign and strength of these alterations and that these effects are caused by both indirect plant trait-mediated and direct trait-mediated interactions.

The response of parasitoids to herbivore-induced plant volatiles was not affected by phloem-feeding non-host herbivores, whereas non-host leaf chewers had a negative effect on the efficiency of locating host-infested plants. After finding the infested plant, the presence of phloem feeders enhanced parasitoid infochemical-based searching efficiency by finding and parasitizing a similar number of hosts but in a shorter time than when non-hosts were absent. The infochemical-based searching efficiency on the plant was, however, not affected by the presence of leaf-chewing non-host herbivores. Although non-hosts from both feeding guilds had direct and indirect trait-mediated effects, they differed in effect size on host location by parasitoids: phloem feeders primarily affected parasitoids through direct trait-mediated effects, whereas leaf chewers had primarily indirect trait-mediated effects through plant volatiles. The resulting host-finding efficiency as tested in the outdoor tent experiment was partly affected by feeding guild of the non-hosts. Although marginally but insignificantly different from the host-only environments, phloem feeders increased the percentage of hosts parasitized in the field tent and they also significantly increased the number of eggs laid per individual host. The percentage of plants with parasitized hosts was significantly higher in situations with phloem feeders than in the presence of leaf-chewing non-hosts. The number of hosts parasitized per plant was not affected by the presence of non-hosts nor by their feeding guild.

Non-hosts will nearly always be present in a parasitoid's natural or agricultural environment. Incorporating the influence of the environment into the conclusions of trait-mediated processes caused by non-hosts, therefore, is essential. From our results, we can compare environments with only non-host phloem feeders to environments with only non-host leaf chewers, whereas in natural systems these species commonly co-occur

(Poelman et al. 2009, Poelman et al. 2010). The results from the outdoor tent and wind tunnel experiments indicate that neighbouring plants infested with only non-host leaf chewers are more distracting to the parasitoid than neighbouring plants infested with only non-host phloem feeders. Our results on leaf-chewing non-hosts confirm previous findings that parasitoids that attack leaf-chewing herbivores prefer volatiles from plants infested with host and non-host leaf chewers over volatiles of plants infested with non-host leaf chewers only (Bukovinszky et al. 2012b). However, in other studies parasitoids did not show a preference for either plants infested with host and non-host leaf chewers or plants infested with non-host leaf chewers only (Vos et al. 2001). This indicates that against a background of non-host leaf chewers, parasitoids can have more difficulties finding their hosts. The underlying mechanism for these findings may be in plant defence signalling. Parasitoids may mistake plants infested by only non-host leaf chewers for plants infested with hosts because both host and non-host leaf chewers induce the JA pathway in plants that will accordingly emit similar volatiles (Turlings et al. 1993, Geervliet et al. 1997a, Stam et al. 2014). In contrast, phloem feeders in general induce the SA pathway, making plants emit different volatiles (Stam et al. 2014) and therefore may not distract the parasitoid as found in our study. However, it is also expected that double infestation by a JA- and an SA-inducing insect would cause crosstalk between these two pathways (Dicke et al. 2009, Pieterse et al. 2009, Thaler et al. 2012). This would result in altered plant volatiles and this in turn would make it more difficult for the parasitoid to find the host-infested plant (Dicke et al. 2009, Zhang et al. 2013). Such an effect of phloem feeders reducing the attraction of parasitoids to chewing hosts on the same plant is not shown in our experiments. This may be explained by specificity in crosstalk between the SA and JA pathways depending on certain combinations of herbivore and plant species (Zhang et al. 2009, Erb et al. 2010, Ponzio et al. 2013, Zhang et al. 2013, Ponzio et al. 2014, Stam et al. 2014).

Our field tent data show that if non-hosts accompany hosts, phloem-feeding non-hosts increase parasitism of hosts and provide associational susceptibility. However, with leaf-chewing non-hosts, the direction of associational resistance is observed. This difference between phloem-feeding and leaf-chewing non-hosts could be caused, to a large extent, by the neighbouring plants that were infested with only non-hosts. At the information level of volatile-based foraging, neighbouring plants caused the parasitoids in the leaf-chewer situation to choose the ‘wrong’, non-host-only-infested plant. Therefore, the guild of non-hosts feeding on plants in the environment of the target plant proves to be at least as important as the guild of non-hosts feeding on the target plant itself. The presence of non-hosts on both host-infested and neighbouring plants may cause intricate interactions between direct and indirect trait-mediated processes. In our behavioural studies, we found that parasitoids spent more time on off-plant excursions when the non-hosts were represented by phloem feeders compared with leaf-chewing non-hosts. While making off-plant excursions induced by direct trait-mediated effects of non-hosts, the parasitoids may perceive volatile information from neighbouring plants. Because they can more easily discriminate host-infested from non-host-infested plants with non-host phloem feeders than with leaf chewers, plant-volatile-based indirect trait-mediated interactions may cause parasitoids in off-plant excursions to more frequently locate host-infested plants in a phloem-feeding non-host background than in a leaf-chewer non-host background. When visiting more host-infested plants in environments with phloem feeding non-hosts, the percentage of plants with parasitized hosts increases as found in our field tent study. Moreover, the same host-infested plants may be visited repeatedly, potentially resulting in superparasitism of these hosts as indicated by the larger number of eggs found in hosts recovered from field tent environments with phloem feeding non-hosts. Additionally, the quality of the hosts available for the parasitoids could have added to the positive effect of phloem feeders in comparison to leaf chewers. It is known that by inducing plant signal-transduction pathways and interactions between them, phloem feeders can positively affect the development of parasitoids through their effect on leaf-chewing hosts (Soler et al. 2012a, Li et al. 2014). This may cause clutch size adjustments in which parasitoids lay more eggs in high-quality hosts. In addition, it is also known that honeydew produced by aphids may serve as a food source for parasitoids and may enhance parasitoid egg production (Faria et al. 2008, Kugimiya et al. 2010). The sum of these indirect and direct trait-mediated processes of non-host aphid presence caused associational susceptibility to caterpillar hosts by enhancing the performance of their parasitoid enemy. Although our study shows that feeding guilds of non-host herbivores may differentially affect parasitoid efficiency in host location, the relative importance of non-host feeding guild compared to other non-host traits (e.g. herbivore feeding position on the plant or their diversity) and interactions among those should still be evaluated. Such evaluations are essential for understanding parasitoid foraging in naturally complex environments.

The effect of non-prey/-host organisms and their characteristics on parasitoid or predator foraging behaviour may regulate the prey community and therefore the strength of the interaction between predator and prey (Kratina et al. 2007, Fan et al. 2010). In the presence of non-prey, predator control of prey could be dampened (Kratina et al. 2007) while in the absence of non-prey, prey communities could be overexploited (Fan et al. 2010). Understanding how naturally occurring and human-introduced non-target species indirectly affect predator-prey interactions and interspecific competition (Fan et al. 2010) could be a step forward towards developing more complex and true-to-nature indirect interaction webs (Ohgushi 2008, Utsumi et al. 2010). Stronger predictions of the effects of global environmental changes on communities and ecosystems can be made using these complex interaction models (Tylianakis et al. 2008, Bascompte 2009).

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Chapter 4

Flexible parasitoid behaviour overcomes constraint resulting from position of host and non-host herbivores

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Parasitoids face several hurdles and distractions during the process of foraging for their hosts, one of which is the presence of non-host herbivores. Non-host herbivores may interfere with plant volatile-mediated location of host-infested plants and reduce encounter rates with hosts on the plant. This results in a lower foraging efficiency. In this study, we tested whether the feeding position of a host and non-host herbivore on the same plant influences foraging decisions and parasitization efficiency of parasitoids. We confined host and non-host herbivores to either higher positions, i.e. younger leaves (preferred by the host) or lower positions on the plant, i.e. older leaves (preferred by the non-host). Host and non-host herbivores were either feeding on separate leaves, or mixed and feeding on the same leaf. Results from laboratory experiments show that during the first phase of foraging when plant volatiles are used to locate a host-infested plant, parasitoids were misled when host and non-host were positioned in an unnatural way on the individual plant (host on the older leaves). The position of host and non-host partly influenced parasitoids during the second phase of foraging, when the host is located on the plant by using host cues. Total host-finding efficiency as tested in a semi-field set-up was not affected by herbivore position. We conclude that parasitoid foraging behaviour has enough flexibility to overcome constraints resulting from an unexpected distribution of herbivores over a plant.

Key words

Foraging efficiency, herbivore position, multi-herbivory, non-host, parasitoid behaviour, semi-field experiment

Foraging is essential for all animals and, therefore, the behaviour when foraging for food or other resources is well-studied in many groups of organisms (Nonacs 2001, Stephens 2008). One of those studied groups are parasitic wasps (parasitoids) of which the behaviour when foraging for hosts is especially interesting. Foraging for hosts is expected to be subject to strong selection pressure, because the success of foraging for hosts is strongly linked to parasitoid fitness (Thiel and Hoffmeister 2009). Parasitoids lay their eggs in or on the host (usually other arthropods) and after emergence the larvae are dependent on this host as their food source (Godfray 1994, Thiel and Hoffmeister 2009). To be able to find their herbivorous hosts, parasitoids have evolved foraging strategies that can be divided into two phases (Van Alphen et al. 2003, De Rijk et al. 2013). During the first phase, plant volatiles are used as cues to locate the plant on which the herbivore host is feeding (Vet and Dicke 1992, Van Alphen et al. 2003, Hare 2011). These so-called herbivore-induced plant volatiles (HIPVs) are known to be produced by plants in response to herbivore attack (Vet and Dicke 1992, Arimura et al. 2011, Stam et al. 2014). During the second phase of foraging, host cues (e.g. from frass or feeding damage) are used to locate the host on the plant after which the host is recognized and examined for its quality before being accepted (Van Alphen et al. 2003, De Rijk et al. 2013, Colazza et al. 2014).

Hosts are ‘hidden’ in an environment full of possible hurdles and distractions for the parasitoid. For example, landscape structure (Nelson and Forbes 2014, Roitberg and Gillespie 2014), vegetation composition (Gols et al. 2005, Bezemer et al. 2010) and on a smaller scale host patch size (Bezemer et al. 2010) or the presence of competitors (Almohamad and Hance 2014, Couchoux and van Nouhuys 2014) could affect the ability of parasitoids to find their host. Furthermore, the presence of herbivores that are not suitable as a host could impair host location (De Rijk et al. 2013, Desurmont et al. 2014, Ponzio et al. 2014). Finding these non-hosts alongside hosts is common, as in both natural and agricultural ecosystems plants are commonly attacked by more than one herbivore species simultaneously as well as sequentially (Vos et al. 2001, Stam et al. 2014). Like hosts, non-hosts also induce the plant to produce volatiles. Non-host feeding could modify the induction of volatiles by the host herbivore (Dicke et al. 2009, Zhang et al. 2009, Soler et al. 2012a, Zhang et al. 2013) and could, therefore, interfere with the detectability of host-induced cues by the parasitoid. In addition, non-hosts that feed on the same plant also produce cues like frass and feeding damage that could lead the parasitoid away from the infochemicals produced by the host (Takabayashi and Takahashi 1990). Here, we use the term ‘misleading’ to describe when non-host-induced plant volatiles and non-host infochemicals lead the parasitoid away from cues induced and released by hosts.

Previously, it was found that the foraging efficiency of parasitoids could be differently affected by non-host herbivores belonging to different feeding guilds (Moujahed et al. 2014, De Rijk et al. 2016b). It is, however, not known whether the position of the host and these non-hosts on the leaves of the same plant would influence decisions and foraging efficiency of parasitoids (Bukovinszky et al. 2012b). It is known that parasitism rates can be affected by plant structure (Romeis et al. 2005, Obermaier et al. 2008), position of the infested leaf (El-Wakeil 2011) and feeding on either leaves or flowers by host herbivores (Lucas-Barbosa et al. 2014). Therefore, adding a non-host herbivore on the same plant but at a separate leaf could possibly also affect parasitoid foraging behaviour and efficiency. When host and non-host herbivores are feeding on different leaves of the same plant, both will induce the plant to defend. Plant defence is often expressed not only locally but also systemically (Heil and Ton 2008, Shah 2009, Gutbrodt et al. 2011, Marti et al. 2013) and systemic signal distribution can be affected by plant architecture (Jones et al. 1993, Orians 2005, Utsumi et al. 2010, Mousavi et al. 2013). It is not clear whether two herbivores feeding on different parts of the plant will induce one cue emitted from the entire plant, or that the different plant parts provide distinct cues that can be used during the first phase of foraging to determine the position of the host herbivore (Utsumi et al. 2010). In either case, when herbivores are feeding on separate leaves, parasitoids would have to choose on which leaf to land. During the second foraging phase the influence of the separated positions of host and non-host on parasitoid behaviour is expected to depend on the leaf selected. When the parasitoid lands on a non-host-infested leaf, it is likely to encounter non-hosts and their products. This may result in either one short visit to the plant without parasitization of hosts (Sato et al. 1999, Shiojiri et al. 2000a, Vos et al. 2001, Bukovinszky et al. 2012b), or prolonged visits during which parasitoids waste time by encountering non-hosts, but eventually find the host-infested leaf. In contrast, when landing on a host-infested leaf, no constraints are present on this smaller scale, so the parasitoid could be as efficient as when no non-hosts would be around.

Here, we present the results of three experiments that studied the effect of herbivore position on the foraging behaviour of the parasitoid *Cotesia glomerata* that is specialized on *Pieris* caterpillars. We selected its most common host, the gregariously feeding *Pieris brassicae*, and a non-host that lays eggs in batches, *Mamestra brassicae,* for this study (Bukovinszky et al. 2012b). These caterpillars feed on brassicaceous plants, including cultivated *Brassica oleracea*. In general, the specialist *P. brassicae* caterpillars feed on the younger, and so higher located, leaves (Gutbrodt et al. 2012), while generalist caterpillars like *M. brassicae* would feed on the older, less defended and lower located leaves of *B. oleracea* (Gutbrodt et al. 2012). First, we tested whether host and non-host position on the plant influenced parasitoid decisions for landing on infested leaves by using a wind tunnel. Second, we observed in a flight chamber set-up how the behaviour of the parasitoid after landing on the plant was affected by herbivore position. Third, we tested in a semi-field set-up whether the total host-finding efficiency of the parasitoid was affected by herbivore position.

Materials and methods

Plants and Insects

Five weeks old *Brassica oleracea* var gemmifera Cyrus plants were used in all three experiments. The plants were grown by Unifarm of Wageningen UR in a greenhouse (20±2°C, 60±10% RH, 16:8 L:D photoperiod, SON-T light 500 μ mol $m^{-2} s^{-1}$) in pots of 0.7 litre filled with potting soil (Lentse potgrond, no. 4; Lent, the Netherlands) and fertilized two to three times per week using a liquid fertilizer (EC 2.1 mS/cm, pH 5.8). No pest control measures were taken.

Pieris brassicae and *Mamestra brassicae* cultures were maintained on *B. oleracea* plants at 20±2°C, 60±10% RH and 16:8 L:D photoperiod for over 10 generations. Both caterpillar species were originally collected from agricultural fields in the Netherlands. In the experiments first instar caterpillars were used. A *Cotesia glomerata* culture was maintained on *P. brassicae* larvae at 20±2°C, 60±10% RH and 16:8 L:D photoperiod for over 10 generations and the culture was renewed with field collected parasitoids yearly. The parasitoids were originally collected from an agricultural field in Wageningen, the Netherlands. *Cotesia glomerata* cocoons were collected from rearing cages and emerged adults were kept in cages with water and honey *ad libitum* at 21°C and 16:8 L:D photoperiod. The parasitoids were allowed to mate and 1-to-10 days old parasitoids inexperienced with herbivores and plants were collected from the cages before being used in the experiments.

Wind tunnel experiment

To study whether host and non-host position on the plant influenced decisions of *C. glomerata* for infested leaves to land on, a wind tunnel as described by Geervliet et al.

(1994) was used in July and August 2013. It was observed which leaf was preferred for landing by *C. glomerata* when the parasitoid was given the choice for all leaves of a single plant. *Brassica oleracea* plants were infested with herbivores according to four treatments: (1) ten first-instar *P. brassicae* larvae on the higher leaf, ten first-instar *M. brassicae* larvae on the lower leaf (host high), (2) ten first-instar *P. brassicae* larvae on the lower leaf, ten first-instar *M. brassicae* larvae on the higher leaf (host low), (3) ten first-instar *P. brassicae* and ten first-instar *M. brassicae* larvae on the higher leaf (mixed high), (4) ten first-instar *P. brassicae* and ten first-instar *M. brassicae* larvae on the lower leaf (mixed low). Numbers of host and non-host individuals attacking the plant as a whole were kept identical between treatments to prevent variation in systemic volatile emission by quantitative effects. Clutch sizes of the two herbivore species in nature are similar, the number of individuals used here are within the lower range of larval numbers found infesting single plants in nature and both herbivores are frequently found sharing a food plant (Poelman et al. 2009). Previous research showed that *C. glomerata* responds well to the used numbers of (non-)hosts (Geervliet et al. 1998a, Bukovinszky et al. 2012b). The ‘higher leaf’ was the youngest fully expanded leaf of the plant, the ‘lower leaf’ was the oldest leaf of the plant. The leaf area of all infested leaves was similar and abundant for feeding by the caterpillars. The infested leaves were positioned in the same half of the plant, so that the infested leaves were pointing in a similar direction. Cotton wool was wrapped around the petiole of the higher and the lower leaf and fine mesh bags (35 x 27cm) were placed around the leaves, to prevent insects from moving to other leaves. In treatments three and four just one leaf of the plant was infested with herbivores, however on the empty leaf also cotton wool and a mesh bag were used to treat all plants equally except for the insect infestations. Five week old plants were infested with herbivores one day before the experiment took place and kept in a greenhouse (23±2 °C, 60±10% RH, 16:8 L:D). Just before testing, the bag and cotton wool were removed from the plant. One plant was placed in the wind tunnel and parasitoids were released 70 cm downwind and tested individually. We observed on which leaf the parasitoids landed: the ‘host infested leaf’, the ‘non-host infested leaf’, the ‘mixed infested leaf’ or any ‘other leaf’. Parasitoids that did not respond within five minutes were considered as unresponsive. Per plant ten parasitoids were tested, every treatment was replicated 13 times (using in total 13 plants per treatment), randomly spread over 14 days. Treatments were never tested twice on one day and per day the treatments were tested in a random order.

Flight chamber experiment

To observe how the behaviour of *C. glomerata* after landing on the plant was affected by herbivore position, a flight chamber experiment was carried out from January 22nd to April 16th 2013. The flight chamber consisted of a tent (290 cm x 200 cm x 230 cm in height) of insect screen (mesh size 0.6 mm) situated in a greenhouse compartment (25±2 °C, 60±10 % RH) (see also Bukovinszky et al. 2012b). Five weeks old plants were infested with herbivores using the same methods and treatments as described for the wind tunnel experiment. Just before the start of the experiment, three plants with the same treatment were placed about 50 cm apart from each other forming a triangle on the table in the tent to generate a high quantity of plant volatiles in order to attract the parasitoids to one of the plants. Cotton wool and mesh bags that had been used to confine the herbivorous insects to a specific leaf, were removed. Per observation, one parasitoid was released from the middle of the triangle of plants. When the parasitoid landed on one of the plants, the observation started. The following behaviours were recorded using the Psion Workabout PRO 3 device (Motorola Solutions, Inc., Schaumburg, Illinois, USA) and Observer XT 10 software (Noldus Information Technology, Wageningen, The Netherlands): flying in the vicinity of the plant (flying), landing on the higher leaf, landing on the lower leaf, landing on any other leaf, standing still, walking, preening, encounter with herbivore product (parasitoids were considered to encounter herbivore products when the antennae were turned sideward while walking around on frass or feeding damage), encounter non-host, attack non-host, attack host, oviposit in non-host, oviposit in host, excursions outside the vicinity of the plant (off-plant excursions). Frequency and duration of all behaviours was recorded, except for 'oviposit in host' and 'oviposit in non-host'. These behaviours were regularly very short, so it was not possible to accurately record the duration. Parasitoids that flew to the tent walls immediately after release and stayed there for more than one minute were considered unresponsive and were removed from the experiment. The observation stopped when the parasitoid stayed for more than five minutes out of the vicinity of the plant it first landed on or after one hour. Before a new observation started, the plant that the previous parasitoid landed on was replaced by a new plant of the same treatment. Caterpillars were dissected under a stereomicroscope to count parasitoid eggs.

Tent experiment

To test whether the total host-finding efficiency of *C. glomerata* was affected by herbivore position, a tent experiment in an agricultural field in Wageningen, the Netherlands, was conducted between April 22nd and September 20th 2013. Eight tents (l x w x h: 3m x 4m x

2m) made out of insect screen (mesh size 0.6 mm) were used. The plants used in the tent experiment were grown in a greenhouse for four weeks and were placed outside to acclimatize during the fifth week. After the fifth week, 16 *B. oleracea* plants were transplanted to each tent in a 4x4 grid. In the sixth week plants were infested with herbivores. The four treatments as described for the wind tunnel experiment were randomly distributed over eight tents (one treatment per tent, two tents per treatment). Per tent, every other plant was infested with herbivores using the methods as described for the wind tunnel experiment, the remaining eight plants were not infested. One day after introducing the herbivores, per tent three female and three male parasitoids were released. In each tent two plastic lids (diameter 3 cm) with drops of honey were placed as a food source for the parasitoids. Two days after releasing the parasitoids, plants were harvested and kept separately in labelled plastic bags at 4°C. All recovered *P. brassicae* caterpillars on the plants were dissected and parasitoid eggs were counted under a stereomicroscope. The experiment was replicated eight times.

Statistical analysis

Data derived from the wind tunnel experiment were analysed in two ways. First, for all three choices that could be made (‘host or mixed infested leaf’, ‘non-host infested leaf’ or ‘other leaf’) separate logistic regression analyses were conducted to test for differences between the four treatments. The models comprised a logit link function and a multiplicative dispersion parameter for the binomial variance function. Second, for all four treatments separate trinomial logistic regression analyses were conducted to test for differences between the three choices that could be made. These models comprised a log link function and a multiplicative dispersion parameter for the Poisson variance function (McCullagh and Nelder 1989). All models comprised main effects for treatments and an explanatory variable for the day the experiment was conducted. If the day of the experiment had no significant ($P > 0.05$) effect, it was subsequently dropped from the model. Inference was based on Wald-tests.

From the flight chamber experiment, the behaviours ‘flying’, ‘landing’, ‘standing still’, ‘walking’, ‘preening’, ‘encounter herbivore product’, ‘encounter non-host’, ‘attack non-host’, ‘attack host’ and ‘off-plant excursions’, were analysed for both the duration of the behaviour expressed as a proportion of residence time as well as for the rate per minute at which the behaviour was performed. Behaviours ‘oviposit in non-host’ and ‘oviposit in host’ were only analysed for the rate per minute at which the behaviour was performed.

The duration of behaviours was analysed with logistic regression, comprising a multiplicative dispersion factor for the binomial variance and main effects for treatment on the logit scale. The 'binomial total' was fixed at value 1. The residence time, the time to first oviposition, and the number of hosts parasitized were analysed similarly. However, for the residence time and the time to first oviposition, the 'binomial total' was fixed at 60 minutes, for the number of hosts parasitized this was fixed at 10 hosts with the number of hosts parasitized as a response variable. The rate per minute at which the behaviours were performed, was analysed with log-linear regression comprising a multiplicative dispersion factor for the Poisson variance function and main effects for treatment on the log scale. The number of eggs per host and per oviposition were analysed similarly, but with an extra offset: log of number of hosts parasitized and log of oviposition frequency. An extra explanatory variable for the age of the parasitoid was added in all models and dropped subsequently when it was not significant ($P > 0.05$). Inference was based on Wald tests. To calculate the duration of behaviours, only those observations were included where the parasitoid actually performed the behaviour. Likewise, the number of hosts parasitized (per minute), the number of eggs per host and per oviposition and the time to the first oviposition were calculated per individual observation only when the parasitoid actually parasitized one or more hosts. Furthermore, to evaluate these last five behaviours, three observations for which the number of hosts parasitized and the eggs could not be counted for various reasons, were excluded from the dataset.

Data from the tent experiment were analysed with generalized linear mixed models (GLMM). To analyse the percentage of parasitized hosts per tent and the percentage of plants with parasitized hosts, the models comprised a logit link function, a multiplicative dispersion factor for the binomial variance and fixed and random effects on the logit scale. To analyse the number of eggs per parasitized host, the model comprised a log link function, a multiplicative dispersion parameter for the Poisson variance function, and an offset: the log of the number of parasitized hosts per tent. All three models comprised main effects for treatments and series in time and random effects (assumed to be independently normally distributed with constant variance) for tent number. It happened twice that no hosts were parasitized in a tent. These observations were not included in the analyses because most likely the parasitoids had died or escaped. Inference was based on penalized quasi-likelihood (PQL) (Breslow and Clayton 1993). Quasi-Wald tests and approximate F-tests (Kenward and Roger 1997) applied to the approximate linear mixed model from the last step of the iterative re-weighted REML algorithm, were used.

Calculations were two tailed and performed using GenStat® 17th.

Results

Wind tunnel experiment

In the wind tunnel set-up 303 parasitoids out of 520 tested responded by landing on one of the leaves of the test plant. Such a response rate was expected based on previous studies (e.g. Geervliet et al. 1998a, Bukovinszky et al. 2012b). When comparing landing preferences within each treatment, the parasitoids in most cases had a distinct preference for the leaf on which the host was feeding compared to all other leaves (host high GLM: $F2,36 = 19.74$, $P < 0.001$, mixed high GLM: $F2,36 = 21.54$, $P < 0.001$, mixed low GLM: $F2,36 = 20.56$, $P < 0.001$) (fig.1). One type of herbivore infestation, however, affected the preference of the parasitoid. When the host was feeding on the lower leaf and the non-host was feeding on the higher leaf, the parasitoids landed as frequently on the higher leaf with the non-host, as on the lower leaf with the host (GLM: $F2,36 = 4.83$, $P = 0.014$; LSD non-host vs host/mixed P=0.301) (fig. 1). This type of herbivore infestation also showed deviating results when landing preferences between the four treatments were compared. When the host was feeding on the lower leaf and the non-host on the higher leaf, the parasitoids landed significantly less frequently on the leaf with the host compared with situations where the host was either feeding alone on the higher leaf, or together with the non-host on the higher or the lower leaf (GLM: $F3,48 = 6.97$, $P < 0.001$). The parasitoids in this situation also landed more often on the leaf infested with the non-host compared with the opposite infestation of the host on the higher and the non-host on the lower leaf (GLM: $F3,48 = 3.07$, $P = 0.036$) (fig. 1).

Flight chamber experiment

The behaviour of 109 *C. glomerata* was observed individually, after they had landed on plants infested with differently positioned herbivores. Complementary to the experiments in the wind tunnel, here we could not only observe the first landing choice, but we recorded all landings during the total observation and analysed these in terms of landing rate per minute on the different leaves. When hosts and non-hosts were feeding on the same leaf (‘mixed high’ and ‘mixed low’) parasitoids hardly ever landed on the non-infested leaves, while parasitoids landed on the non-host-infested leaf as well when hosts and non-hosts were feeding separately (‘host high’ and ‘host low’) (comparing all treatments for landing on higher leaf GLM: $F3,105 = 4.31$, $P = 0.007$ (fig. 2A), comparing all treatments for landing on lower leaf GLM: $F3,105 = 7.42$, $P < 0.001$ (fig. 2B)). In addition,

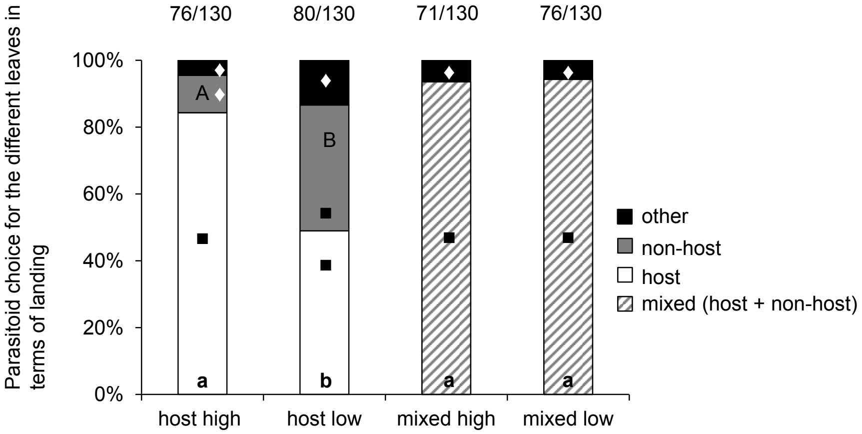

Figure 1. First-choice preference of *Cotesia glomerata* parasitoids to *Brassica oleracea* plants infested with herbivores at different positions in the wind tunnel experiment. Parasitoids could choose for the host-infested leaf (infested by ten first-instar *Pieris brassicae* caterpillars), the non-host-infested leaf (infested by ten first-instar *Mamestra brassicae* caterpillars) or the other, non-infested leaves in the treatments 'host high' and 'host low'. In the treatments 'mixed high' and 'mixed low', the parasitoids could choose for the mixed infested leaf (infested by ten first-instar *P. brassicae* and ten first-instar *M. brassicae* caterpillars) or the other, non-infested leaves. Similar letters indicate no difference between treatments within a choice (horizontally), similar symbols indicate no difference between choices within a treatment (vertically), all based on generalized linear models and least significant differences (α = 0.05). Numbers above the bars indicate the number of responding parasitoids/the number of tested parasitoids.

when the non-host was feeding on the higher leaf ('host low'), parasitoids landed on the higher leaf as frequently as when the host was feeding on that higher leaf ('host high') (GLM: F3,105 = 4.31, P = 0.007, LSD host low vs host high P = 0.930) (fig. 2A), which was also found for the times the parasitoids landed on the lower leaf (GLM: F3,105 = 7.42, P < 0.001, LSD host low vs host high P = 0.060) (fig. 2B). From the start of the observation, it took the parasitoids on average 4.8 ± 1.0 minutes to parasitize the first host, which was not affected by treatment (GLM: F3,69 = 0.65, P = 0.584) (fig. S1, graph X, Appendices). In more detail, in the treatment 'host low', parasitoids that first landed on the host-infested leaf did not significantly differ in the time until first oviposition from parasitoids that first landed on the non-host-infested leaf, despite of a threefold longer average time spent by the latter group (mean ± SE first landing high: 7.12 ± 3.6 minutes, mean ± SE first landing low: 2.34 ± 2.7 minutes) (GLM: F1,13 = 1.77, P = 0.207)(fig. S1, graph AC, Appendices). This is likely a result of the low number of replicates for this analysis, only 15 (eight parasitoids on the higher leaf, seven on the lower leaf), and a high variation in parasitoid behaviour.

The total time spent on the plant since the first landing, for all treatments, was on average (±SE) 38.0 ± 2.4 minutes. This was not affected by herbivore position (GLM: *F*3,105 = 1.37, *P* = 0.257) (fig. S1, graph W, Appendices). During this period, the number of hosts that was parasitized per minute did not differ between herbivore positions (mean ± SE: 0.18 ± 0.01) (GLM: *F*3,69 = 7.795, *P* = 0.059) (fig. S1, graph AB, Appendices). The total number of hosts that was parasitized during the observation was significantly larger when host and non-host were feeding on the same leaf (GLM: *F*3,69 = 3.08, *P* = 0.033) (fig. S1, graph Y, Appendices). Non-hosts were rarely parasitized (fig. S1, graph T, Appendices). Previous research showed that during oviposition of *M. brassicae* by *C. glomerata* occasionally a few eggs were laid that never developed into parasitoid larvae (Bukovinszky et al. 2012b). Of the other observed behaviours, the rate per minute of ‘encounter herbivore product’ was higher in the treatment where both herbivores were positioned on the lower leaf (fig. S1, graph I, Appendices). The duration of walking was shorter when both herbivores were feeding together on the higher leaf, compared to when they were feeding on the lower leaf and to when the host was positioned on the lower leaf and the non-host on the higher leaf (fig. S1, graph F, Appendices). A smaller number of eggs per host was recorded for the situation where the host and non-host were infesting the lower leaf together, compared to both herbivores infesting the higher leaf (fig. S1, graph AA, Appendices).

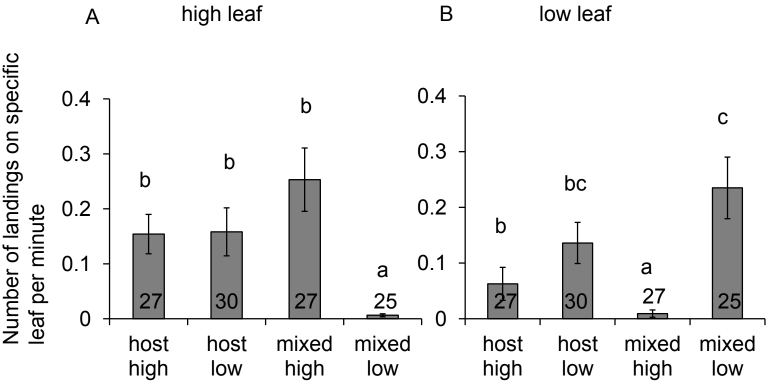

Figure 2. Landing rates of *Cotesia glomerata* parasitoids per minute on the higher leaf (A) and the lower leaf (B) ± SE in the flight chamber experiment on *Brassica oleracea* plants infested with herbivores according to the treatments: ‘host high’ (ten first-instar *Pieris brassicae* caterpillars on the higher leaf, ten first-instar *Mamestra brassicae* larvae on the lower leaf), ‘host low’ (ten first-instar *P. brassicae* larvae on the lower leaf, ten first-instar *M. brassicae* larvae on the higher leaf), ‘mixed high’ (ten first-instar *P. brassicae* and ten first-instar *M. brassicae* larvae on the higher leaf) and ‘mixed low’ (ten first-instar *P. brassicae* and ten first-instar *M. brassicae* larvae on the lower leaf). Similar letters indicate similar means based on generalized linear models and least significant differences (α = 0.05). Numbers in bars indicate the number of analysed parasitoids per treatment.

Tent experiment

In the tent set-up to test the parasitization efficiency of *C. glomerata*, in total 3597 hosts (73% of the released hosts) were found back on the plants of which on average (± SE) 50.0% (± 3.5%) was parasitized. The moment in time at which the experiments were conducted affected the percentage of hosts parasitized, likely because of weather conditions (GLMM: F7,51 = 21.31, P < 0.001), as well as the percentage of plants with parasitized hosts (GLMM: *F7,45.4* = 8.00, P < 0.001) and the number of eggs per parasitized host (GLMM: F7,46 = 5.38, P < 0.001,). The percentage of parasitized hosts was not affected by the different positions of hosts and non-hosts (mean ± SE: 50.2 ± 6.9) (GLMM: F3,51 = 2.01, P = 0.124) (fig. S2, graph A, Appendices), nor was the mean percentage of plants with parasitized hosts (mean ± SE: 61.5% ± 4.1%) (GLMM: F3,45.7 = 1.38, P = 0.260), nor the mean number of eggs per parasitized host (mean ± SE: 19.1 ± 1.2) (GLMM: F3,34.6 = 0.42, P = 0.742) (fig. S2, graph B, Appendices).

Discussion

With this study we show that parasitoids have an innate preference for landing on the younger leaves of a plant, where their hosts prefer to feed. This innate preference caused parasitoids to also land on the younger leaves when those leaves were infested with non-hosts while the host was feeding on the plant's older leaves. Although landing on the non-host-infested leaf could result in a longer time to find their first host, the number of hosts that was parasitized per time unit was similar for all parasitoids. However, separation of host and non-host over leaves made parasitoids parasitize fewer hosts than when hosts and non-hosts were feeding on the same leaf. Even though position of host and non-host on the plant affected host location during the two phases of parasitoid foraging, the parasitoids compensated for these effects during foraging in the short term as well as over longer time when foraging in a plant community.

Parasitoids did not show an innate preference for landing on the younger leaves of a plant when these leaves were free of herbivores, but in the presence of either host, non-host or both, plant cues were produced that attracted the parasitoids to the younger leaves (Takabayashi et al. 1994, Brilli et al. 2009). When the hosts and non-hosts were separately positioned in an unnatural way (the host on the lower leaf and the non-host on the higher leaf), parasitoids more often made the 'mistake' of choosing the non-host-infested leaf first, compared to the choice for the non-host-infested leaf when offered the natural distribution. Additionally, on the younger and higher positioned leaf the number of landings per minute was equal for the situation where only the host was feeding on this higher leaf (natural) and for the situation where only the non-host was infesting the higher leaf (unnatural). An unnatural distribution of herbivores therefore misleads the parasitoids during the first phase of foraging and makes non-host-infested leaves as attractive as host-infested leaves.

Previous studies show that parasitoids spend less time on plants infested with only non-hosts, compared to the time spent on plants infested with either the host alone (Sato et al. 1999, Shiojiri et al. 2000a, Bukovinszky et al. 2012b, De Rijk et al. 2016b) or a dual infestation of host and non-host (Vos et al. 2001, Bukovinszky et al. 2012b, De Rijk et al. 2016b). In our study all plants were dually infested with hosts and non-hosts, the distribution of herbivores over more than one leaf did not make a difference for the time spent on the plant by the parasitoids. Therefore, we assume that parasitoids consider the whole plant as an object of interest (or patch), rather than single leaves. This is supported by the observation that parasitoids spent a short amount of time on non-host-infested leaves (personal observation), after which they flew close to the plant to further search for the host-infested leaf. The parasitoids, therefore, seemed to have information that the plant was in fact attacked by the host, but the right leaf was not found yet and, consequently, the plant was not abandoned. We hypothesize that the parasitoids kept searching on the same plant based on the perception of specific host(-induced) cues, rather than utilizing general cues of leaf-chewing herbivores, because general cues induced by non-host herbivores do not arrest the parasitoid as long as the specific host(-induced) cues do (Sato et al. 1999, Shiojiri et al. 2000a, Vos et al. 2001, Bukovinszky et al. 2012b, De Rijk et al. 2016b). The total number of parasitized hosts was higher on dual infested leaves compared to single infested leaves. This could indicate a positive effect of the presence of non-hosts on finding hosts, although parasitoid efficiency was not affected since the number of hosts parasitized per minute was similar for both herbivore distributions. The time spent searching on the plant before the first host was parasitized was similar for all herbivore distributions. Even when comparing the parasitoids that first chose the non-host-infested leaf and those that directly chose the host-infested leaf in the situation with an unnatural herbivore distribution, the time until parasitizing the first host was similar. Possibly though, when more parasitoids would be tested in this treatment, the observed threefold increase in time needed until the first oviposition by parasitoids that landed first on the non-host-infested leaf would be significantly higher. We conclude that herbivore distribution does not affect parasitoid perseverance to examine a potentially rewarding plant. At the same time, even though parasitoid behaviour on the plant can be affected by the distribution of host and non-host, herbivore position does not interfere with the host-finding efficiency of parasitoids once they initiated searching on the plant.

The effect of herbivore position on the selection of a leaf to land on was not translated into a lower host-finding efficiency in a semi-field plant community. Most likely this was already compensated for in the second phase of foraging, when the time wasted by landing on the ‘wrong’ leaf seemed relatively unimportant compared to the total amount of time spent on the plant (Liu et al. 2009). Previously, the effect of non-host presence on host finding efficiency was shown to be negative (Shiojiri et al. 2002, Bukovinszky et al. 2012b), neutral (De Rijk et al. 2016b) or positive (Shiojiri et al. 2002, De Rijk et al. 2016b). Predicting the direction of the effects seems difficult because various factors might be involved (De Rijk et al. 2013). Our study indicates that at least the position of non-hosts on the plant is not important for the efficiency of foraging parasitoids.

Cotesia glomerata parasitoids do not randomly land on a plant that is infested with hosts. Instead, they land on a specific leaf that is attacked by herbivores, preferably one of the younger, higher located leaves. If the selected leaf is not attacked by hosts, the parasitoids continue searching and effects of the wasted time on foraging efficiency are negligible. Arriving at the host at a later time point, however, could negatively affect a parasitoids competitiveness and that of its offspring within the host (Harvey et al. 2013b). Non-host herbivore densities can affect parasitoid foraging behaviour through density-mediated indirect interactions (Werner and Peacor 2003). However, we previously found that non-host density does not influence parasitoid foraging efficiency in this study system (De Rijk et al. 2016c). During the searching process, parasitoids are known to learn where to find their host (Hoedjes et al. 2011). This may have had an effect in our outdoor experiment and so on the results of the host-finding efficiency of the parasitoid. This, however, does not affect the conclusion that the foraging behaviour of the parasitoid has enough flexibility to overcome the constraint of an unnatural distribution of herbivores over a plant. This conclusion would have been different if we would have based it on one laboratory experiment exclusively, instead of using results from two laboratory experiments plus a semi-field experiment. Extrapolating findings from the laboratory to natural situations in the field should, therefore, be done with care (Hunter 2002). The essential and supporting roles that (semi-)field experiments can play, is shown by studies on e.g. ants (Resasco et al. 2014), hoverflies (Easley and Hassall 2014), parasitoids (Gols et al. 2005, Bukovinszky et al. 2012b, Adams and Morse 2014, De Rijk et al. 2016b), mosquitoes (Verhulst et al. 2011), caterpillars (Nielsen and Papaj 2015) and flies (Helms et al. 2013). These experiences about the importance of (semi-)field studies should lead to further development and improvement of our methods of studying ecological phenomena.

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5

Chapter 5

Density- and trait-mediated indirect interactions alter host foraging behaviour of parasitoids without altering foraging efficiency

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Under review

Foraging decisions of parasitoids are influenced by host density via density-mediated indirect interactions. However, in the parasitoid's environment also non-suitable herbivores are present. These non-hosts also occur in different densities, which can affect a parasitoid's foraging behaviour. The influence of non-host traits and densities can be expressed during the first phase of the foraging process, when parasitoids use plant volatiles to locate plants infested by their host. They may also play a role during the second phase, when parasitoids use infochemicals from the host and plant to locate, recognize and accept the host. By using laboratory and field set-ups we studied whether the density of non-host herbivores influences these two phases of the foraging behaviour of the parasitoid Cotesia glomerata as well as the parasitoid's efficiency to find its host, Pieris brassicae caterpillars. Our data show that a high non-host density negatively affected parasitoid preference for host-infested plants, but that the behaviour on the plant and the total host-finding efficiency of the parasitoids was not influenced by non-host density. We discuss our results in the context of density-mediated and trait-mediated indirect interactions.

Key words
DMII, field experiment, foraging efficiency, multi-herbivory, non-host

Arthropod herbivores in both natural and (agri)cultural environments occur in different densities, depending on, for example, climate conditions (Pramanik and Dey 2012) and plant traits (Stam et al. 2014, Zvereva et al. 2014). Whenever these herbivore densities change, this can influence the foraging behaviour of their enemies by so-called density-mediated indirect interactions (DMII) (Abrams 1995, Werner and Peacor 2003). In general, as a consequence of changing plant traits (Maeda and Takabayashi 2001, Horiuchi et al. 2003, Shiojiri et al. 2010, Kroes et al. 2015), an increasing herbivore density has a positive effect on the behavioural response of their carnivorous enemies (Maeda and Takabayashi 2001, Gols et al. 2003, Horiuchi et al. 2003, Shiojiri et al. 2010, Girling et al. 2011). Next to DMII, also trait-mediated indirect interactions (TMII) can influence carnivorous insects. In TMII, changes in traits of one species, induced by a second species, indirectly influence interactions between the second and a third species (Werner and Peacor 2003, Utsumi et al. 2010). The two types of interactions, DMII and TMII, can be intertwined and may interact with each other within the interaction web that herbivores and their enemies are part of (Werner and Peacor 2003).

One group of carnivores that is thoroughly studied for their density- and trait-mediated indirect interactions with the environment are parasitic wasps, or parasitoids. Parasitoids lay their eggs in or on herbivorous hosts (usually other arthropods). The larvae emerging from these eggs depend on this host as their food source and eventually the host dies (Godfray 1994, Thiel and Hoffmeister 2009). Because plants in (agri)cultural and natural environments are often attacked by several herbivore species simultaneously (Vos et al. 2001, Stam et al. 2014), the hosts of parasitoids normally share their food plants with other herbivores. These other herbivores that are not suitable for the development of the offspring of parasitoids, so-called non-hosts, can be part of the interaction web that links a parasitoid to its host (Ohgushi 2008, Utsumi et al. 2010, Eubanks and Finke 2014, Hammill et al. 2015). Several non-host herbivore features can play a role in the interaction web of parasitoids, e.g. feeding guild (Soler et al. 2012a, Zhang et al. 2013), origin (Desurmont et al. 2014, Chabaane et al. 2015) and feeding location (De Rijk et al. 2016a) of the non-host herbivores, the diversity of the non-host species (Vos et al. 2001) and possibly also the density of the non-host herbivores (Kratina et al. 2007, Zhang et al. 2009, Yamamoto et al. 2011, Ponzio et al. 2014).

The influence of non-host traits and densities can be expressed during the foraging process of parasitoids that can be devided into two phases: 1. finding the host infested plant from a distance and 2. finding, recognizing and accepting the host after landing on the plant (Van Alphen et al. 2003, De Rijk et al. 2013). During the first phase, parasitoids use plant volatiles emitted in response to feeding of the host to locate host infested plants (Vet and Dicke 1992, Hare 2011). However, feeding of non-hosts also induces the emission of plant volatiles. Plants attacked by both hosts and non-hosts, therefore, produce an altered volatile blend which can hamper recognition of host presence by the foraging parasitoid (Dicke et al. 2009, Arimura et al. 2011, De Rijk et al. 2013, Zhang et al. 2013). During the second phase of foraging, parasitoids have arrived on the plant and use infochemicals from the host and the plant to locate, recognize and accept the host (Van Alphen et al. 2003, Colazza et al. 2014).

The physical presence of non-hosts, for example as obstacle on a leaf (Hauzy et al. 2010), as well as their infochemicals (Takabayashi and Takahashi 1990) can make parasitoids less efficient in host finding and even to mistakenly attack non-hosts (Bukovinszky et al. 2012b, Chabaane et al. 2015). Here, we studied whether the density of non-host herbivores influences both phases of parasitoid foraging as well as the total host-finding efficiency. An increase in non-host density was hypothesized to enhance the effects of their presence on the parasitoid.

We created environments in which every plant was infested with non-host herbivores while only half of the plants were infested with the host. This imitated as closely as feasible the natural situation for the gregarious parasitoid *Cotesia glomerata*, because its most common and gregarious host, *Pieris brassicae*, has a patchy distribution (Vos et al. 1998) while non-host herbivores are everywhere in the environment. To study the specificity of non-host identity in density-mediated effects on parasitoid behaviour we tested two densities of four species of non-hosts (*Spodoptera exigua*, *Autographa gamma*, *Plutella xylostella* and *Mamestra brassicae*). First, the flight response of the parasitoid during the initial phase of foraging was studied by using a wind tunnel. Second, the behaviour of the parasitoid on the plant during the subsequent foraging phase was studied by using an on-plant experiment. Finally, to study the effect of non-host density on the total host-finding efficiency that combined the first and second phase of host location, an outdoor tent experiment was performed. We discuss in what phase of foraging non-host density plays a role and how this affects the total foraging efficiency.

Materials and methods

Plants

Brassica oleracea var gemmifera Cyrus plants (Brassicales: Brassicaceae) were grown in a greenhouse (20±2°C, 60±10% RH, 16:8 L:D photoperiod) in standard potting soil (Lentse potgrond, 0.7 litre) and fertilized two to three times a week using a liquid fertilizer (EC 2.1 mS/cm, pH 5.8). Five-week-old plants were used in all experiments.

Insects

Pieris brassicae (Lepidoptera: Pieridae), *Autographa gamma* (Lepidoptera: Noctuidae), *Plutella xylostella* (Lepidoptera: Plutellidae) and *Mamestra brassicae* (Lepidoptera: Noctuidae) caterpillars were cultured on *B. oleracea* var gemmifera Cyrus plants in a greenhouse (20±2°C, 60±10% RH, 16:8 L:D photoperiod). *Spodoptera exigua* (Lepidoptera: Noctuidae) caterpillars were cultured on an artificial diet mainly composed of polenta, beer yeast and wheat-germs in a climate cell (25.5°C, 50% RH and 16:8 L:D photoperiod) and kindly provided to us by the Laboratory of Virology of Wageningen University. First-to-second-instar caterpillars were used, all equally sized. The parasitoid *Cotesia glomerata* (Hymenoptera: Braconidae) was cultured on *Pieris brassicae* caterpillars in a greenhouse (20±2°C, 60±10% RH, 16:8 L:D photoperiod). Parasitoid cocoons were daily collected from the rearing cages and kept in a climate cabinet (21°C, 16:8 L:D photoperiod). Emerged parasitoids were provided with honey and water *ad libitum* and were allowed to mate. One-to-seven-days-old naive parasitoids were tested in the experiments.

Wind-tunnel experiment

A wind tunnel as described by Geervliet et al. (1994) (wind tunnel conditions: 0.1 m/s air speed, 22-24°C, 60-72% RH) was used to test the preference of *C. glomerata* for plants infested with either a combination of hosts and non-hosts or non-hosts only in a two-choice situation. Before the start of the experiment a pair of plants was placed upwind in the wind tunnel. Both plants were infested with the same number of first-to-second-instar caterpillars of one of the non-host species, where only one of the plants received ten first-instar hosts in addition to the non-hosts. Two densities of four non-host herbivore species (*S. exigua*, *A. gamma*, *P. xylostella*, *M. brassicae*) were tested; low (ten non-host caterpillars) and high (50 non-host caterpillars). As a control for parasitoid attraction to host-infested plants without non-host presence, we tested the response to a plant pair consisting of a clean plant and a plant infested with ten hosts. To test if the difference in herbivore numbers feeding on the two plants in each treatment affected the preference of parasitoids, two extra control treatments were performed. In the low-density control treatment, both plants were infested with 20 caterpillars (10 hosts + 10 *M. brassicae* caterpillars vs 20 *M. brassicae* caterpillars). In the high-density control treatment, both plants were infested with 60 caterpillars (10 hosts + 50 *M. brassicae* caterpillars vs 60 *M. brassicae* caterpillars). Preparation of plants was done one day prior to the experiment by gently transferring caterpillars to the youngest fully expanded leaf. To restrict the caterpillars to a single leaf, cotton wool was wrapped around the petiole and the leaf was enclosed in a fine gauze bag to exclude any effect of herbivore position on the plant. Right before the experiment on the next day, both cotton wool and bag were removed. After placing a set of plants in the wind tunnel, ten female parasitoids were tested individually per plant pair. The first plant the parasitoid landed on was considered the plant of choice. Parasitoids that did not land on a plant within five minutes after their release were considered unresponsive. After testing five parasitoids, the position of the two plants was switched in order to limit any effect of plant position. Per treatment eight plant pairs were tested, in a random order divided over 36 days.

On-plant experiment

An on-plant experiment was used to observe parasitoid behaviour after landing on a plant infested with a low or a high density of non-hosts. The on-plant experiment was conducted in a gauze tent (LxWxH: 2.9 x 2.0 x 2.3m) positioned inside a greenhouse compartment (25±2°C, 60±10% RH) (see also Bukovinszky et al. 2012b). Due to the time-consuming nature of the experiment only one species of non-host was tested. Three treatments were used: plants infested with ten first-instar *M. brassicae* plus ten first-instar *P. brassicae* caterpillars (low density), plants infested with 50 first-instar *M. brassicae* plus ten first-instar *P. brassicae* caterpillars (high density) and plants infested with ten first-instar *P. brassicae* caterpillars (host only). Plants were prepared as described for the wind-tunnel experiment. On a table inside the gauze tent three plants of the same treatment were placed forming a triangle with approximately 50 cm in between. One parasitoid at a time was released in the middle of this triangle. The experiment started at the moment the parasitoid landed on one of the plants. Using a Psion Workabout PRO 3 device and Observer XT 10 software, the behaviour of the parasitoid was manually recorded. The following behaviours were distinguished: flying in the vicinity of the plant (flying), landing, standing still, walking, preening, encounter herbivore product (parasitoids were considered to encounter herbivore products when the antennae were turned sidewards while walking around on frass or feeding damage), encounter non-host, attack host, attack non-host, oviposit in host, oviposit in non-host, excursions outside the vicinity of the plant (off-plant excursions). Except for two behaviours, both duration and frequency were recorded. For the behaviours ‘oviposit in host’ and ‘oviposit in non-host’ only the frequency was recorded, because these behaviours were sometimes too short to accurately measure the duration. Parasitoids were considered unresponsive when they flew immediately to the tent after being released and stayed there for more than one minute. The observation stopped after the maximum observation time of one hour, or when the parasitoid had spent more than five minutes outside the vicinity of the plant. Before starting the next observation, the plant that had been visited by the parasitoid was replaced by a new plant. Host caterpillars were dissected under a stereomicroscope to count parasitoid eggs. Per treatment 22 parasitoids were observed.

Outdoor tent experiment

To test the host-finding efficiency of *C. glomerata* in environments with low or high non-host densities an outdoor set-up was used. Tents (LxWxH: 3 x 4 x 2m) made out of insect screen (0.6 mm mesh size) positioned in an agricultural field in Wageningen, The Netherlands were divided in three groups of seven tents. The tents were used for 12 series of experiments, per series one group of tents was used, each tent in the group receiving one of the seven unique treatments that were randomly divided for each series. The *B. oleracea* plants used in this experiment were grown in a greenhouse for four weeks and were transferred to gauze tents outside to acclimatize during the fifth week. After the fifth week, sixteen plants were planted in a four-by-four grid per experimental tent. In the sixth week, every plant was infested with one species of first-to-second-instar non-host caterpillars in a low (ten caterpillars) or a high (50 caterpillars) density and every other plant was at the same time also infested with ten first-instar hosts. Using this method three non-host species (*S. exigua*, *P. xylostella* and *M. brassicae*) were tested in two densities. In addition to these six treatments, a control treatment was applied in which only eight plants (every other plant) were infested with host caterpillars and the other plants were left uninfested. Except for one, every treatment was replicated 12 times. Due to unexpected collapse of the *P. xylostella* culture the treatment *P. xylostella* in high densities was replicated 11 times. Plants were infested with herbivores as described for the wind-tunnel experiment one day prior to parasitoid release. Three female and three male parasitoids were released in each tent and were supplied with drops of honey on two plastic lids (diameter 3 cm). Three days after releasing the parasitoids, host-infested plants were harvested and kept in labelled plastic bags at 4°C. All hosts recovered from these plants were dissected under a stereomicroscope and parasitoid eggs were counted.

Statistical analysis

The data derived from the wind-tunnel experiment were analysed by a binomial test per treatment (i.e. combination of density and non-host species), per density and per species to test whether parasitoid responses differed from equal preferences for plants of a pair. Treatments, densities and species were compared for the number of parasitoids that chose the host- and non-host-infested plant out of the total number of responding parasitoids per plant pair using logistic regression analyses. The models comprised an extra multiplicative dispersion factor for the binomial variance, an explanatory variable for the age of the parasitoids and main effects for treatment/density/species on the logit scale. Because there was no significant effect of parasitoid age, it was subsequently dropped from the model. The low and high-density control treatments were compared to the treatments '*M. brassicae* low density' and '*M. brassicae* high density' using a separate and similar logistic regression analysis as previously described to test for differences in parasitoid response between treatments with equal or unequal numbers of caterpillars on both plants of a pair.

Separate analyses were performed for the different behaviours observed in the on-plant experiment with parasitoids as experimental units. Durations of the different behaviours were expressed per parasitoid as a proportion of residence time. Duration was only included in the analysis when the particular behaviour was performed, i.e. only non-zero proportions were included. Using a logistic regression model comprising a multiplicative dispersion factor for the binomial variance and main effects for treatments on the logit scale, the duration of behaviours was analysed. The 'binomial total' was fixed at value 1. Residence time and the number of hosts parasitized were analysed in the same way, with 60 minutes and 10 hosts as 'binomial totals'. The rate per minute of the different observed behaviours was analysed for all observations (also when the particular behaviour was not performed during the observation). Analyses were done using a generalized linear model comprising an unknown multiplicative dispersion factor for the Poisson variance and main effects for treatment on the log scale. The number of eggs per oviposition and the number of eggs per host were analyzed with a similar model, with number of eggs as the response and an extra offset: log of oviposition frequency or log of number of hosts parasitized. All models comprised an explanatory variable for the age of the parasitoid, this was dropped from the model whenever it was not significant.

Data derived from the outdoor tent experiment were analysed with generalized linear mixed models (GLMM). The models to analyse the percentage of parasitized hosts per tent and the percentage of plants with parasitized hosts per tent comprised fixed effects for series in time and either treatment (i.e. combination of density and non-host species) or density, and random effects for tent number on the logit scale, and a multiplicative dispersion parameter for the binomial variance. The models to analyse the number of parasitized hosts per plant on which parasitized hosts were found per tent and the number of eggs per parasitized host per tent comprised fixed effects for series in time and either treatment or density, and random effects for tent number on the log scale, and a multiplicative dispersion parameter for the Poisson variance function. The response was the number of parasitized hosts per tent with the log of the number of plants with parasitized hosts per tent as an offset, respectively the number of eggs per tent with the log of the number of parasitized hosts per tent as an offset. Inference was based on penalized quasi-likelihood (PQL) (Breslow and Clayton 1993). Quasi-Wald tests and approximate F-tests (Kenward and Roger 1997) applied to the approximate linear mixed model from the last step of the iterative re-weighted REML algorithm, were used. Calculations were performed using GenStat® 17th edition.

Results

Wind-tunnel experiment

In the different treatments, 402 out of 640 parasitoids made a choice to land on one of the plants within the experimental time. The parasitoids in general preferred to land on plants infested with hosts and non-hosts over plants with non-hosts only (binomial tests: all $P < 0.05$). Only in one situation, in the treatment where plants were infested with a high density of *S. exigua* non-hosts (*S. exigua* high), the parasitoids had no preference for either plant type (binomial test: $P = 0.461$) (fig. 1A). However, the distribution of preferences did not differ significantly among the individual treatments of four non-host species in two densities and the control treatment without non-hosts (GLM, $P = 0.333$) (fig. 1A). When combining all treatments of the same non-host density, parasitoids still preferred the plants infested with hosts and non-hosts over those infested with non-hosts only (binomial tests: all $P < 0.001$) (fig.1B). However, the preference for plants infested by hosts and non-hosts was lower when non-hosts were present in a high density compared with a situation without non-hosts (GLM: $P = 0.05$ (overall), LSD: $P = 0.043$ (high vs control)) (fig. 1B). No effect of non-host species identity on this preference was found (GLM, $P = 0.401$) (fig. 1C); in all four situations a significant preference for dual-infested plants was recorded

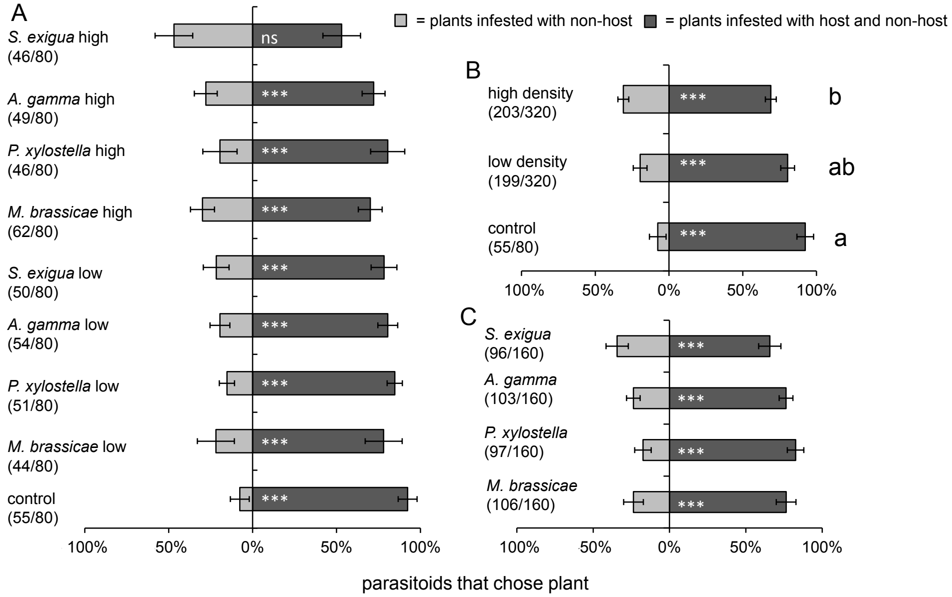

Figure 1. Preference of *C. glomerata* parasitoids in a wind tunnel for plants infested with hosts and non-hosts (right, dark grey) or plants infested with only non-hosts (left, light grey). Four non-host species (*S. exigua*, *A. gamma*, *P. xylostella*, *M. brassicae*) in two densities (low = 10 first-to-second-instar caterpillars and high = 50 first-to-second-instar caterpillars) were tested. For example, in the treatment '*S. exigua* high', the first plant was infested with 50 first-to-second-instar *S. exigua* caterpillars, while the second plant was infested with ten first-instar *P. brassicae* + 50 first-to-second-instar *S. exigua* caterpillars. In the control treatments, the first plant was clean, while the second plant was infested with ten first-instar *P. brassicae* caterpillars. A) effects of the four non-host species in two densities, B) effects of the combined species in two densities and C) effects of the combined densities for the four species. Error bars indicate standard errors of different plant pairs (n = 8 plant pairs per treatment) and asterisks indicate significance levels based on binomial tests, ***: P ≤ 0.001, ns: not significant (α = 0.05). Numbers between brackets indicate the number of parasitoids that responded/the number of parasitoids tested. Different letters in b) indicate significantly different means based on a generalized linear model (α = 0.05) and least significant differences (α = 0.05).

(binomial tests: all P ≤ 0.001). We found that correcting treatments for equal numbers of caterpillars, i.e. by having the same numbers of non-host on one plant as the total number of hosts plus non-hosts on the other, did not yield different results. In the high-density control treatment 49 out of 67 responding parasitoids preferred the plants infested with hosts and non-hosts over non-host infested plants that both had equal total numbers of caterpillars (binomial test: P < 0.001). Similarly, in the low-density control treatment 39 out of 56 responding parasitoids preferred the plants infested with hosts and non-hosts (binomial test: P = 0.005). Comparing parasitoid responses to these treatments in which we applied equal herbivore numbers with responses to our treatments that had received hosts in addition to non-hosts shows no effect of caterpillar numbers (GLM, P = 0.746). These data support the conclusion that parasitoid attraction in our main treatments (fig. 1) was not mediated by a larger number of caterpillars on plants infested with both hosts and non-hosts compared with plants only infested by non-hosts.

On-plant experiment

The behaviour of 66 parasitoids was observed after landing on plants infested with only hosts and on plants with hosts with an additional low or a high density of the non-host *M. brassicae*. The time the parasitoids spent on the plant (residence time) was on average (±SE) 48.5 (±2.1) minutes; this was not affected by the different herbivore infestations (GLM, P = 0.198) (fig. 2A). Neither was the number of hosts that was parasitized by each parasitoid (GLM, P = 0.63) (fig. 2B), nor the number of hosts that was parasitized per minute (GLM, P = 0.497) (fig. 2C). Parasitoids more often attacked and oviposited in non-hosts on plants with a high density of non-hosts compared with plants with a low non-host density (attack non-host rate GLM: P < 0.001, oviposit non-host rate GLM: P = 0.037)(fig. 3). The duration of walking was also affected by non-host density; the parasitoids walked longer on plants with a high non-host density compared with walking on host-only infested control plants (GLM: P = 0.028) (fig. S1A, Appendices). The rate of landing on the other hand, was negatively affected by a high non-host density compared with a low density (GLM, P = 0.05) (fig. S1B, Appendices). All other observed behaviours were neither affected by non-host presence nor by density (fig. S2, Appendices).

Outdoor tent experiment

In total 4303 (64.8%) host caterpillars were found back on the plants, of which 2244 (52.1%) were parasitized. The timing of the experiment influenced parasitism rates, likely because of weather conditions (percentage of parasitized hosts, series effect, GLMM: P < 0.001; percentage of plants with parasitized hosts, series effect, GLMM: P < 0.001; number of hosts per parasitized plant, series effect, GLMM: P < 0.001; number of eggs per plant, series effect, GLMM: P < 0.001). The percentage of parasitized hosts was not affected by non-host density (GLMM: P = 0.224), nor was the percentage of plants with parasitized hosts (GLMM: P = 0.418), nor the number of hosts per parasitized plant (GLMM: P = 0.190) (fig. 4ABC). However, non-host density did have an influence on the number of eggs found per parasitized host; more eggs were found in an environment with a high non-host density compared with a low non-host density (GLMM = 0.047 (overall), LSD: P = 0.0138 (high vs low)). Nevertheless, neither of these treatments differed from the control treatment with only hosts (fig. 4D).

Discussion

Non-host herbivores are found in varying densities in the environment where parasitoids forage for their hosts. We found that an increased density of non-host herbivores hampered *Cotesia glomerata* in locating plants on which their host was feeding, in particular with the non-host *Spodoptera exigua*, while the behaviour on the plant and the total host-finding efficiency of the parasitoid were not affected.

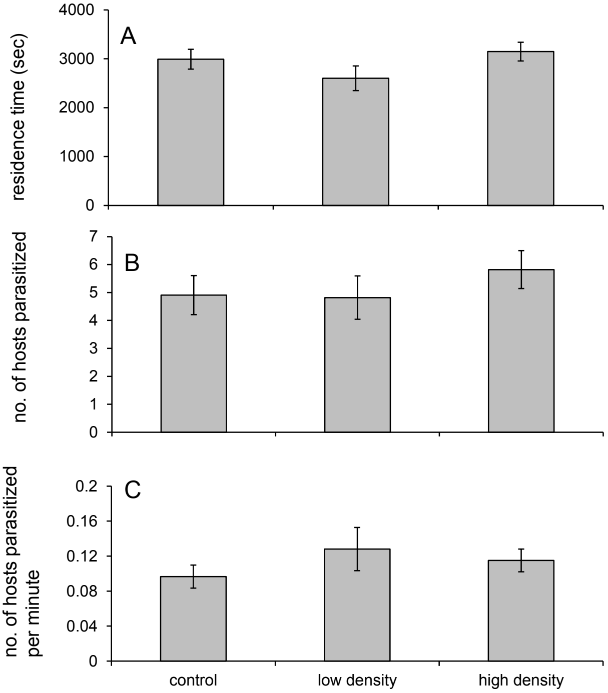

Figure 2. Behaviour of *C. glomerata* parasitoids on plants infested with ten first-instar *P. brassicae* host caterpillars (control), with ten first-instar *P. brassicae* plus ten first-to-second-instar *M. brassicae* non-host caterpillars (low density) or with ten first-instar *P. brassicae* plus 50 first-to-second-instar *M. brassicae* caterpillars (high density) as observed in the on-plant experiment. A) residence time ± SE of the parasitoids on the plant, B) number of hosts ± SE parasitized, C) number of hosts ± SE parasitized per minute. N = 22 per treatment. Based on generalized linear models no significant effects of non-host density were observed ($\alpha = 0.05$).

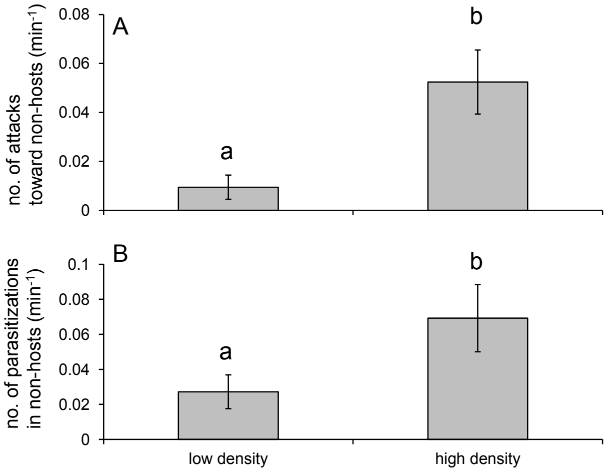

Figure 3. Behaviour of *C. glomerata* parasitoids on plants infested with ten first-instar *P. brassicae* host caterpillars plus ten first-to-second-instar *M. brassicae* non-host caterpillars (low density) or with ten first-instar *P. brassicae* plus 50 first-to-second-instar *M. brassicae* caterpillars (high density) as observed in the on-plant experiment. A) number of non-host attacks per minute (mean ± SE), B) number of oviposition events in non-hosts (mean ± SE). N = 22 per treatment. Different letters indicate significant differences based on generalized linear models ($P < 0.05$).

The non-host density-mediated effects on parasitoid flight response towards plants could originate from both quantitatively and qualitatively altered herbivore-induced plant volatiles. Quantitatively, HIPV blends can be influenced by the density of feeding herbivores, therefore, a high non-host density could have resulted in high quantities of emitted volatiles (Maeda and Takabayashi 2001, Horiuchi et al. 2003, Shiojiri et al. 2010, Girling et al. 2011). These large amounts of volatiles emitted by plants infested with a high density of only non-hosts could have increased the attractiveness of these plants, like has been shown for high host/prey herbivore densities (Maeda and Takabayashi 2001, Gols et al. 2003, Horiuchi et al. 2003, Shiojiri et al. 2010, Girling et al. 2011), resulting in a weaker preference for the host-infested plants. Moreover, the increase in noise caused by neighbouring infested plants may have diluted cues that are associated with host presence used by parasitoids (Bezemer et al. 2010, Wäschke et al. 2013). This phenomenon is known as associational resistance when neighbouring plants reduce the likelihood of detection of the host plant by herbivores (Barbosa et al. 2009). By having the 'right' neighbours, plants can (partly) escape herbivory, which can also be indirectly caused by an increased presence of natural enemies (Barbosa et al. 2009). From the perspective of the

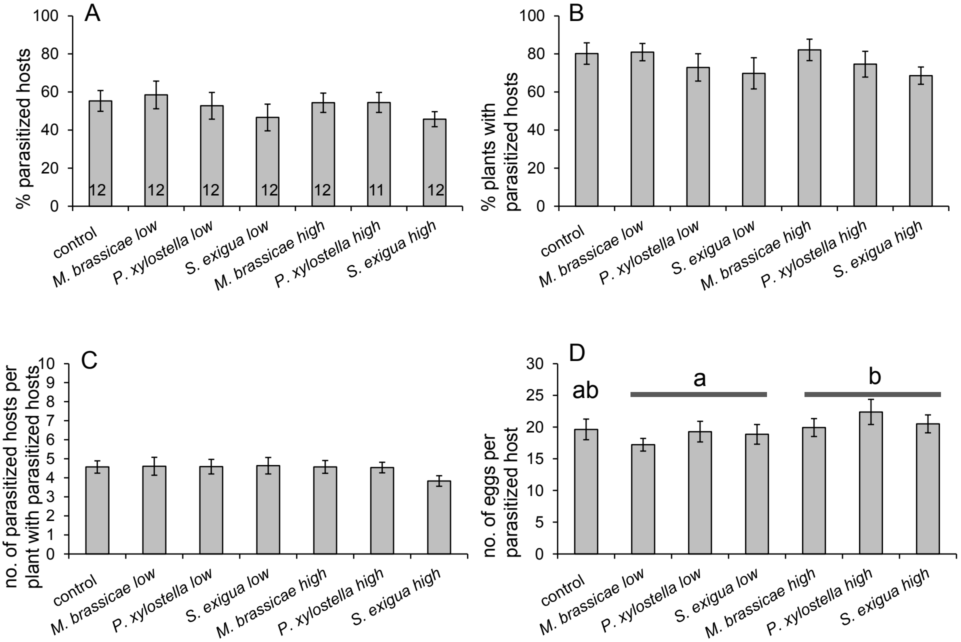

Figure 4. Foraging efficiency of *C. glomerata* parasitoids on plants infested with ten first-instar *P. brassicae* host caterpillars and ten (low density) or 50 (high density) first-to-second-instar non-host caterpillars (either *M. brassicae*, *P. xylostella* or *S. exigua*) in a background of plants infested with the same density of the same non-host species in the outdoor tent experiment. A) mean percentage ± SE of hosts parasitized, B) mean percentage ± SE of plants with parasitized hosts, C) mean number ± SE of parasitized hosts per plant with parasitized hosts, D) mean number ± SE of eggs per parasitized host. Numbers in bars indicate the number of tents per treatment, equal for all four analyses. Based on generalized linear mixed models no significant effect of non-host density was found for A), B) and C) ($\alpha = 0.05$). For D) a significant effect of non-host density was found based on a generalized linear mixed model ($P = 0.047$), different letters indicate differences based on least significant differences ($\alpha = 0.05$).

host caterpillars in our study, high densities of non-hosts in the environment could have provided associational resistance to parasitism. This might even apply to the small scale of high non-host densities on only the host and non-host-infested plant.

The blend of plant volatiles emitted in response to herbivory can also be qualitatively influenced by the density of non-host herbivores (Zhang et al. 2009, Ponzio et al. 2015). On plants infested by host and non-host herbivores in the high-density treatments, the ratio of host:non-host was 1:5, while in the low-density treatments this was 1:1. The combination of herbivores in the high-density ratio could have affected the induction of plant defences and therefore the composition of the volatile blend (Zhang et al. 2009).

Such a qualitative effect was, for example, also caused by high densities of non-prey that, in a dual attack with prey, negatively affected the emission of a volatile that attracts predators and consequently affected the behaviour of the predator (Zhang et al. 2009). Either via quantitative or qualitative effects, density-mediated interactions may additionally interact with trait-mediated interactions since our results show the strongest effects of a high density for one particular non-host species. The presence of a high density of *S. exigua,* with its species-specific characteristics, could have induced the production of a specific volatile blend (DeMoraes et al. 1998, Hare 2011) that affected the parasitoid so that it could not distinguish the plant infested with hosts from its background of non-host infestations.

After choosing a plant and landing on it, an increased non-host density did not change the number of hosts that *C. glomerata* parasitized per time unit. However, in the presence of a high density of non-hosts, parasitoids spent more time on walking compared with the time spent on walking on host-only infested plants, while a low non-host density showed an intermediate effect. Moreover, the rate of attacking non-hosts and oviposition in them was higher for a situation with a high density of non-hosts compared with the low-density situation. That numbers of parasitized hosts per time unit for host only and host plus non-host infested plants were similar, is in contrast with previous findings of Bukovinszky et al. (2012b) who presented data of *C. glomerata* that parasitized more hosts per time unit on plants infested by hosts only compared with plants infested by both hosts and non-hosts (Bukovinszky et al. 2012b). However, in that study the less preferred solitary caterpillar *Pieris rapae* was used as host, while we used a more preferred host, the gregarious caterpillar host *P. brassicae.* The behaviour of parasitoids foraging for solitary and gregarious hosts differs, particularly regarding patch leaving decisions and therefore the time available to parasitize hosts (Vos et al. 1998, Vos and Hemerik 2003). The presence of many non-hosts in our study may have interfered with the accessibility of the host and consequently more time for walking was needed (Hauzy et al. 2010). Additionally, logically resulting from the presence of more non-hosts, the parasitoids encountered more of these herbivores and wasted time on them by attack and oviposition (Takabayashi and Takahashi 1990, Vos et al. 2001, Bukovinszky et al. 2012b, Chabaane et al. 2015). Oviposition in non-hosts could even have resulted in a lowered parasitoid fitness, because the parasitoids wasted eggs on herbivores in which their offspring cannot develop (Bukovinszky et al. 2012b, Chabaane et al. 2015). Even though high non-host densities alter parasitoid behaviour on the plant and potentially parasitoid fitness, the residence

time and the total number of parasitized hosts (per time unit) as well as the eggs found in these were not affected. We, therefore, conclude that density of non-hosts had no influence on the efficiency of the parasitoids when searching for hosts on the plant.
The total host-finding efficiency of the parasitoids in the outdoor tents, that included both the flight response and the on-plant behaviour, was not affected by non-host herbivore density. However, environments with a high non-host density positively affected the number of eggs laid per host, compared with environments with a low non-host density. This could be caused by the insertion of more eggs during one oviposition or by superparasitism by the same or a different parasitoid individual. Because the on-plant experiment showed that parasitoids spent more time on walking in high non-host density situations, in the outdoor experiment the parasitoids may have encountered the same host individuals multiple times and subsequently oviposited in these hosts multiple times. In the wind-tunnel experiment parasitoids landed less frequently on plants containing hosts in a background of a high density of non-hosts. This did, however, not translate into lower parasitism rates in tents with high non-host densities in the outdoor experiment. Our study shows that overall foraging efficiency of *C. glomerata* was not hampered by increased densities of non-host herbivores.

We conclude that even though the density of non-host caterpillars affects the flight response of parasitoids towards infested plants, non-host density does not affect the foraging efficiency of *C. glomerata*. The parasitoids do not distinguish between plants infested by host caterpillars or non-host caterpillars (Geervliet et al. 1996, Shiojiri et al. 2000b, Vos et al. 2001, Bukovinszky et al. 2012b, but see Chabaane et al. 2015). Preferences, however, are shown when one of the plants is attacked by both host and non-host herbivores, which is common in nature (Shiojiri et al. 2000b, Vos et al. 2001, Soler et al. 2007b, Bukovinszky et al. 2012b, Kruidhof et al. 2012a, Soler et al. 2012b, Ponzio et al. 2014, Chabaane et al. 2015). We showed that parasitoid preferences for dually attacked plants are affected by the density of non-host herbivores (Zhang et al. 2009, Yamamoto et al. 2011). Ponzio et al. (2014) however, showed that parasitoid flight preferences are not influenced by increasing non-host densities when host and non-host were feeding from separate leaves (Ponzio et al. 2014). Increasing densities of these non-hosts when feeding together with the host from the same leaf, on the other hand, did affect parasitoid preferences like it also (non-linearly) affected plant responses (Ponzio et al. 2015). Yet, the relative importance of alterations in flight responses may be limited, because the total foraging efficiency of the parasitoids was not altered by non-host density. Studying the total foraging process, preferably outside of the laboratory, therefore, is important (Hunter 2002).

The impact of density-mediated indirect interactions on ecological communities is found to be lower than that of trait-mediated indirect interactions (Werner and Peacor 2003, Křivan and Schmitz 2004, Schmitz et al. 2004, Trussell et al. 2006), although this can depend on resource levels, life history stages and whether a whole season or only a small time frame is studied (Luttbeg et al. 2003). Despite unequal individual importance, DMII's and TMII's together could synergistically contribute to stable ecosystems (Schmitz et al. 2004, Van Veen et al. 2005, Paixão et al. 2014). In our study system the TMII between plant, host and parasitoid in which parasitoids utilize plant volatiles induced by host feeding, is combined with the TMII between plant, non-host and parasitoid in which non-hosts interfere with this utilization. An extension with different densities of non-hosts shows that DMII's play a role in this system of combined TMIIs. Multiple DMII's and TMII's can be intertwined (Werner and Peacor 2003, Utsumi et al. 2010) and should be studied in that context to expand the knowledge of non-trophic interactions that form the basis of complex interaction webs (Eubanks and Finke 2014, Hammill et al. 2015).

Acknowledgements

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Chapter 6

Herbivore species identity rather than diversity of the non-host community determines parasitoid foraging behaviour

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Under review

Extensive research has been executed to reveal how species diversity affects ecosystem functions and services. Yet, consequences of diversity loss for ecosystems as a whole as well as for single community members are still difficult to predict. A suitable system for studies into diversity effects is that consisting of arthropod herbivores and their natural enemies; parasitoids. Parasitoids forage for herbivorous hosts by using herbivore-induced plant volatiles (indirect cues) and cues produced by their host (direct cues). However, in addition to hosts, non-suitable herbivores are also present in a parasitoid's environment and they may complicate the foraging process for the parasitoid. Therefore, whenever the species diversity of herbivores, including both hosts and non-hosts, changes, this could affect the behaviour of parasitoids. Either by means of species numbers per se, or by specific species traits, or by both. To investigate how diversity and identity of non-host herbivores influence the behaviour of parasitoids, we created environments with different levels of non-host diversity, where hosts were complemented with herbivores from one to four non-host species. We subsequently studied the behaviour of the gregarious endoparasitoid Cotesia glomerata while foraging for its gregarious host Pieris brassicae throught indirect plant cues or direct host cues. Our results show that neither non-host species diversity, nor non-host identity influences the preference of the parasitoid for herbivore-infested plants. However, after landing on the plant, non-host species identity does affect parasitoid behaviour whereas non-host diversity does not. One of the non-host species investigated, i.e. Trichoplusia ni, negatively affected the time the parasitoid spent on the plant and the number of hosts it parasitized. We conclude that non-host herbivore species identity has a larger influence on C. glomerata foraging behaviour than non-host species diversity. Our study shows the importance of species identity over species diversity in a multi-trophic interaction of plants, herbivores and parasitoids.

Key words

Cotesia glomerata, HIPV, multi-herbivory, non-host, patch residence time, species traits, *Trichoplusia ni*

Biodiversity on Earth rapidly declines and this affects ecosystem functions and services (Cardinale et al. 2012). Extensive research has been executed to investigate the underlying factors explaining the stabilising effects of species diversity on ecosystems (Cardinale et al. 2012). Unravelled factors are e.g. the asynchrony in how and how rapidly different species respond to environmental changes and the reduction in competition strength between species in diverse environments (Cardinale et al. 2012, Loreau and de Mazancourt 2013). Additionally, the presence of key species has been shown to contribute to the functioning of ecosystems by their unique species- or group identity (Mouillot et al. 2011, Cardinale et al. 2012, Harvey et al. 2013a). Studies on the role of diversity incorporated effects of altered diversity within and between trophic levels to observe the contribution of diversity to ecosystem functions in complex trophic interaction webs (e.g. Thébault and Loreau 2006, Duffy et al. 2007 , Long et al. 2007, Narwani and Mazumder 2012, Jabiol et al. 2013, Abdala-Roberts and Mooney 2014 , Knop et al. 2014). Despite these efforts, it is complicated to generalize and so predict the consequences of diversity loss for particular ecosystems (Long et al. 2007, Cardinale et al. 2012, Harvey et al. 2013a, Loreau and de Mazancourt 2013). Moreover, to predict how diversity affects the performance of a single community member, and which components is challenging.

A suitable biological system for diversity studies that is intensively studied for the interactions between trophic levels is that of arthropod herbivores and their natural enemies; parasitic wasps, or parasitoids (e.g. Vet and Dicke 1992, Takabayashi et al. 2006, Wajnberg 2006, De Rijk et al. 2013, Ponzio et al. 2013, Wäschke et al. 2013 , Desurmont et al. 2014). Parasitoids lay their eggs in or on a host, usually another arthropod. The larvae that emerge from these eggs feed on the host resulting in its (eventual) death (Godfray 1994, Thiel and Hoffmeister 2009). In order to find their herbivorous hosts, parasitoids employ foraging strategies that can be divided into two phases (Van Alphen et al. 2003, De Rijk et al. 2013). First, parasitoids utilize plant volatiles to locate the plant that the host is feeding from (Vet and Dicke 1992, Hare 2011, Clavijo McCormick et al. 2012). Volatile blends are emitted by plants in response to feeding by herbivores (herbivore-induced plant volatiles (HIPV)) and their different composition from undamaged plants makes HIPVs reliable cues of host presence (Arimura et al. 2011, Stam et al. 2014). Second, parasitoids make use of infochemicals such as host frass and feeding damage to locate, recognize and accept the host on the plant (Van Alphen et al. 2003, De Rijk et al. 2013, Colazza et al. 2014).

Plants in (agro)ecosystems are generally attacked by multiple herbivore species (Vos et al. 2001, Stam et al. 2014) and hosts thus share their environment and food plants with other herbivores. The presence of these other herbivores that are not suitable for parasitoid offspring development, so-called non-hosts, may complicate the foraging process for the parasitoid (De Rijk et al. 2013, Desurmont et al. 2014, Ponzio et al. 2014). First, feeding by non-host herbivores also induces plants to produce volatiles. Plant responses to non-host feeding therefore can dilute host-induced plant volatiles and even alter the plant's response to host feeding (Dicke et al. 2009, Arimura et al. 2011, Soler et al. 2012a, Zhang et al. 2013). Second, the physical presence of non-host herbivores and their infochemicals (e.g. emitted from frass or from the body of the non-host) can distract the parasitoid while foraging on the plant (Takabayashi and Takahashi 1990, Bukovinszky et al. 2012b, Chabaane et al. 2015). Therefore, whenever the species diversity within the trophic level of herbivores, including both hosts and non-hosts, changes, this could affect the behaviour of parasitoids in the adjacent trophic level (Vos et al. 2001, Thébault and Loreau 2006, Kratina et al. 2007, Knop et al. 2014, Hammill et al. 2015). Either by means of species numbers per se, or by specific species traits, or by both (Mouillot et al. 2011, Cardinale et al. 2012, Narwani and Mazumder 2012, Harvey et al. 2013a). Vos et al. (2001) mathematically modelled the effects of increased herbivore diversity on parasitoid-host interactions. They found that non-host presence negatively affected parasitoid behaviour and thereby weakened interaction strengths between parasitoids and hosts, eventually resulting in a positive effect of non-host diversity on parasitoid community persistence (Vos et al. 2001). Similar results were found when the diversity of non-prey species was experimentally manipulated and predation rates (Kratina et al. 2007) and species abundances (Hammill et al. 2015) were measured. Non-prey presence reduced predation rates (Kratina et al. 2007), increased the persistence of susceptible prey species (Hammill et al. 2015) and non-prey acted synergistically to increase prey persistence (Hammill et al. 2015). This in turn dampens predator-prey interactions (Kratina et al. 2007) and strengthens food web persistence (Hammill et al. 2015).

To experimentally show how non-host herbivore diversity and identity influence the behaviour of parasitoids, we here created environments with different levels of non-host diversity by using five species of non-host herbivores. In these environments, the gregarious endoparasitoid *Cotesia glomerata* (Hymenoptera: Braconidae) was introduced to forage for its gregarious host, *Pieris brassicae* (Lepidoptera: Pieridae). The lepidopteran *P. brassicae* is in natural situations found in a patchy distribution (Vos et al. 1998), while non-host herbivores can be found everywhere in the environment. For this reason we offered the parasitoid hosts in a background of non-host infestations. We studied the influence of non-host diversity and species identity on both phases of parasitoid foraging separately, using a wind-tunnel set-up to observe the flight response towards plants and an additional set-up to observe the behaviour of the parasitoid on the plant. We hypothesized that a low non-host diversity would simplify the environment, leading to a more efficient host-finding behaviour during both phases of foraging (Wäschke et al. 2013). Effects of non-host species identity on parasitoid behaviour were expected to be revealed irrespective of herbivore diversity (Mouillot et al. 2011, Narwani and Mazumder 2012, Harvey et al. 2013a).

Material and methods

Plants and insects

Brassica oleracea var gemmifera Cyrus plants (Brassicales: Brassicaceae) of five to six weeks old were used in all experiments (greenhouse conditions for plant rearing: 22±2°C, 60±10% RH, 16:8 L:D photoperiod). *Plutella xylostella* (Lepidoptera: Plutellidae)*, Mamestra brassicae* (Lepidoptera: Noctuidae), *Autographa gamma* (Lepidoptera: Noctuidae) and *P. brassicae* caterpillars were reared on *Brassica oleracea* plants (greenhouse conditions: 20±2°C, 60±10% RH and 16:8 L:D photoperiod), *Spodoptera exigua* (Lepidoptera: Noctuidae) and *Trichoplusia ni* (Lepidoptera: Noctuidae) caterpillars were reared on artificial diet mainly composed of polenta, beer yeast and wheat-germs (climate cell conditions: 25.5°C, 50%RH and 16:8 L:D photoperiod) and kindly provided to us by the Laboratory of Virology of Wageningen University. *Cotesia glomerata* parasitoids were reared on *P. brassicae* caterpillars (greenhouse conditions: 20±2°C, 60±10% RH and 16:8 L:D photoperiod). Parasitoid cocoons were daily collected from rearing cages and kept in a climate cabinet (21°C, 16:8 L:D photoperiod). Emerged parasitoids were given honey and water *ad libitum*, were allowed to mate and two-to-eight-day old naive females were used in the experiments.

Wind-tunnel experiments

In a two-choice wind-tunnel set-up as described by Geervliet et al. (1994) the preference of *Cotesia glomerata* for plants infested with different herbivore combinations was tested. In the wind tunnel (23-25°C, 55-70% RH, 0.1 m/s wind speed) two sets of experiments were performed. The first set of experiments was conducted from October 2013 to February 2014, the second set of experiments was conducted from October 2014 to

February 2015. In each set, four species of non-host herbivores were used next to the host *P. brassicae*: in the first set *P. xylostella*, *M. brassicae*, *S. exigua* and *T. ni*, in the second set *P. xylostella*, *M. brassicae*, *S. exigua* and *A. gamma*. Plants were prepared one day before conducting the wind-tunnel experiment by gently transferring first-to-second instar caterpillars (all equal sized) to the youngest fully expanded leaf of the plant. To restrict caterpillars to the leaf and so exclude any effect of herbivore position on the plant, cotton wool was wrapped around the petiole of the leaf and a gauze bag was placed around the leaf. Plants were kept in a greenhouse (21±2°C, 50±10% RH, 16:8 L:D) and just before testing on the next day the bag and cotton wool were removed. After placing a set of plants in the wind tunnel, parasitoids were released individually to test their plant preference. The first plant the parasitoid landed on was considered the preferred plant. Parasitoids that did not make a choice within five minutes after release were considered unresponsive. In the first set of experiments, ten responding parasitoids were tested. In the second set of experiments, ten parasitoids were tested irrespective of their response. The position of the plants was switched after testing half of the group of parasitoids, to limit any effect of plant position.

In the first wind-tunnel experiment (experiment A) we tested whether parasitoids discriminate between plants infested with both hosts and non-hosts and plants infested with only non-hosts, whereby the level of diversity of non-host species was manipulated. The levels of non-host diversity ranged from level 1 (one non-host) to level 4 (four non-hosts), and all possible combinations of species within a set of experiments were used (table 1). Level 4 was only tested in the second set of experiments. Within a level of diversity, the numbers of caterpillars used were equal, both between plants of a set and between treatments. The host:non-host ratio on a plant was always 1:2, or as close to this as possible (table 1). Every treatment was replicated four times in a random order within a set of experiments.

In the second wind-tunnel experiment (experiment B) it was tested if an effect seen in wind-tunnel experiment A was caused by non-host species diversity or identity. Parasitoids were tested for their preference for plants infested with hosts and one species of non-hosts, or for plants infested with hosts and four species of non-hosts. Per set of experiments four treatments were used and the number of caterpillars was equal, both between plants of a set and between treatments. The host:non-host ratio within a treatment was always 1:2 (table 2). Every treatment was replicated eight (first set of experiments) or four (second set of experiments) times in a random order.

Table 1. Treatments as used in wind-tunnel experiment A. Non-host species *T. ni* was only used in the first set of experiments, non-host species *A. gamma* and diversity level 4 were only used in the second set of experiments. Host: *P. brassicae*, Px: *P. xylostella*, Mb: *M. brassicae*, Se: *S. exigua*, Tn: *T. ni*, Ag: *A. gamma*.

diversity level	**set of experiments**	**plant one**	**vs**	**plant two**
1	1st and 2nd	host + non-host Px		non-host PX
	1st and 2nd	host + non-host Mb		non-host Mb
	1st and 2nd	host + non-host Se		non-host Se
	1st	host + non-host Tn		non-host Tn
	2nd	host + non-host Ag		non-host Ag
number of individuals		10 hosts + 20 non-hosts		30 non-hosts
2	1st and 2nd	host + non-host Px+Mb		non-host Px+Mb
	1st and 2nd	host + non-host Px+Se		non-host Px+Se
	1st	host + non-host Px+Tn		non-host Px+Tn
	2nd	host + non-host Px+Ag		non-host Px+Ag
	1st and 2nd	host + non-host Mb+Se		non-host Mb+Se
	1st	host + non-host Mb+Tn		non-host Mb+Tn
	2nd	host + non-host Mb+Ag		non-host Mb+Ag
	1st	host + non-host Se+Tn		non-host Se+Tn
	2nd	host + non-host Se+Ag		non-host Se+Ag
number of individuals		10 hosts + 10 non-hosts A + 10 non-hosts B		15 non-hosts A + 15 non-hosts B
3	1st and 2nd	host + non-host Px+Mb+Se		non-host Px+Mb+Se
	1st	host + non-host Px+Mb+Tn		non-host Px+Mb+Tn
	2nd	host + non-host Px+Mb+Ag		non-host Px+Mb+Ag
	1st	host + non-host Px+Se+Tn		non-host Px+Se+Tn
	2nd	host + non-host Px+Se+Ag		non-host Px+Se+Ag
	1st	host + non-host Mb+Se+Tn		non-host Mb+Se+Tn
	2nd	host + non-host Mb+Se+Ag		non-host Mb+Se+Ag
number of individuals		9 hosts + 6 non-hosts A + 6 non-hosts B + 6 non-hosts C		9 non-hosts A + 9 non-hosts B + 9 non-hosts C
4	2nd	host + non-host Px+Mb+Se+Ag		non-host Px+Mb+Se+Ag
number of individuals		8 hosts + 5 non-hosts A + 5 non-hosts B + 5 non-hosts C + 5 non-hosts D		7 non-hosts A + 7 non-hosts B + 7 non-hosts C + 7 non-hosts D

Table 2. Treatments as used in wind-tunnel experiment B. Non-host species *T. ni* was only used in the first set of experiments, non-host species *A. gamma* was only used in the second set of experiments. Host: *P. brassicae*, Px: *P. xylostella*, Mb: *M. brassicae*, Se: *S. exigua*, Tn: *T. ni*, Ag: *A. gamma*.

combination	plant one	vs	plant two
1	host + non-host Px		host + non-host Px+Mb+Se+Tn/Ag
2	host + non-host Mb		host + non-host Px+Mb+Se+Tn/Ag
3	host + non-host Se		host + non-host Px+Mb+Se+Tn/Ag
4	host + non-host Tn/Ag		host + non-host Px+Mb+Se+Tn/Ag
number of individuals	10 hosts + 20 non-hosts		10 hosts + 5 non-hosts A + 5 non-hosts B + 5 non-hosts C + 5 non-hosts D

On-plant experiment

An on-plant experiment in a gauze tent (L x W x H: 290 x 200 x 230 cm, mesh size 0.6 mm) inside a greenhouse compartment (25±2°C, 60±10%) was used to observe the behaviour of *Cotesia glomerata* after landing on an herbivore-infested plant (see also Bukovinszky et al. 2012b). In this no-choice experiment the response of the parasitoid to plants infested with hosts and non-hosts with a low level of diversity (one species) and to plants infested with hosts and non-hosts with a high level of diversity (four species) was observed. The remaining levels of non-host diversity were not tested due to the time-consuming nature of the experiment. Two experiments were conducted with different combinations of herbivore species. Experiment one was conducted from November 2013 to February 2014 and used the combinations: host + *P. xylostella*, host + *M. brassicae*, host + *S. exigua,* host + *T. ni* and host + *P. xylostella* + *M. brassicae* + *S. exigua* + *T. ni*. Experiment two was conducted from October 2014 to February 2015 and used the combinations: host, host + *S. exigua*, host + *A. gamma*, host + *P. xylostella* + *M. brassicae* + *S. exigua* + *A. gamma*. Plants were prepared using the same methodology a described for the wind-tunnel experiments. The numbers of caterpillars were equal to the numbers used in the second wind-tunnel experiment (table 2), the treatment 'host' included only ten host caterpillars.

Before the experiment started, three plants of the same treatment were placed approximately 50 cm from each other on a table inside the tent, forming a triangle. In the centre of the triangle parasitoids were released individually. Whenever the parasitoid landed on one of the plants the observation started. Using a Psion Workabout PRO 3 device (Motorola Solutions, Inc., Schaumburg, Illinois, USA) and Observer XT 10 software (Noldus Information Technology, Wageningen, The Netherlands), the following behaviours of the parasitoid were recorded: excursions outside the vicinity of the plant (off-plant excursions), flying in the vicinity of the plant (flying), landing on the plant (landing), walking, preening, standing still, attacking a host, oviposition in a host, encounter host/ non-host products (i.e. frass, silk, feeding damage), encounter with a non-host, attacking a non-host, oviposition in a non-host. Of all but two behavioural components, both the duration and the frequency were recorded. Of the behavioural components 'oviposition in a host' and 'oviposition in a non-host' only the frequency was recorded, because it was not possible to accurately record the duration of these - sometimes very short - behaviours. An observation lasted for maximum one hour and was terminated earlier when the parasitoid showed an off-plant excursion for over five minutes. Parasitoids that flew to the walls of the tent immediately after release and stayed there for more than one minute were considered unresponsive. Before a new observation started, the plant that had been visited by the parasitoid was replaced by a new plant. Host caterpillars were dissected and the number of parasitoid eggs that had been injected was counted by using a stereomicroscope.

Statistical analysis

To combine the two sets of experiments conducted in wind-tunnel experiment A, we first tested if the overlapping treatments (those that did not include *T. ni* or *A. gamma*) showed similar results in both sets. For this, logistic regression analyses were conducted on the number of parasitoids choosing the host- and non-host-infested plant out of the total number of responding parasitoids per plant pair. The model comprised an unknown multiplicative dispersion factor for the binomial variance, an explanatory variable for the age of the parasitoid and main effects for experimental set on the logit scale. None of the seven overlapping treatments showed significant differences ($\alpha = 0.05$) between sets of experiments and, therefore, the results from both sets were combined. Next, all treatments (22 in total) were compared using a logistic regression analysis as described above, with treatment as main effect. Also the four diversity levels were compared using this model, with diversity level as main effect. Parasitoid age was not significant in these models and, therefore, subsequently dropped. To test for preferences for infested plants, binomial tests were conducted per treatment/diversity level for equal preference of parasitoids for host- and non-host-infested and non-host-only infested plants. Analysis for the second wind-tunnel experiment were performed similarly: overlapping treatments were compared using logistic regression analysis and combined, because no effects of experimental set were found. After that, logistic regression was used to compare treatments and we used binomial tests to test for preferences for plant treatments within a choice test.

To combine the two on-plant experiments, the overlapping treatment 'host + *S. exigua*' was tested for differences between the two experiments for all behaviours and derived findings (e.g. residence time, eggs per oviposition) separately. For that purpose, generalized linear models were used comprising an unknown multiplicative dispersion factor for the binomial (duration of behaviour) or Poisson (rate of behaviour) variance, an explanatory variable for the age of the parasitoid and main effects for experiment on the logit (duration) or log (rate) scale. For 14 out of 26 behaviours and derived findings no significant differences ($\alpha = 0.05$) were found between the overlapping treatments, so data of these from both experiments were combined. Again, generalized linear models were used. For the duration of each behaviour expressed as a proportion of residence time, only those observations were included where the particular behaviour was actually performed. The model comprised a multiplicative dispersion factor for the binomial variance, an explanatory variable for the age of the parasitoid and main effects for treatment on the logit scale. The 'binomial total' was fixed at value 1. A similar model was used to analyse the residence time and the number of hosts parasitized, but with 'binomial totals' of 60 minutes or ten hosts. The rate per minute with which behaviours were performed were analysed for all observations using logistic regression models comprising a multiplicative dispersion factor for the Poisson variance, an explanatory variable for the age of the parasitoid and main effects for treatment on the log scale. An extra offset was added to analyse the number of eggs per oviposition and the number of eggs per host. The offset was set as the log of oviposition frequency or the log of the number of parasitized hosts respectively. In all models the age of the parasitoids was dropped if it showed to have a non-significant effect. Calculations were performed using GenStat® 17th.

Results

Wind-tunnel experiments

In wind-tunnel experiment A, five combinations of plants infested with single non-host species were tested, nine combinations of double non-host infestations, seven combinations of triple non-host infestations and one combination of four non-host species (fig. 1). The combined results show that the 399 responding parasitoids significantly preferred plants infested with both host and non-hosts over plants infested with only non-hosts, irrespective of the level of non-host diversity (all binomial tests: $P < 0.05$) (fig.

2A). The distribution of this preference was equal for all non-host diversity levels (GLM: P = 0.524) (fig. 2A).

The effect of non-host species identity on parasitoid preference for plants infested with hosts and a low or high non-host diversity was tested in wind-tunnel experiment B for five non-host species. Parasitoids did not show preferences for either plants containing a single non-host species or four non-hosts species (all binomial tests: P > 0.07) (fig. 2B) and this was similar for each of the five non-host species identities (GLM: P = 0.839) (fig. 2B).

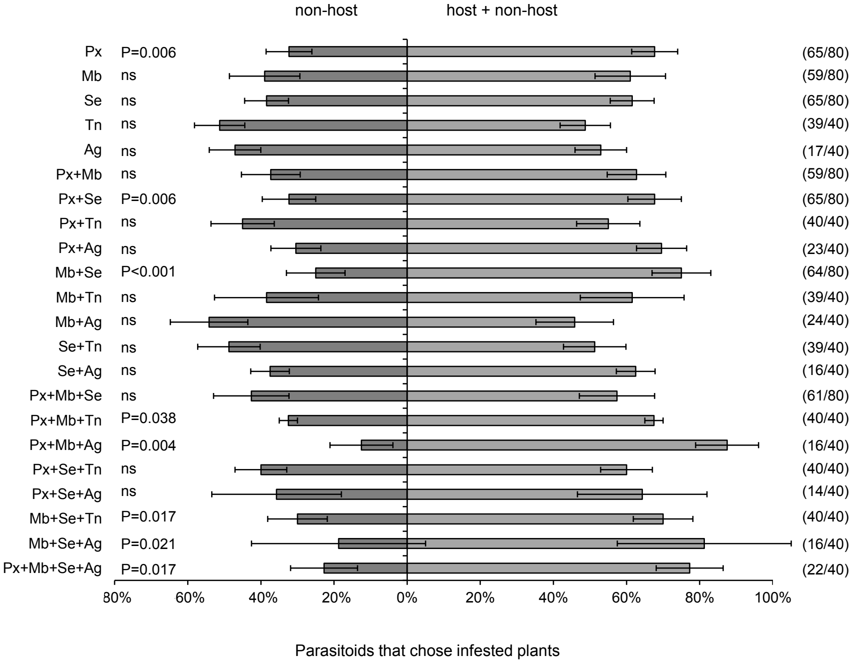

Figure 1. Landing preference of parasitoids. Percentage of *C. glomerata* parasitoids that chose *B. oleracea* plants infested with non-hosts (left) or *P. brassicae* hosts + non-hosts (right). First-to-second-instar caterpillars were used according to the amounts as presented in table 1. Error bars indicate standard errors of different plant pairs, ns: not significant (α = 0.05). Numbers between brackets indicate the number of parasitoids that exhibited a response/the number of parasitoids tested. Px: *P. xylostella*, Mb: *M.brassicae*, Se: *S. exigua*, Tn: *T. ni*, Ag: *A. gamma*.

On-plant experiment

The behaviour of 187 individual *C. glomerata* parasitoids was observed on plants either infested with only hosts, or on plants infested with hosts and a single non-host species (five combinations) or on plants infested with hosts and four non-host species (two

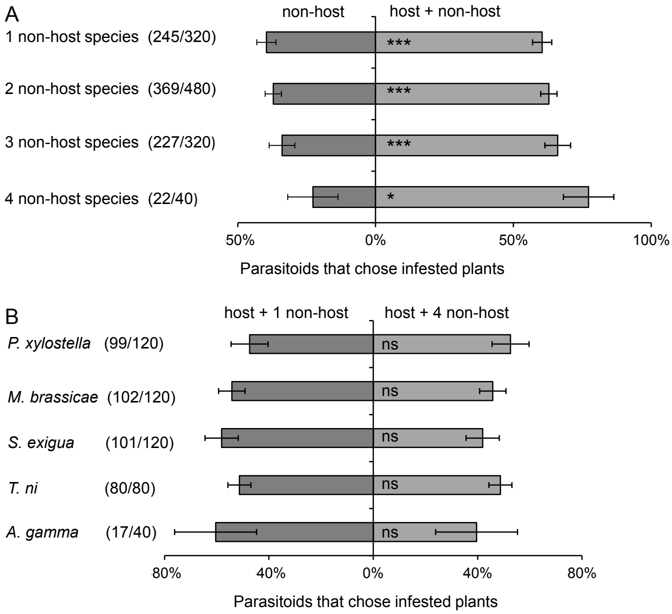

Figure 2. Landing preference of parasitoids. Percentage of *C. glomerata* parasitoids that chose *B. oleracea* plants infested with (A) non-hosts (left) or hosts (*P. brassicae*) + non-hosts (right) and (B) hosts + one species of non-hosts (left) or hosts + four species of non-hosts (right). Treatments differed in the number of non-host species present (A) and the species identity of the non-host (B). First-to-second-instar caterpillars were used according to the treatments and amounts as presented in tables 1 and 2. Error bars indicate standard errors of different plant pairs and asterisks indicate significance levels based on binomial tests, ns: not significant (α = 0.05), *:$P < 0.05$, ***:$P < 0.001$. Numbers between brackets indicate the number of parasitoids that exhibited a response/the number of parasitoids tested.

combinations). The time spent by the parasitoids on the infested plants was lowest in the presence of *T. ni*, either alone or in combination with three other non-host species, compared with all other treatments except for the infestation by hosts and *M. brassicae* (GLM: P = 0.003) (fig. 3A). The number of hosts parasitized was lowest in the presence of *T. ni* alone or in combination with three other non-host species, compared with all other treatments except for an infestation by hosts and *A. gamma*. On plants infested with hosts and *A. gamma* the number of hosts parasitized was only lower compared with an infestation by hosts only (GLM: P = 0.004) (fig. 3B).

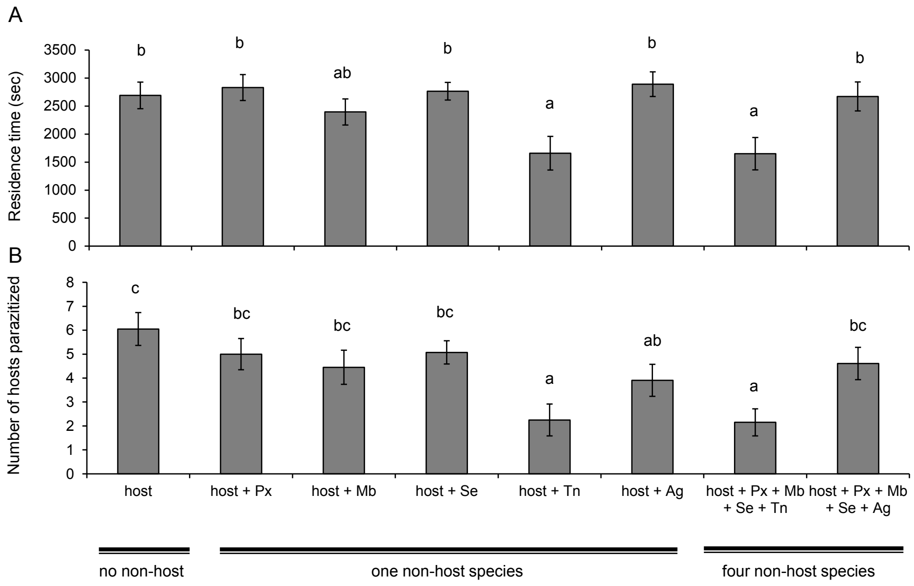

Figure 3. On-plant behaviour of parasitoids. (A) Residence time ± SE of *C. glomerata* and (B) number of *P. brassicae* hosts ± SE parasitized by the parasitoid on *B. oleracea* plants infested with only hosts, hosts + one species of non-hosts or hosts + four species of non-hosts. Plants were infested with (combinations of): host: *P. brassicae*, Px: *P. xylostella*, Mb: *M. brassicae*, Se: *S. exigua*, Tn: *T. ni*, Ag: *A. gamma* in amounts as presented in table 2. For the treatment 'host', ten *P. brassicae* caterpillars were used. Different letters indicate significantly different means based on a generalized linear model ($P < 0.05$) and least significant differences ($\alpha = 0.05$).

The non-host species showed diverging effects on the behaviours 'duration of attack host' (fig. S1, graph A, Appendices), 'duration of attack non-host' (fig. S1, graph B, Appendices), 'duration of standing still' (fig. S1, graph C, Appendices), 'rate of encounter products' (fig. S1, graph H, Appendices), 'rate of flying' (fig. S1, graph E, Appendices), 'rate of landing' (fig. S1, graph F, Appendices) and 'rate of preening' (fig. S1, graph G, Appendices). Overall, none of the species nor the combinations of four species clearly stood out in affecting the behaviour of the parasitoid (all GLM: $P < 0.02$) (fig. S1, Appendices). The presence of the five non-host species either alone or in combinations of four species did not affect the behaviours 'duration of off plant excursions' (fig. S1, graph D, Appendices), 'rate of attack non-host' (fig. S1, graph I, Appendices), 'rate of oviposit non-host' (fig. S1, graph J, Appendices), 'eggs per oviposition' (fig. S1, graph K, Appendices) and 'eggs per parasitized host' (fig. S1, graph L, Appendices) (all GLM: $P > 0.05$).

Discussion

Rather than non-host herbivore species diversity, we found that non-host species identity affects foraging behaviour of parasitoids. Presence of the non-host caterpillar *Trichoplusia ni*, either alone or in combination with other non-host species, reduced the time the parasitoid *Cotesia glomerata* spent on a plant and the number of *P. brassicae* hosts it parasitized.

In contrast to the behaviour of the parasitoid on the plant, parasitoid flight response to herbivore-induced plant volatiles emitted by plants infested with host and non-hosts was neither influenced by non-host identity nor by non-host diversity. For parasitoids in chemically complex environments three foraging strategies have been defined: ignoring, avoiding and preferring chemical complexity caused by, for example, herbivore induction (Wäschke et al. 2013). Avoiding and preferring complexity do not correspond to the behaviour we observed, because the parasitoids showed neither preference nor aversion towards plants infested with diverse herbivore communities. The remaining strategy, ignoring complexity, could apply to *C. glomerata*. When ignoring chemical complexity of plants, parasitoids might change to using visual or chemical cues directly derived from the host (Wäschke, Meiners & Rostás 2013). If this was the foraging strategy of *C. glomerata* in our study, the parasitoids distinguished host cues from a mix of non-host cues. These non-host cues represented herbivores that were, to a certain extent, similar to the host because of equivalent size and feeding guild. This contrasts with previous research in which naive *C. glomerata* showed no preference for *P. brassicae* infested or non-host caterpillar infested plants in a chemically simple environment (Geervliet et al. 1996, Vos et al. 2001). However, in chemically complex multi-herbivore environments the parasitoid preferred *P. brassicae* plus non-host infested plants over plants infested with only non-hosts (De Rijk et al. 2016b, De Rijk et al. 2016c), like in our current study. Parasitoid foraging strategies, therefore, might differ between chemically simple and complex environments (Wäschke et al. 2013). During foraging in chemically simple environments the parasitoids might mainly rely on plant volatiles, while in complex environments direct visual or chemical host cues could be of increased value.

Although plant volatiles induced by a diverse non-host community did not influence parasitoid preferences for infested plants, the parasitoids were affected in their behaviour after landing on the plant by the presence of the non-host *Trichoplusia ni*. The residence time and, most likely as a result of that, the number of hosts parasitized, were both low when this species was feeding on the plant either with or without other non-host species. The presence of *T. ni* in addition increased the time the parasitoids spent on attacking non-hosts. The parasitoid also spent an increased amount of time on attacking *A. gamma* when this was the only non-host present, but the residence time on plants with this infestation was not affected. Besides the effects of *T. ni* on residence time, hosts parasitized and time spent on non-host attack, no clear effects on the behaviour of *C. glomerat*a were observed. This complicates pinpointing the exact non-host characteristics that altered the behaviour of the parasitoid. *Trichoplusia ni* is an exotic species that is not found in the natural environment of *C. glomerata* in The Netherlands. Exotic herbivores have the potential to affect parasitoid behaviour (Chabaane et al. 2015) and by that, interactions within the ecological community (Desurmont et al. 2014, Desurmont and Pearse 2014). The parasitoids might have responded differently to *T. ni* because of its deviating, exotic, characteristics. However, one of the other tested non-host species, *Spodoptera exigua*, is an exotic species as well, but no contrasting parasitoid behaviours were seen in response to its presence. Instead of a species-specific effect of *T. ni*, the combination of this non-host and the host *P. brassicae* feeding together on the plant might has been the source of altered parasitoid behaviour. Different foraging strategies are employed by *Cotesia glomerata,* dependent on the host species for which the parasitoid is foraging (Vos et al. 1998, Vos and Hemerik 2003). Therefore, whenever *T. ni* would feed on a plant together with another host species (e.g. *Pieris rapae*), a different behavioural response might be elicited from the parasitoid. Previous studies showed that, in mathematical models, increased non-host diversity promotes persistence of parasitoid-herbivore communities (Vos et al. 2001). Additionally, in communities with predators, the presence of multiple non-prey species weakened predator-prey interactions (Kratina et al. 2007, Hammill et al. 2015) and synergistically increased persistence of prey (Hammill et al. 2015). Deviating from this, in our system non-host diversity per se did not affect parasitoid-host interactions, but non-host species identity did. This resembles earlier research that showed the importance of species- or group identity (Mouillot et al. 2011, Harvey et al. 2013a, Hammill et al. 2015), group composition (Narwani and Mazumder 2012) or a combination of both diversity and identity (Cardinale et al. 2012) on ecosystem functioning.

The level of species diversity within a trophic level can influence processes in other trophic levels, which is shown for example for the diversity of plants (Cardinale et al. 2011), plant genotypes (e.g. Abdala-Roberts and Mooney 2014)ÿ, predatory arthropods (e.g. Knop et al. 2014), microbial decomposers and detritivorous invertebrates (e.g. Jabiol et al. 2013). In diversity-dependent interactions between trophic levels, edibility of resources can play an important part (Thébault and Loreau 2006, Duffy et al. 2007). Incorporating non-suitable resources in studies on the role of diversity therefore increases the biological relevance (Hammill et al. 2015). Our study combined the effects of species diversity on multi-trophic interactions with the presence of (inedible) non-host species in the interaction web. After separating species identity and species diversity factors, we conclude that non-host herbivore species identity has a larger influence on *C. glomerata* foraging behaviour than non-host species diversity. However, our work shows non-host effects on a short period of time for two separate parts of parasitoid foraging in laboratory settings. Over a longer time span in a field environment results could be different (Bukovinszky et al. 2012b, Chabaane et al. 2015) and, therefore, it is too early to extrapolate our conclusions to natural situations. Nevertheless, our study shows the importance of species identity over species diversity in a multi-trophic interaction of plants, herbivores and parasitoids. Knowledge of the influence of declining species diversity on functioning of complex interaction webs is necessary to develop suitable management strategies to handle global ecosystem change (Duffy et al. 2007, Bascompte 2009, Cardinale et al. 2012, Loreau and de Mazancourt 2013). Also in these processes, species traits may be more important than diversity per se.

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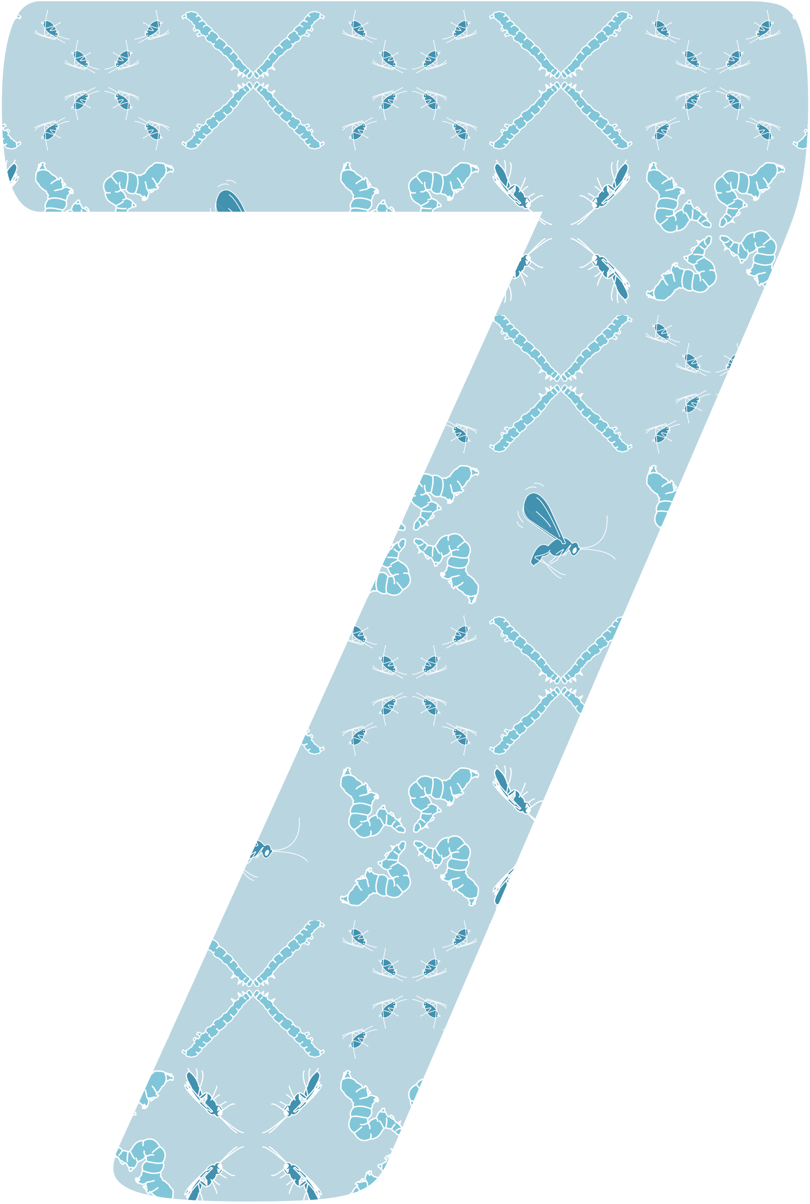

Chapter 7

Associative learning of host presence in non-host environments influences parasitoid foraging

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Under review

Parasitic wasps are known to utilize learning when foraging for their herbivorous host. In natural situations these hosts share food plants with other, non-suitable herbivores (non-hosts). By the production of non-host related (in)direct cues, the presence of non-host herbivores partly determines the environment of the parasitoid. Parasitoids may learn to associate cues related to simultaneous infestations of hosts and non-hosts with the presence of their hosts. We used a laboratory and a field-tent set-up to study the adaptive nature of learning by a foraging parasitoid that had acquired oviposition experience on a plant-host-non-host complex with its associated odours in the laboratory. Our laboratory results show that parasitoids can indeed learn to prefer plant cues induced by simultaneous feeding of hosts and non-hosts. However, the learned preference found in the laboratory did not translate into parasitization preferences in a field-tent situation. We suspect that the incomplete information given to the parasitoids and the environment that was saturated with hosts in the field-tent experiment, could have played a major role in the observed absence of effects of previous oviposition experience on parasitization preference.

Keywords
Associative learning, *Cotesia glomerata*, field-tent experiment, multi-herbivory, non-host, parasitization preference, parasitoid behaviour

The ability to learn to associate certain cues with the presence of a food reward is important for animals to optimize their foraging success and parasitic wasps are no exception to this (Ishii and Shimada 2010, Hoedjes et al. 2011, Mery 2013). Parasitic wasps, or parasitoids, lay their eggs in or on other arthropods, their hosts. The larvae emerging from these eggs feed on this host and eventually the host dies (Godfray 1994). To find the herbivorous hosts in the complex environment in which they forage, parasitoids use direct cues from the host (e.g. from frass) (Van Alphen et al. 2003, Colazza et al. 2014) and indirect cues produced by the food plant of the host in response to herbivore feeding (herbivore-induced plant volatiles, HIPVs) (Vet and Dicke 1992, Van Alphen et al. 2003, Hare 2011). By learning to associate such cues with the presence of hosts, parasitoids can increase their foraging efficiency (Ishii and Shimada 2010, Hoedjes et al. 2011, Mery 2013).

In natural situations, next to hosts, other herbivores are present that are not suitable for parasitoid larval development. These non-host herbivores share food plants with the host or feed on neighbouring plants, consequently producing and inducing the production of non-host related (in)direct cues (De Rijk et al. 2013). Similarities between host and non-host cues depend to a large extent on the feeding habits of the herbivores. At the level of indirect cues, phloem-feeding herbivores like aphids generally induce the emission of volatile compounds regulated by the salicylic acid signal-transduction pathway of the plant, whereas leaf-chewing herbivores such as caterpillars generally induce the emission of a different group of volatiles regulated by the activation of the plant jasmonic acid pathway (Arimura et al. 2011). In fact, when phloem feeders and leaf chewers attack the same plant, the plant signal-transduction pathways can interact (cross-talk) and consequently an even different volatile blend is emitted (Dicke et al. 2009, Thaler et al. 2012). In addition, the direct cues produced by phloem feeders and leaf chewers are dissimilar from each other, e.g. different body odours, frass, and patterns of feeding damage. By the induction of different volatile blends and the production of different herbivore-related direct cues, the presence of non-host herbivores with various feeding habits partly determines the environment of the parasitoid. Parasitoids may therefore learn to associate cues related to simultaneous infestations of hosts and non-hosts, with the presence of their hosts.

Although thorough laboratory studies have identified the potential of learning by parasitoids as well as some of the underlying mechanisms (Tumlinson et al. 1993, Turlings et al. 1993, Vet et al. 1995, Hoedjes et al. 2011, Wäschke et al. 2013), the significance of learning in foraging under complex field conditions has seldom been studied (Lewis and Martin 1990, Mery 2013, but see Hare et al. 1997; Kruidhof et al. 2015 and Papaj and Vet 1990). A study into the adaptive value of learning by predators has been conducted by Janssen et al. (2014) who tested if predatory arthropods in the field associated the presence of food with a volatile blend of mint oil. The food supplemented with mint oil was preyed upon increasingly over four to five days, which suggests that the predators in the field associated the volatiles with the presence of food (Janssen et al. 2014). Similarly, *Drosophila melanogaster* can learn to associate apple- or orange-flavoured food with (un) palatability of the food in semi-natural environments (Zrelec et al. 2013). The fruit flies showed this ability both after having a learning experience in the laboratory, and when the learning occurred under free-flying conditions (Zrelec et al. 2013). Kruidhof and colleagues (2015) studied in a semi-field set-up how surrounding vegetation influences the foraging behaviour of a parasitoid experienced with oviposition on the host plant. They found that foraging efficiency was lowered by the presence of surrounding vegetation, but that the vegetation did not hamper the parasitoid in showing its learned preference. Moreover, they found an increased proportion of parasitized host patches when parasitoids previously experienced an associative learning event (Kruidhof et al. 2015). Here, we studied in a field-tent set-up the adaptive nature of learning by a foraging parasitoid that had acquired oviposition experience on a plant-host-non-host complex with its associated odours in the laboratory.

The parasitoid *Cotesia glomerata* is specialized on caterpillars of the genus *Pieris*, but is a generalist within this genus with *Pieris brassicae* as its preferred host (Geervliet et al. 2000). *Pieris* caterpillars are oligophagous, feeding on plants within the Brassicaceae family and both the parasitoid and the caterpillar are gregarious species. In laboratory settings, *Cotesia glomerata* is able to associate volatiles produced by plants in response to host feeding with the presence of the host (Geervliet et al. 1998a, Geervliet et al. 1998b, Bleeker et al. 2006, Smid et al. 2007, Kruidhof et al. 2012a, Kruidhof et al. 2012b). In this study we tested if *C. glomerata* could learn to associate cues induced and produced by simultaneous feeding of host and non-host herbivores with the presence of the host. For that, we used both a wind-tunnel set-up in the laboratory and a tent set-up in an agricultural field. We conditioned the parasitoids by giving them an oviposition experience on plants infested by both leaf-chewing hosts and leaf-chewing non-hosts and/or on plants infested by both leaf-chewing hosts and phloem-feeding non-hosts. The next day, the conditioned parasitoids were offered a foraging situation with both types of plant infestation. We hypothesized that parasitoids in the wind-tunnel experiment would show a preference for the plant infestation type previously experienced, or no preference when both plant infestation types were experienced, as a consequence of associative learning (Vet et al. 1990). Plants infested by host and non-host leaf-chewers harboured a higher total number of leaf-chewing herbivores compared with the plant infestation type that harboured hosts and phloem-feeding non-hosts. Inexperienced parasitoids were hypothesized to prefer plants infested by a high number of leaf-chewing herbivores, based on expected quantitative (Geervliet et al. 1998a) and qualitative (Bukovinszky et al. 2012b) differences between leaf-chewer-induced cues produced by the two plant infestation types. In the field-tent set-up, we expected to find a larger number of plants with parasitized hosts of the plant infestation type previously experienced by the parasitoid compared with the number of plants with parasitism of the inexperienced infestation type. Equal numbers of plants with parasitism were expected when both plant infestation types had been previously experienced by the parasitoid. A larger number of plants infested with host and non-host leaf-chewers over phloem-feeding non-hosts was expected to harbour hosts parasitized by inexperienced parasitoids. Previously, we found that *C. glomerata* was more efficient in finding its host in the presence of non-hosts phloem feeders compared with the presence of non-host leaf chewers (De Rijk et al. 2016b). Based on this finding, we additionally hypothesized that parasitoids that previously experienced a plant infested by hosts plus non-host aphids would have a higher parasitization efficiency (number of hosts parasitized per time unit) compared with those that previously experienced a plant infested by hosts plus non-host caterpillars. Parasitoids that previously experienced both types of plant infestation were expected to show an intermediate parasitization efficiency.

Materials and methods

Plants and insects

Brassica oleracea var gemmifera Cyrus plants were used in the experiments. The plants were grown in a greenhouse (20±2°C, 60±10% RH, 16:8 L:D photoperiod) in pots of 0.7 litre containing potting soil (Lentse potgrond, no. 4; Lent, The Netherlands). Two to three times per week the plants were fertilized with a liquid fertilizer. Cultures of the host caterpillar *Pieris brassicae*, non-host caterpillar *Mamestra brassicae* and non-host aphid *Myzus persicae* (strain 2 of De Rijk et al. 2016b) were maintained on *B. oleracea* plants in a greenhouse (20±2°C, 60±10% RH, 16:8 L:D photoperiod). For both experiments, first-instar caterpillars and wingless adult aphids were used. A culture of *Cotesia glomerata* was maintained on *P. brassicae* caterpillars in a greenhouse (20±2°C, 60±10% RH, 16:8 L:D photoperiod). Parasitoid cocoons were kept in a climate cabinet (21°C, 16:8 L:D photoperiod) and emerged adults were provided with water and honey *ad libitum*. The parasitoids were allowed to mate and one-to-six days old inexperienced parasitoids were conditioned and tested in the experiments.

Preparation of plants

Plants used for the conditioning of parasitoids were prepared three days in advance in order to allow plants to fully deploy their response to the inducing herbivores. To create host plus non-host aphid-infested plants, ten *P. brassicae* and 40 *M. persicae* individuals were transferred to the youngest fully expanded leaf of a five weeks old plant. Of those, all aphids and five caterpillars were placed in a clip cage (diameter 3 cm) to ensure concentrated feeding damage by both herbivore species. Because of limited leaf area inside the clip cage, the remaining five caterpillars were placed outside of the clip cage. Host plus non-host caterpillar-infested plants were prepared in a similar way, but instead of 40 aphids, 10 *M. brassicae* caterpillars were transferred to the plant. Of those, five were placed inside the clip cage together with the five *P. brassicae* caterpillars. To prevent the herbivores outside of the clip cages from moving to other leaves, cotton wool was wrapped around the stalk of the leaf and a fine mesh bag (35 x 27 cm) was placed around the leaf. Prepared plants were kept in a greenhouse (23±2°C, 60%RH, 16:8 L:D). Before conditioning the parasitoids, plants were brought to a laboratory environment and mesh bags, cotton wool, clip cages and herbivores were removed. Both host and non-host herbivores were removed, but not their excretions, to prevent that the learning experiences would greatly differ among parasitoids (e.g. when one parasitoid would encounter a non-host individual and the other parasitoid would not). First instar *P. brassicae* caterpillars were placed on the previously infested leaf, on the spot where the clip cage had been. New hosts were offered to the parasitoid because the three-day-old hosts would better defend themselves against oviposition that could negatively affect the conditioning, whereas younger caterpillars defend themselves less well (Gross 1993). To encourage the parasitoids to search for their host, frass of first-instar *P. brassicae* larvae was spread on the leaf. Plants used in the wind-tunnel and field-tent experiment were prepared the same way, but without the use of clip cages. For the wind-tunnel experiment, plants were prepared four days in advance because of logistic reasons.

Conditioning of parasitoids

Parasitoids were inexperienced (inexperienced) or were given an experience by allowing them one oviposition in a host on a plant that was infested by 1) the host in combination with the non-host caterpillar *M. brassicae* (caterpillar conditioning), 2) the host and the non-host aphid *M. persicae* (aphid conditioning) or 3) oviposition on a plant infested by the host and non-host caterpillar immediately followed by oviposition on a plant infested by the host and non-host aphid (dual conditioning). Each conditioning treatment was given two times with ten minutes in between, so-called spaced training (Smid et al. 2007), to create long-term memory that would last for at least two days. Immediately after collecting an individual parasitoid in a 1.5 ml Eppendorf tube, the parasitoid was allowed to walk on the infested part of the leaf and after parasitizing one host, the parasitoid was collected back in the tube. After ten minutes, the parasitoid underwent the second part of the conditioning, which was the same as the first part in the case of caterpillar or aphid conditioning. In the dual conditioning, the parasitoid first experienced one oviposition on a caterpillar infested plant, immediately followed by an oviposition on an aphid-infested plant. After the ten minute interval, the parasitoid experienced these two ovipositions again but in reversed order. The two possible orders of oviposition experiences for this treatment were equally divided over the parasitoids of this treatment. Attacked hosts were replaced by new ones after removal of the parasitoid. Per set of two plants, 12 parasitoids were conditioned. Inexperienced parasitoids were kept ten minutes in the collection tube. Parasitoids of all treatments were kept in cages with honey and water *ad libitum* after conditioning. One day after conditioning, the parasitoids were released individually (wind-tunnel experiment) or in groups of three (field-tent experiment). Each parasitoid was tested only once.

Experimental set-up

The preference of inexperienced and conditioned *C. glomerata* parasitoids towards plants infested with hosts plus non-host caterpillars and plants infested with hosts plus non-host aphids was tested in the laboratory using a wind tunnel as described by Geervliet et al. (1994). Plants were prepared as described above. Before placing one plant of each type in the wind tunnel, the cotton wool and mesh bags to confine herbivores on the leaves were removed. In the wind tunnel (25±1°C, 64-70% RH, wind speed 0.1 m/s) plant pairs were placed upwind and individual (un)conditioned parasitoids were released from a distance of 70 cm from the plants. The first plant the parasitoid landed on was considered as the preferred plant; when a parasitoid did not land on one of the plants within five minutes this parasitoid was considered unresponsive. After every two responding parasitoids, the position of the plants was swapped to control for unforeseen asymmetry in the set-up. For each treatment, 13 or 14 sets of plants were used to test a maximum of 12 responding parasitoids per plant pair in a random order spread over seven weeks.

The parasitization preference of inexperienced and conditioned *C. glomerata* parasitoids was tested using a field-tent experiment conducted from May 1st 2013 until October 4th 2013. Two sets of eight tents were used (l x b x h: 3m x 4m x 2m) made out of insect screen (mesh diameter 0.6 mm) and positioned in an agricultural field in the vicinity of Wageningen, the Netherlands. The two sets of tents were used alternately with at least 11 days between using the same set of tents to exclude the presence of previously released insects. After growing in a greenhouse for four weeks, *B. oleracea* plants were transferred outside to acclimatize for one week. Five-week-old plants were planted in the tents, per tent 16 plants in a 4x4 grid with ± 60 cm distance between plants. During the sixth week, all of the plants in the tents were infested with ten *P. brassicae* caterpillars. Every other plant was additionally infested with 40 *M. persicae* aphids, whereas the remaining eight plants were additionally infested with ten *M. brassicae* caterpillars using methods as described above. Three days after herbivore introduction, three female (un)conditioned parasitoids and three male parasitoids were released after removal of the mesh bags to confine insects to plants. The parasitoid conditioning treatments were randomly distributed over the tents (one conditioning treatment per tent, two tents per conditioning treatment). Two plastic lids (diameter 3 cm) with drops of honey were placed in the tents as a food source for the parasitoids. After 24 hours the plants in the tents were harvested and kept individually in labelled plastic bags at 4°C. All *P. brassicae* caterpillars found back on the plants were dissected under a stereomicroscope to check for parasitization. The experiment was replicated eight times.

Statistical analysis

To test for equal preference for the two plants offered to the parasitoids in the wind-tunnel experiment, initially, per treatment, a logistic regression model was fitted, with just a constant on the logit scale, and a multiplicative dispersion factor for the binomial variance, to account for extra variation between pairs of plants. An approximate t-test was used to check whether the constant differed from 0, i.e. whether parasitoids showed a preference. When the estimated dispersion factor was smaller than 1, the binomial test was used instead, assuming independence of choices of parasitoids within and between pairs of plants. To analyse if the preference and the response rate of the parasitoids were affected by conditioning treatment, a logistic regression analysis was conducted comprising an extra unknown multiplicative dispersion factor for the binomial variance, an explanatory variable for the day of testing and main effects for conditioning treatment on the logit scale. There was no significant effect of the day of testing, so it was subsequently dropped from the model. Statistical inference was based on the Wald test.

To test for equal preference for the two plant types offered to the parasitoids in the field-tent experiment, initially, per treatment, a logistic regression model was fitted, comprising just a constant as a fixed effect, in addition to random effects for tents, on the logit scale, and a multiplicative dispersion factor for the binomial variance, to account for extra variation between groups of plants within the same tent. An approximate t-test was used to check whether the constant differed from 0, i.e. whether parasitoids showed a preference. When the estimated variance component for tents was negligible and the estimated dispersion factor was smaller than 1, the binomial test was used instead, assuming independence of choices of parasitoids within and between groups of plants within the same tent. To analyse if the preference for the two plant types was affected by conditioning treatment, a generalized linear mixed model was used comprising an extra unknown multiplicative dispersion factor for the binomial variance, a random factor for tent number, an explanatory variable for the moment in time of testing and main effects for conditioning treatment on the logit scale. There was no significant effect of the moment of testing, so it was subsequently dropped. To analyse the percentage of parasitized hosts out of the total number of hosts per tent, a similar generalized linear mixed model was used with the moment of testing included. In addition to an overall comparison of the four treatments, a test was performed for the contrast between the inexperienced and the average of the experienced parasitoids, all on the logit scale. One of the replicates was excluded from all analyses because the parasitoids did not fly out of the release tubes, most likely because of low temperatures, bringing the total number of replicates per treatment to eight. Inference was based on penalized quasi-likelihood (PQL) (Breslow and Clayton 1993). Quasi-Wald tests and approximate F-tests (Kenward and Roger 1997) applied to the approximate linear mixed model from the last step of the iterative re-weighted REML algorithm, were used. Calculations were performed using GenStat® 17th edition.

Results

Wind-tunnel experiment

The percentage of parasitoids that responded in the wind tunnel varied between 83% and 95% and was not affected by conditioning treatment (GLM, d.f. = 3, F = 2.34, P = 0.089). Inexperienced parasitoids preferred to land on plants infested with host plus non-host caterpillars over plants infested with hosts plus non-host aphids (approximate t-test, P = 0.007). Likewise, parasitoids with an oviposition experience on plants infested with hosts plus non-host caterpillars (caterpillar conditioning) also preferred this plant type (binomial test, P = 0.011). However, when parasitoids had experienced oviposition on plants infested with hosts plus aphids (aphid conditioning), or in combination with an oviposition on plants infested with host plus non-host caterpillars (dual conditioning), the distribution of preference shifted from host plus non-host caterpillar-infested plants to a no-preference situation (approximate t-test, resp. P = 0.561 and P = 0.504) (fig. 1). The percentage of parasitoids choosing the host plus non-host caterpillar-infested plant was highest for the inexperienced parasitoids compared to aphid- and dual-conditioned parasitoids. In addition, the percentage of parasitoids choosing the host plus non-host aphid-infested plant was higher for the aphid-conditioned parasitoids compared to the caterpillar-conditioned parasitoids (GLM, d.f. = 3, F = 4.07, P = 0.013) (fig. 1).

Field-tent experiment

Of the 10240 hosts transferred to the plants in the tents, 7517 hosts were recollected (73%). Out of the recollected, 2137 hosts were parasitized (28%). The moment in time of performing the separate replicates of the experiment influenced the percentage of hosts parasitized (GLMM, n.d.f. = 7, d.d.f = 41.7, P < 0.001), most likely because of variable weather conditions. Conditioning of parasitoids did not affect the number of host plus aphid-infested or host plus non-host caterpillar-infested plants with parasitized hosts (GLMM, n.d.f. = 3, d.d.f. = 60.0, P = 0.515) (fig. 2). Parasitized hosts were equally divided over host plus non-host aphid-infested and host plus non-host caterpillar-infested plants in all treatments (binomial test: all P > 0.2) (fig. 2). On average (± SE) 28.8 % (± 1.75) of the hosts in the tents were parasitized. This was not affected by conditioning in general (inexperienced vs conditioned, GLMM, n.d.f. = 1, d.d.f = 50.6, P = 0.155), neither by the different types of conditioning (GLMM, n.d.f. = 3, d.d.f = 52.0, P = 0.320).

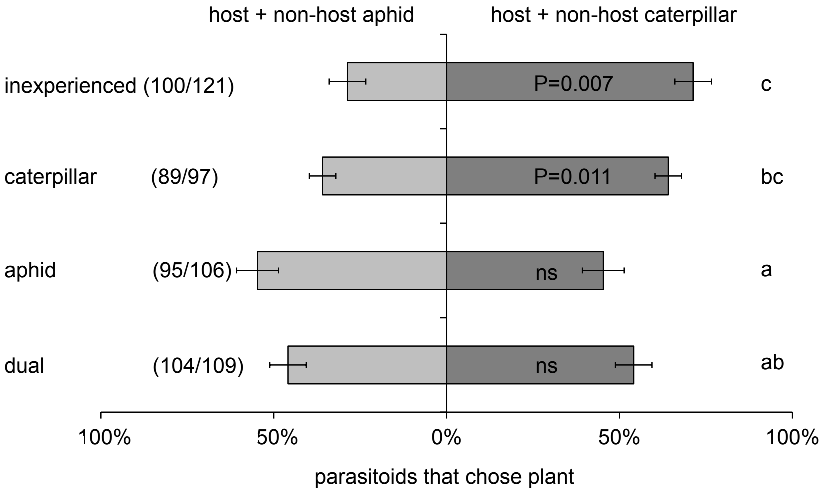

Figure 1. Mean percentages of parasitoids in the wind-tunnel experiment that chose to land on a plant infested with hosts plus non-host aphids (left) and mean percentage of parasitoids that chose to land on a plant infested with hosts plus non-host caterpillars (right). Error bars indicate the standard errors of different plant pairs (n = 13 or 14 plant pairs per conditioning treatment). Parasitoids were inexperienced (inexperienced), experienced with host oviposition on a plant infested with hosts plus non-host caterpillars (caterpillar), experienced with host oviposition on a plant infested with hosts plus non-host aphids (aphid) or experienced with host oviposition on a plant infested with host plus non-host caterpillar as well as on a plant infested with host plus non-host aphids (dual). Values in bars show results of approximate t-tests (for inexperienced, aphid and dual) or binomial tests (for caterpillar) for equal preference (α = 0.05, ns = not significant), dissimilar letters indicate significant differences between treatments based on logistic regression (P = 0.013) and least significant differences (α = 0.05). Values between brackets indicate the number of responding parasitoids/the number of parasitoids tested.

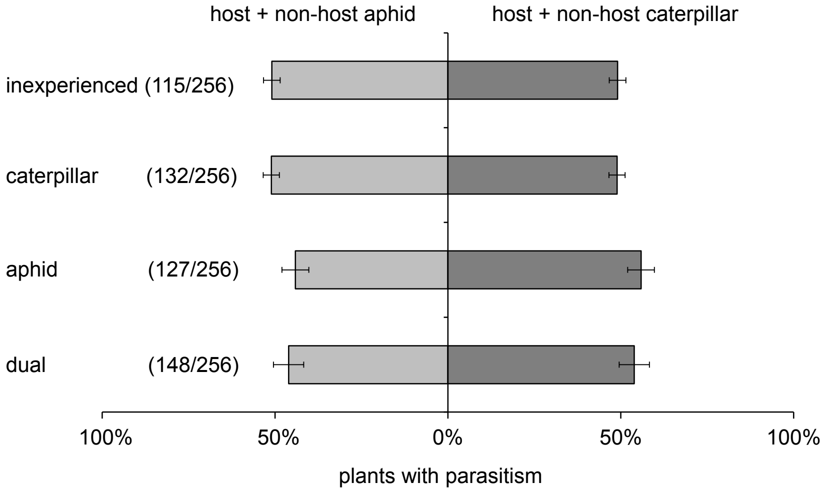

Figure 2. Mean percentages of plants with parasitized hosts in the field-tent experiment. Plants were infested by hosts plus non-hosts aphids (left) or hosts plus non-host caterpillars (right). Error bars indicate the standard errors of different tents (n = 16 tents per conditioning treatment). Parasitoids were inexperienced (inexperienced), experienced with host oviposition on a plant infested with hosts plus non-host caterpillars (caterpillar), experienced with host oviposition on a plant infested with hosts plus non-host aphids (aphid) or experienced with host oviposition on a plant infested with host plus non-host caterpillar as well as on a plant infested with host plus non-host aphids (dual). Based on generalized linear mixed models (α = 0.05) no significant differences were found between treatments. Based on binomial tests (α = 0.05) no significant differences were found between numbers of plants harbouring parasitized hosts of the two different plant infestation types. Values between brackets indicate the total number of plants with parasitized hosts/the number of plants with hosts present.

Discussion

In this study we showed that parasitoids learn to associate cues of non-host identity with the presence of hosts. In a laboratory setting, the landing preference of *C. glomerata* parasitoids shifted in the direction of plants infested by hosts plus non-host aphids if previously cues from non-host aphids were experienced in the presence of hosts. This learned association did, however, not translate into parasitization preferences in a field-tent situation. In the field setting, *C. glomerata* parasitized hosts on similar numbers of plants infested by hosts plus non-host caterpillars as on plants infested by hosts plus non-host aphids, irrespective of their previous experience.

It was hypothesized that parasitoids would prefer to land and to parasitize hosts on plants with the type of herbivore infestation that they previously experienced. When both of the plant infestation types were previously experienced, no landing preference or parasitization preference was expected. Inexperienced parasitoids were hypothesized to prefer host plus non-host caterpillar-induced plants. Supporting our hypothesis, inexperienced parasitoids preferred host plus non-host caterpillar-induced plants over host plus non-host aphid-induced plants in the wind-tunnel experiment. This preference shifted in the direction of host plus non-host aphid-induced plants when parasitoids experienced at least host oviposition on plants infested by hosts plus non-host aphids. The preference of inexperienced parasitoids for host plus non-host caterpillar-damaged plants could be caused by a quantitative difference in emitted volatiles related to caterpillar feeding, as a result of unequal numbers of caterpillars on the two plant infestation types (Geervliet et al. 1998a). However, previous research showed that differences in density of host and non-host caterpillars between two plants do not affect the preference of a parasitoid (Bukovinszky et al. 2012b). Quantitative differences could also have occurred because of changed emission rates of caterpillar-related volatile compounds due to co-infestation by phloem-feeding insects such as aphids (Rodriguez-Saona et al. 2003, Zhang et al. 2013, Li et al. 2015, Ponzio et al. 2015). Next to quantitative differences, also qualitative differences are hypothesized to occur between the volatile blends of plants dually attacked by aphids and caterpillars and plants attacked by only caterpillars (host and non-host) (Dicke et al. 2009). An attack by aphids can affect a plant’s response to an attack by caterpillars via crosstalk of the signal transduction pathways of the plant, which could lead to a qualitative difference in the volatile blend (Thaler et al. 2012, Kroes et al. 2015). In our study, parasitoids conditioned on host plus non-host aphid-infested plants might have experienced, while foraging in the tent, that hosts can also be found on plants emitting volatiles quantitatively and/or qualitatively different from their initially preferred plant infestation type. This new experience levelled out the preference for plants induced by only host caterpillars in the wind-tunnel experiment.

Only few previous studies showed that insects that can learn to associate cues with the presence of their host/food can accordingly show altered behaviour in (semi-)field situations (Lewis and Martin 1990, Papaj and Vet 1990, Hare et al. 1997, Raine and Chittka 2008, Zrelec et al. 2013, Janssen et al. 2014, Kruidhof et al. 2015). Although the landing preference of the parasitoid that frequently showed to be able to learn in the laboratory, *C. glomerata* (Geervliet et al. 1998a, Geervliet et al. 1998b, Bleeker et al. 2006, Smid et al. 2007, Kruidhof et al. 2012a, Kruidhof et al. 2012b), was indeed affected by conditioning in our wind-tunnel experiment, its parasitization preference in field-tent situations was not influenced by previous oviposition experience. An explanation for this mismatch between laboratory and field observations could be found in the incompleteness of the information that we offered to the parasitoids. In natural situations, parasitoids experience not only rewarding situations, e.g. being attracted by an odour followed by finding a host, but also unrewarding situations, e.g. being attracted by an odour followed by not finding hosts. By facing both rewarding and unrewarding situations, parasitoids get complete information about their environment (Vet et al. 1998). Vet and colleagues (1998) showed that when parasitoids are provided with complete information, the response to the rewarding situation increases. In our experiment, the conditioning of the parasitoids was incomplete for the complex situation the parasitoids faced in the field tents. If we would have given the parasitoids both a rewarding (e.g. oviposition on a host plus non-host caterpillar-infested plant) and an unrewarding experience (e.g. no oviposition on a host plus non-host aphid-infested plant), the parasitoids would have had complete information and the response to the 'rewarding plant' may have been stronger (Kruidhof et al. 2015).

Additionally, our field-tent set-up in which host caterpillars were feeding on all plants did not penalize parasitoids that landed on plants other than the plant infestation type they had learned to associate with host presence. Based on the results from our wind-tunnel experiment and on preference tests from literature (e.g. Geervliet et al. 1998a, 1998b and Kruidhof et al. 2012a, 2012b) it is expected that the preference of *C. glomerata* would not be 100% for either of the two plant infestation types. This would mean that an individual parasitoid in the field-tent experiment would mostly fly to its preferred plant, but sometimes to its non-preferred plant that harboured equal numbers of hosts as compared to the preferred plant. In the field-tent experiment the parasitoids, therefore, may have gained oviposition experience on the non-preferred plants in addition to the conditioned cue of host presence. After every oviposition experience on a non-preferred plant, the search image (temporary specialization on a single host type while ignoring alternatives (Ishii and Shimada 2010)) based on laboratory conditioning could have been gradually weakened, resulting in equal parasitism preference for both plant infestation types.

We speculate that in our field-tent experiment the incomplete information given to the parasitoids and the environment that was saturated with hosts, have played a major role in the observed absence of effects of previous oviposition experience on parasitization preference. Because the parasitoids were provided with incomplete information, they possibly made more 'mistakes' by landing on the plant treatment they were not conditioned on. An oviposition experience on this non-conditioned plant treatment added to the experience the parasitoids acquired in the laboratory, leading to a less specific search image. As a result, parasitized hosts were found on similar numbers of plants that were infested with hosts plus non-host caterpillars as on plants that were infested with hosts plus non-host aphids, irrespective of the experience the parasitoids received in the laboratory. In previous work, we found that parasitoids exploited aphid co-infested host patches faster than patches with non-host caterpillars by parasitizing more caterpillars per time unit (De Rijk et al. 2016b). Because of the absence of parasitization preferences, we also did not observe that parasitoids that had previously experienced oviposition on host plus non-host aphid infested plants showed an increased parasitization efficiency.

Our study shows that an initial associative learning experience may not translate into a long-term effect on parasitization preferences of parasitoids in habitats with abundant hosts. It remains to be tested if this is still the case when parasitoids are given complete information about the environment in which they will forage for hosts and how a match, or a mismatch of this experience with the foraging environment, affects their parasitization efficiency.

Acknowledgements

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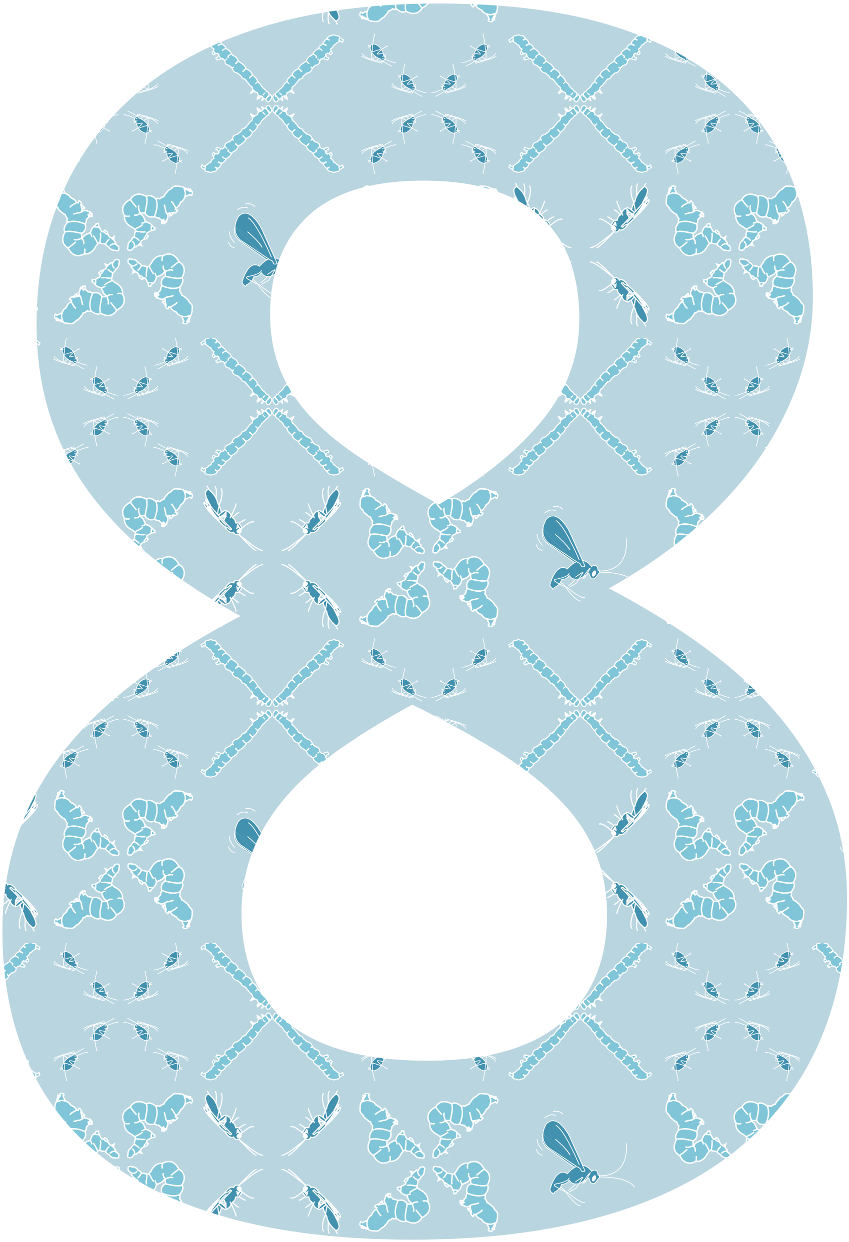

Chapter 8

General discussion

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While foraging in natural or agricultural environments, parasitoids face several hurdles that increase the complexity of the foraging process. This could be, for example, a diverse plant community (Gols et al. 2005, Bezemer et al. 2010), an area of no interest to cross (Nelson and Forbes 2014, Roitberg and Gillespie 2014), or the presence of herbivores that are not suitable for parasitoid larval development (De Rijk et al. 2013, Desurmont et al. 2014, Ponzio et al. 2014). The latter, so-called non-host herbivores, can interfere with the interaction between parasitoid and host on two levels. First, parasitoids use herbivore-induced plant volatiles (HIPVs) to locate plants on which hosts are feeding (Vet and Dicke 1992, Van Alphen et al. 2003, Arimura et al. 2011, Hare 2011, Stam et al. 2014). Feeding by non-host herbivores also induces plants to produce volatiles, which makes the environment of the parasitoid chemically complex (Dicke et al. 2009, Soler et al. 2012a, Zhang et al. 2013). Second, to locate hosts on a host-infested plant, parasitoids make use of host cues, e.g. chemicals in frass and silk (Van Alphen et al. 2003, Colazza et al. 2014). When host and non-host share a food plant, the infochemicals of both herbivores are present which creates a complex environment for the parasitoid at a small, on-plant scale (Takabayashi and Takahashi 1990, Bukovinszky et al. 2012b, Chabaane et al. 2015). Several characteristics of non-host herbivores can have an effect on the foraging process of parasitoids, either negatively or positively affecting parasitoid host location (De Rijk et al. 2013). In this thesis, I studied how four of these non-host characteristics affected the different foraging phases of *Cotesia glomerat*a when searching for its host *Pieris brassicae* on the food plant *Brassica oleracea* and how this influenced the foraging efficiency of the parasitoid. I additionally studied how *C. glomerata* parasitoids adjusted their foraging behaviour when they had previously experienced oviposition in a *P. brassicae* host that shared its food plant with a non-host. The results show that the presence of non-host herbivores can both positively and negatively influence plant-volatile-based and host-infochemical-based host location by *C. glomerata* in the study system. The total foraging efficiency can consequently be altered or remain unaffected. Figure 1 gives a schematic overview of the results described in this thesis. In this chapter I will discuss how the presence of non-host herbivores influences parasitoid individuals, parasitoid-host dynamics and parasitoids on a landscape level. Additionally, I will discuss the importance of studying the total foraging process instead of only fragments and how the knowledge gained about non-host interference could be used in biocontrol practices. Finally, I present the main conclusions of the thesis and suggestions for future research.

Overview

Non-host effects

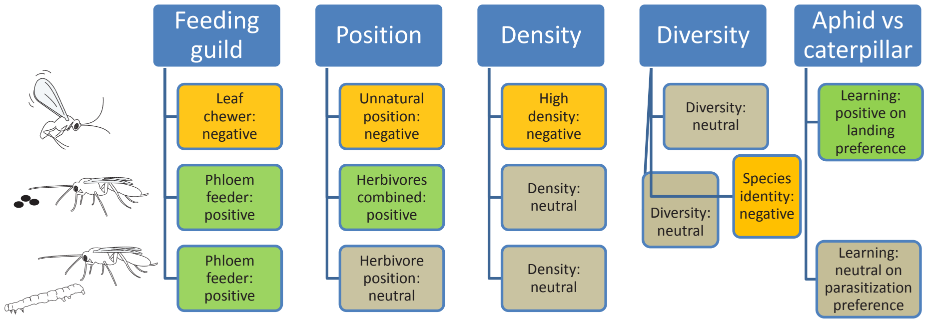

Figure 1. Schematic overview of the results of this thesis. Vertically, the text boxes show the conclusions from the separate experiments. Top: plant-volatile-based searching behaviour in wind tunnel experiments, middle: host-infochemical-based searching behaviour in on-plant experiments, bottom: total foraging process in field-tent experiments. Horizontally, the text boxes show the conclusions from the separate non-host traits tested. Feeding guild: results chapter 3, position: results chapter 4, density: results chapter 5, diversity: results chapter 6, aphid vs caterpillar: results chapter 7.

Non-host influence on individual parasitoid

Foraging process

Before determining the exact location of their host, parasitoids first select a plant on which their host could be feeding. The selection of the host-infested plant is differently affected in an environment with non-host leaf chewers compared with a non-host phloem-feeder-rich environment. The presence of non-host leaf chewers had a negative influence on the preference of *C. glomerata* for *P. brassicae*-infested *B. oleracea* plants as compared to the influence of non-host phloem feeders or the absence of non-hosts (chapter 3; feeding guild). The position of host and non-host caterpillars on the plant also influenced the plant-volatile-based searching behaviour of the parasitoid. In an environment in which the host was feeding from an old leaf and the non-host from a young leaf (an uncommon situation in nature), the parasitoid landed more often on the non-host infested leaf compared with the preference for this leaf in an environment with the opposite, natural distribution of herbivores (chapter 4; position). Finally, a high density of non-host caterpillars in the environment also negatively influenced the selection of host-infested plants by *C. glomerata* when compared with an environment free of non-hosts (chapter 5; density). However, the majority (chapters 3 & 5) or about 50% (chapter 4) of parasitoid individuals still landed, at first choice, on the plant/leaf on which the host was feeding. Non-host herbivores, therefore, can only partly distract *C. glomerata* during the plant-volatile-based foraging phase when *P. brassicae* host-infested plants/leaves are located.

After landing on the plant/leaf of choice, parasitoids use host infochemicals to exactly locate the host herbivore. Compared with the absence of non-hosts on the plant, the presence of non-host phloem feeders with the host *P. brassicae* on the plant positively influenced the host-infochemical-based foraging behaviour of *C. glomerata* resulting in a reduced residence time; yet the number of hosts parasitized was not affected (chapter 3; feeding guild). The position of herbivores on the plant also influenced the host-infochemical-based foraging of the parasitoid. On plants with a shared feeding location of host and non-host herbivores (both on an old leaf, or both on a young leaf), more hosts were parasitized while the residence time was unaffected, compared with the number of parasitized hosts on plants with separated feeding locations of hosts and non-host (one on an old leaf, the other on a young leaf) (chapter 4; position). Finally, when the identity of non-host caterpillars was varied in the context of non-host species diversity, the non-host *Trichoplusia ni* negatively affected the on-plant behaviour of the parasitoid by a reduced residence time resulting in a lower number of hosts that was parasitized (chapter 6; diversity). Therefore, non-host herbivores can both positively and negatively affect the behaviour of *C. glomerata* during the host-infochemical-based foraging phase when *P. brassicae* hosts are located on a plant.

Adding up the effects of a certain non-host characteristic on the first and the second foraging phase of *C. glomerata*, does not necessarily result in the total effect on the foraging efficiency of the parasitoid. The neutral influence of phloem feeders on the first phase of foraging and their positive influence on the second phase did translate into a positively affected foraging efficiency of *C. glomerata* in an environment with non-host phloem feeders (chapter 3; feeding guild). However, the negative effect of an environment with non-host leaf chewers on the first phase and its neutral effect on the second phase of foraging, resulted in a neutral effect of an environment with leaf chewers on the total foraging efficiency of *C. glomerata* (chapter 3; feeding guild). Likewise, the negative effects of an environment with an uncommon distribution of herbivores over the plant (chapter 4; position) or the negative effects of an environment with a high non-host density (chapter 5; density) during the first phase of foraging by *C. glomerata*, did not translate into any influences on the parasitoid's foraging efficiency in these environments (chapters 4 & 5). Therefore, even if *C. glomerata* parasitoids are somehow distracted by non-hosts and consequently make altered decisions during the first foraging phase, the remaining part of the process of foraging for *P. brassicae* can show enough flexibility to compensate for the altered behaviour. Systems with other combinations of species could show a similar flexibility (Chabaane et al. 2015) or may show a different response toward the presence of non-host herbivores (Bukovinszky et al. 2012b). Vos et al. (2001) suggested that non-hosts can interrupt the utilization of herbivore-induced plant volatiles that are used to solve the reliability-detectability problem that foraging parasitoids face (Vet and Dicke 1992). Based on my findings, I conclude that this hypothesis does not hold for the parasitoid-host complex *C. glomerata - P. brassicae*. The impact of non-host presence on parasitoid fitness, however, could still be relevant. The small amount of time wasted during separate foraging processes did not affect the outcome of foraging. However, over the total life span of the parasitoid, the total time wasted could add up to a significant waste of time that might affect the fitness of the parasitoid (Vos et al. 2001). Additionally, insertion of a large number of eggs in individual hosts as a response to non-host presence (chapters 3 & 5) could result in egg limitation later in life (Ikawa and Okabe 1985).

Performance of parasitoids

Non-host herbivores can interfere with parasitoid foraging on different levels and into different directions. By sharing a food plant with hosts, non-host herbivores can also indirectly have an impact on the development of the parasitoid larvae inside the host. A positive effect of aphids on the foraging behaviour of *C. glomerata* (chapter 3) could have resulted in, and have been directed by, a positive influence of aphids on parasitoid performance. Such a situation was shown for non-host aphids that positively influenced the development of parasitoids through their effect on the caterpillar host via altered plant defences (Soler et al. 2012a, Li et al. 2014). In a similar way, non-host caterpillars influenced parasitoid development, but in a negative sense (Bukovinszky et al. 2012a). Likewise, the preference of inexperienced parasitoids for herbivore-infested young leaves as opposed to infested old leaves (chapter 4), could have resulted in, and have been directed by, a better performance of the parasitoid. The specialist host caterpillar *Pieris* *brassicae* prefers to feed on young, well-defended leaves over old, less defended leaves of brassicaceous plants (Gutbrodt et al. 2012). Because the young leaves have a higher nutritional value than the older ones, the performance of a specialist caterpillar such as *P. brassicae* feeding on these young leaves and accordingly that of the parasitoid larvae inside this host, is expected to be better compared with the performance when feeding on old leaves (Smallegange et al. 2007, Gutbrodt et al. 2012). The influence of non-host herbivore traits on parasitoid performance could be an underlying factor driving non-host effects on parasitoid foraging behaviour.

Possible mechanisms of non-host influence

Parasitoid foraging behaviour can be affected by non-host herbivore presence, which may be driven by a changing performance of parasitoid offspring. However, the exact mechanisms behind the observed non-host effects on parasitoid behaviour are not identified in this thesis. Here, I speculate about two possible mechanisms.

Plant defences toward herbivore attack are generally expressed systemically (Heil and Ton 2008, Shah 2009, Gutbrodt et al. 2011, Marti et al. 2013). The distribution of the systemic defence can however be determined by plant architecture (Orians 2005, Utsumi et al. 2010) and time elapsed since induction (Gutbrodt et al. 2011), whereas composition of emitted volatiles can depend on leaf age (Takabayashi et al. 1994). *Cotesia glomerata* landed half of the time on host-infested old leaves and the other half on non-host-infested young leaves (unnatural herbivore distribution, chapter 4). The reason why the parasitoids did not always land on the young leaves where their hosts were expected to feed, may lay in a possible difference in the cues emitted by infested leaves of a plant (Orians 2005). Young leaves infested with herbivores may have emitted a volatile blend different from old infested leaves. This would have been in accordance with the landing preference of parasitoids on plants with differently distributed herbivores (chapter 4); combined feeding of herbivores on one leaf would have induced the emission of one blend and consequently attracted the parasitoids to this one leaf; separated feeding in a natural position of herbivores (host on young leaf, non-host on old leaf) would have induced the emission of two blends of which the host-induced blend directed the parasitoids to the young leaf that was also innately preferred; separated feeding in an unnatural position (host on old leaf, non-host on young leaf), on the other hand, would have resulted in the emission of a host-induced blend that directed the parasitoids to the old leaf. In combination with an innate preference for young leaves, this could have resulted in equal preferences for both leaves. Even though the exact mechanism is unknown, the hypothesis that parasitoids are able to make a distinction between differently infested leaves is supported by the literature. Geervliet et al. (1997b) showed that *C. glomerata* and *Cotesia rubecula* parasitoids both preferred to land on leaves infested by their preferred host species over leaves of the same plant infested by a less preferred host species (Geervliet et al. 1997b). Additionally, *C. glomerata* responded differently to volatiles emitted by plants when *P. brassicae* and non-host aphids were feeding on the same leaf or from separate leaves of a plant (Ponzio et al. 2014, 2015). In response to attack of one leaf by both herbivores, the parasitoid preferred either dually infested plants over host-infested plants or host-infested plants over dually infested plants, depending on aphid density (Ponzio et al. 2015). Whereas the parasitoid equally preferred host-infested and dually infested plants when host and non-host were feeding on separate leaves (Ponzio et al. 2014). Plant defence responses may depend on spatial distance between attackers (Orians 2005), e.g. cross-talk between signal-transduction pathways may become more apparent when both attackers feed on the same leaf (Ponzio et al. 2014).

After locating a host-infested plant and the host-infested leaf of that plant, parasitoids forage for hosts on the leaf by using herbivore-derived infochemicals. This behaviour can be disturbed by non-hosts with a specific species identity (chapter 6). The non-host *T. ni* had a negative impact on foraging *C. glomerata* (chapter 6), possibly because its defence behaviour in response to parasitoid attack was more aggressive from that of the other tested non-host species (Gross 1993). The parasitoid spent more time on attacking *T. ni* compared with the time spent on attacking most of the other tested non-hosts (chapter 6). If indeed the defence method of *T. ni* was uncommon, the increased exposure to this defence may have led to the relatively low residence time of the parasitoid. However, the question remains why the parasitoid spent more time on attacking *T. ni* (and *A. gamma*) compared with the time spent on attacking other non-hosts. The answer to this question might be found in the way the parasitoid perceives and interprets cues from herbivores on the plant. To locate their host at close range, parasitoids could use host-associated infochemicals (Colazza et al. 2014) and plant volatiles (Uefune et al. 2012), they could drum the substrate with their antennae or probe the substrate with their ovipositor (Van Alphen et al. 2003). *Cotesia glomerata* in particular needs a combination of olfactory, visual and physical cues to make foraging decisions (Geervliet et al. 1997b). When cues from a specific non-host closely resemble one or more of these host-related cues, the parasitoid might be misled and mistake the non-host for a host (Takabayashi and Takahashi

1990). Here, this would have meant that *T. ni*-associated cues misled *C. glomerata,* resulting in non-host attack. If indeed *T. ni* responded in a deviating way to this attack, the parasitoid might have reacted to this by leaving the plant sooner than it did in response to other non-host species. It would be interesting to specifically study how non-host cues interfere with close-range host cues and how this could lead parasitoids to accept non-host herbivores as if it were suitable hosts.

Non-host influence on parasitoid-host dynamics

Through the effect on parasitoid behaviour, the presence of non-host herbivores might influence parasitoid-host dynamics. Previous research showed that the presence of non-hosts can be essential for the persistence of herbivore and parasitoid communities (Vos et al. 2001, Van Veen et al. 2005, Van Veen et al. 2009, Fan et al. 2010). For example, by the creation of enemy-free space for hosts (Shiojiri et al. 2000b). Also in other systems, non-prey species could be of importance for population persistence of predator and prey (Kratina et al. 2007, Hammill et al. 2015).

Here, the presence of non-hosts might have created environments in which hosts became associational susceptible or associational resistant to parasitoids (chapter 3). For example, parasitoids foraged more efficiently in environments with non-host aphids, which made the host caterpillar more susceptible to parasitism (chapter 3). In this case, non-host presence could negatively affect the host population, which in turn could have its effects on the parasitoid population. Assuming that non-hosts could have the ability to affect parasitoid populations (Vos et al. 2001, Van Veen et al. 2005, Van Veen et al. 2009, Fan et al. 2010), non-host species identity is one of the factors that is expected to be of importance (chapter 6). The introduction of new herbivore species, for example due to migration in response to changing environmental conditions, might therefore play a role in parasitoid-host dynamics. Parasitoid behaviour could directly be affected by these newly introduced non-hosts, whereas indirectly, new non-host species could have an impact on parasitoid-host dynamics through their influences on the interaction web (Desurmont et al. 2014, Chabaane et al. 2015).

Non-host influence on landscape level

The influence of non-host herbivores on parasitoid foraging might turn out differently in different landscapes. Both the diversity of the landscape on plant level and the distribution of herbivores in the landscape could determine the outcome of non-host interference.

Monocultures versus diverse landscapes

The influence of non-hosts on parasitoid foraging behaviour was hypothesized to be different in agricultural landscapes with a low diversity compared to diverse, natural environments (De Rijk et al. 2013). In diverse environments the diversity of HIPVs is high. Volatile plumes, consisting of odour packets in clean air, disperse depending on habitat characteristics (e.g. open area or forest) and on atmospheric conditions (Murlis et al. 1992, Cardé and Willis 2008). In environments with a diverse mix of odours, parasitoids are expected to use general herbivore-induced odour profiles of their host's food plants, instead of specific host induced volatile cues. Because general cues are used, non-hosts would not interfere to a large extent with this first phase of foraging. However, after landing on a plant that is attacked by herbivores but not necessarily by hosts, the parasitoid is expected to rely heavily on direct host cues to locate the host. Non-hosts would therefore interfere to a large extent on this second phase of foraging. Contrary, in less diverse environments such as monocultures, the diversity of HIPVs is low. Parasitoids consequently could focus on plant volatiles induced by their host herbivores. Because specific HIPVs are used, non-hosts could interfere with the volatile-mediated searching of the parasitoid by affecting these specific plant volatiles. However, after landing on the plant that is most likely infested with hosts, the parasitoid is not expected to rely heavily on direct host cues to locate the host on the plant. Non-hosts would, therefore, not interfere to a large extent with this second phase of foraging (De Rijk et al. 2013). This thesis supports the hypothesis regarding the low diverse, monoculture situations. Non-hosts indeed negatively affected the plant-volatile-based foraging behaviour of the parasitoid in three types of herbivore communities (chapters 3, 4 & 5, fig. 1). Non-hosts, however, as well influenced the host-infochemical-based searching on the plant, though two of these effects were positive (chapter 3 & 4), while only one was negative (chapter 6) (fig. 1). In monoculture situations in which parasitoids do not rely strongly on close-range host cues, a positive effect of non-hosts could for example occur when non-host cues lead the parasitoid toward the host. Non-hosts could, on the other hand, obstruct the way to the host (Hauzy et al. 2010) or occupying time of the parasitoid when it wrongfully attacks non-hosts and so negatively influence the host-infochemical-based foraging on the plant (Bukovinszky et al. 2012b, Chabaane et al. 2015). To extend the original hypothesis as in De Rijk et al. (2013): in monocultures, non-hosts are expected to have a negative influence on plant-volatile-based searching of parasitoids. By altering the combination of herbivore-related cues present on the plant, non-host presence could be expected to have a positive or a negative influence on host-infochemical-based searching.

Solitary hosts versus gregarious hosts

The influence of non-host herbivores on parasitoid foraging was hypothesized to depend on the distribution of host herbivores in the landscape (De Rijk et al. 2013). Parasitoids searching for solitarily distributed hosts would be time-limited, because hosts have to be found at multiple locations. Handling non-host (cues) would cost valuable time that cannot be spent on finding hosts. On the other hand, parasitoids in search for hosts with a clumped distribution would be egg-limited, because many eggs can be inserted in one group of hosts. The time wasted by these egg-limited parasitoids on handling non-host (cues) will not affect their fitness (De Rijk et al. 2013). The host studied in this thesis, *P. brassicae*, has a clumped distribution. Its parasitoid, *C. glomerata*, is egg-limited when foraging for this host and is not expected to suffer large fitness consequences from wasting time on non-hosts or their cues. The data presented in this thesis support the hypothesis. The parasitoid was observed to spend time on attacking non-hosts while foraging on the plant (chapters 4, 5 & 6) and this indeed did not negatively affect the efficiency of the parasitoid. However, besides wasting time, parasitoids could also waste their eggs by inserting them in non-hosts (Chabaane et al. 2015). In this way, non-hosts could affect the fitness of parasitoids searching for clumped distributed hosts. *Cotesia glomerata* indeed oviposited in non-hosts, though this occurred by far less frequently than ovipositions in hosts (chapters 3, 4, 5 and 6). The number of eggs that was inserted in the non-hosts was not systematically counted, but occasionally observed to be a few per parasitized non-host. Based on these observations and a previous study (Bukovinszky et al. 2012b), it seems that the number of eggs inserted in non-hosts in this study system was low. Chabaane et al. (2015), however, observed that *C. glomerata* inserted as many eggs in a non-host (*S. littoralis*) as in its host (*P. brassicae*). To extend the original hypothesis as in De Rijk et al. (2013): the addition of non-host herbivores in a landscape with host herbivores with a clumped distribution, is not expected to affect parasitoid fitness regarding time-limitations. However, regarding egg-limitations, parasitoid fitness is expected either to be negatively or not to be affected by non-host herbivores.

The importance of studying the total foraging processes

The study of parasitoid foraging behaviour can be performed by using different experimental set-ups. The foraging process could be observed as a whole or as separate phases and experiments could be performed in a laboratory or a field setting. This thesis shows that observed effects of individual non-host characteristics on parasitoid behaviour do not necessarily occur in all phases of parasitoid foraging (e.g. ‘density’ in fig. 1). It also shows that conclusions from the two phases of foraging are not necessarily additive, i.e. to predict effects on the total foraging efficiency, the separate conclusions of both phases cannot be simple added up (e.g. for ‘position’ in fig. 1). To be able to draw conclusions on parasitoid foraging efficiency it is, therefore, important to study all phases of foraging, preferably in one experiment that covers the whole process. To explain the different outcomes of the separate experiments in this thesis, I discuss three possible arguments. First, the two tested phases of foraging are different in temporal scales and for that reason the relative value given to the two phases should not be equal. During the first foraging phase parasitoids decide on which plant/leaf to land. Although it is far from a natural situation, in the wind-tunnel experiments the parasitoids made this decision within five minutes. During the second foraging phase parasitoids make numerous decisions while foraging on the plant. In the on-plant experiments, the parasitoids spent on average about 45 minutes on plants infested with host herbivores. Obviously, decisions made during the first phase of the foraging process determine the environment of the second phase. Choices influenced by non-host presence made during the first phase, therefore, can be argued to have a high impact on the efficiency during subsequent foraging. If parasitoids chose for a plant of low quality during the first phase, time might be wasted during the second phase. According to this argumentation, effects of non-host presence would not depend on the endurance of the impact. However, the time spent by *C. glomerata* searching on plants infested with only non-hosts (chapter 3) or those infested with hosts and a specific non-host species (chapter 6), was short. In fact, the residence time on highly rewarding plants was also short (chapter 3). A less profitable decision made during plant-volatile-based searching may be corrected by a shortened host-infochemical-based searching, after which plant-volatile-based searching starts again. The transfer of non-host effects from one foraging phase to the other might, therefore, not be highly significant. The endurance of non-host interference on the separate phases of foraging is unequal and should not be valued equally. It will, however, be complex to determine how the results of both types of experiments should be rated. A combination with a study of the total foraging efficiency will, therefore, be inevitable.

Second, circumstances in a laboratory are deviating from those in nature and for that reason results from laboratory experiments should not be directly extrapolated to natural situations (Hunter 2002, Heimpel and Casas 2008). The parasitoids in the wind-tunnel experiments in this thesis were released 70 cm from the plants they were supposed to land on and they were given a choice between two plants or between the leaves of one plant. This is a reliable method to study the process of plant selection (Geervliet et al. 2000), but it is not resembling nature in which the parasitoid would have to choose between several plant individuals and species, from several distances. Laboratory experiments, therefore, can elucidate (part of) the foraging process, while only more realistic field experiments could show the actual effects of the studied factors on the total foraging efficiency (Heimpel and Casas 2008). Previous studies made the comparison between parasitoid preferences in laboratory and (semi-)field experiments (Janssen 1999, Kappers et al. 2005, Halitschke et al. 2008) or combined results from laboratory and (semi-)field to draw conclusions on parasitoid foraging efficiency (Bukovinszky et al. 2012b, Chabaane et al. 2015). The field-tent experiment used in this thesis is an example of a more realistic field experiment, however, it could be improved by including e.g. multiple plant species, a more complex insect community and a host distribution that is more close to the natural situation.

Third, in the laboratory experiments presented in this thesis, inexperienced parasitoids were tested (except for chapter 7), while the parasitoids in the field-tent experiment were given the time to learn during the experiment. For that reason, results from laboratory and field cannot directly be compared. The field-tent experiments lasted for at least 24 hours. In this time frame *C. glomerata* is able to learn to associate cues in the environment with the presence of hosts (Smid et al. 2007). Acquired experience can change the foraging behaviour of the parasitoid and, thereby, foraging outcomes (Geervliet et al. 1998b). However, a single spaced learning experience did not change the behaviour of *C. glomerata* in a field tent set-up (chapter 7). Yet, these parasitoids were given incomplete information about their environment, that was probably completed while foraging. The parasitoids with different previous learning experiences foraged in equal environments and thereby gained the same information, resulting in equal foraging outcomes. In contrast, parasitoids in the laboratory experiments were not given the opportunity to make use of experiences due to the design of the experiments. Laboratory experiments such as used in this thesis are, therefore, suitable for studying behavioural responses of parasitoids in isolated situations, whereas long lasting experiments, preferably in a field set-up, are preferred to study parasitoid behaviour over time.

The relevance of non-host herbivores for biological control

Parasitoids are key biological control agents widely used in greenhouses and open agricultural fields. A good understanding of their behaviour can contribute to further optimization of biocontrol methods, leading to raised successes of biological control programs (Giunti et al. 2015). The behaviour of the parasitoid *C. glomerata* is altered in a positive sense by the presence of non-host aphids (chapter 3) and the parasitoid is able to learn to associate aphid(-induced) cues with the presence of the host *P. brassicae* (chapter 7). From a biocontrol perspective, it might be worthwhile to give parasitoids an experience with such an advantageous non-host prior to parasitoid release (Benelli et al. 2014, Giunti et al. 2015), provided that this non-host is abundantly present in the greenhouse or agricultural field and correlates with host presence. An experience with non-hosts might also be profitable when this increases the foraging efficiency of parasitoids of which the host is 'hidden' in a multi-herbivore environment. Depending on the species of the biocontrol agent, experience might be given by using olfactory or visual cues, with or without oviposition of hosts and during adult or immature stage (Giunti et al. 2015). After their release, parasitoids most likely will experience new combinations of herbivores attacking the crop, which could weaken the initially learned preference (chapter 7; Giunti et al. 2015). Providing the parasitoids with complete information about their environment could strengthen the preference for the target infestation type (Vet et al. 1998). The implementation of complete information supply to biocontrol agents could, therefore, be worthwhile. However, an efficient information supply method should be developed which is expected to be time consuming. One should, for example, consider if biocontrol agents should be given the learning experience before or after shipping from supplier to agricultural field, or how the specific circumstances of each individual agricultural field should be implemented in the training of mass-reared biocontrol agents (personal communication M. Kruidhof). For this reason, the costs and benefits of such a method should be well considered.

Conclusions and future perspectives

Herbivores that are not suitable for parasitoid larval development are generally present in environments in which parasitoids search for hosts. The extent to which these non-hosts have an impact on the searching behaviour of parasitoids is dependent on non-host characteristics and might differ between parasitoid-host communities. Based on this thesis, I conclude that the foraging efficiency of the parasitoid *C. glomerata* when searching for its host *P. brassicae* is not strongly affected by non-host herbivore presence. Here, I list additional conclusions and future perspectives.

Non-host herbivores do not interrupt the use of herbivore-induced plant volatiles by *C. glomerata* foraging for *P. brassicae*, that is used to solve the reliability-detectability problem that parasitoids face during the foraging process. Non-hosts in some cases distracted parasitoids, but this was not consequently observed throughout the entire foraging process. Most likely, the time wasted because of the distraction by non-host herbivore presence was compensated for during the remaining phases of foraging. However, the impact of non-hosts on parasitoid fitness remains unstudied.

All phases of the foraging process should be considered in foraging-behaviour studies. Laboratory studies similar to the ones used in this thesis are highly suitable for detailed observation of separate parts of the foraging behaviour. These studies should, therefore, not be criticized for their value regarding gaining detailed knowledge on foraging mechanisms. However, the extrapolation of information about the separate foraging parts to the entire foraging process should be performed carefully. Translating laboratory findings to conclusions about natural situations should also be done with care. All direct and indirect interactions, either trait-mediated or density-mediated, together make up an interaction web. Some of these interactions are obvious and could be included in a controlled experimental environment, whereas other interactions are overlooked and will be absent from such controlled experiments. It is, therefore, preferred to use close-to-nature experiments that cover the entire foraging process in one go. Using complex environments with e.g. different plant species and multiple non-host factors might reveal if non-host effects as seen in the laboratory stay of equal magnitude or are reduced in natural environments.

Parasitoids appear to be able to make a distinction not only between differentially infested plants, but also between differentially infested leaves of a single plant. A combination of research into the systemic responses of plants and research into parasitoid responses to differentially infested leaves might give more insight in the interaction between both these components of the interaction web. If plants give detailed cues that inform parasitoids about the exact location of their host, the interrupting effect of non-hosts might even be weaker than shown in this thesis.

Parasitoids can learn to associate non-host cues with the presence of hosts and alter their foraging behaviour accordingly. Field studies are needed to unravel if previous experience with non-host cues is used by parasitoids to alter succeeding foraging behaviour in a multi-herbivore community. If so, experience with non-host cues could possibly decrease the initial effects of non-host presence on parasitoid foraging behaviour and thereby levelling out the distraction by non-hosts. This information could potentially be used in biocontrol programs to prepare parasitoids for the environment they will face after release.

Studies on animal foraging do not just elucidate on the behaviour of the specific forager, but can also give insight in population dynamics and community-wide effects (Lotka 1925, Volterra 1928, Utsumi et al. 2010). By giving a detailed overview of non-host effects on the foraging behaviour of a parasitoid, this thesis contributes to the fundamental knowledge of insect foraging in a multi-herbivore context and thereby extends our understanding of the dynamics between insects and their natural environment.

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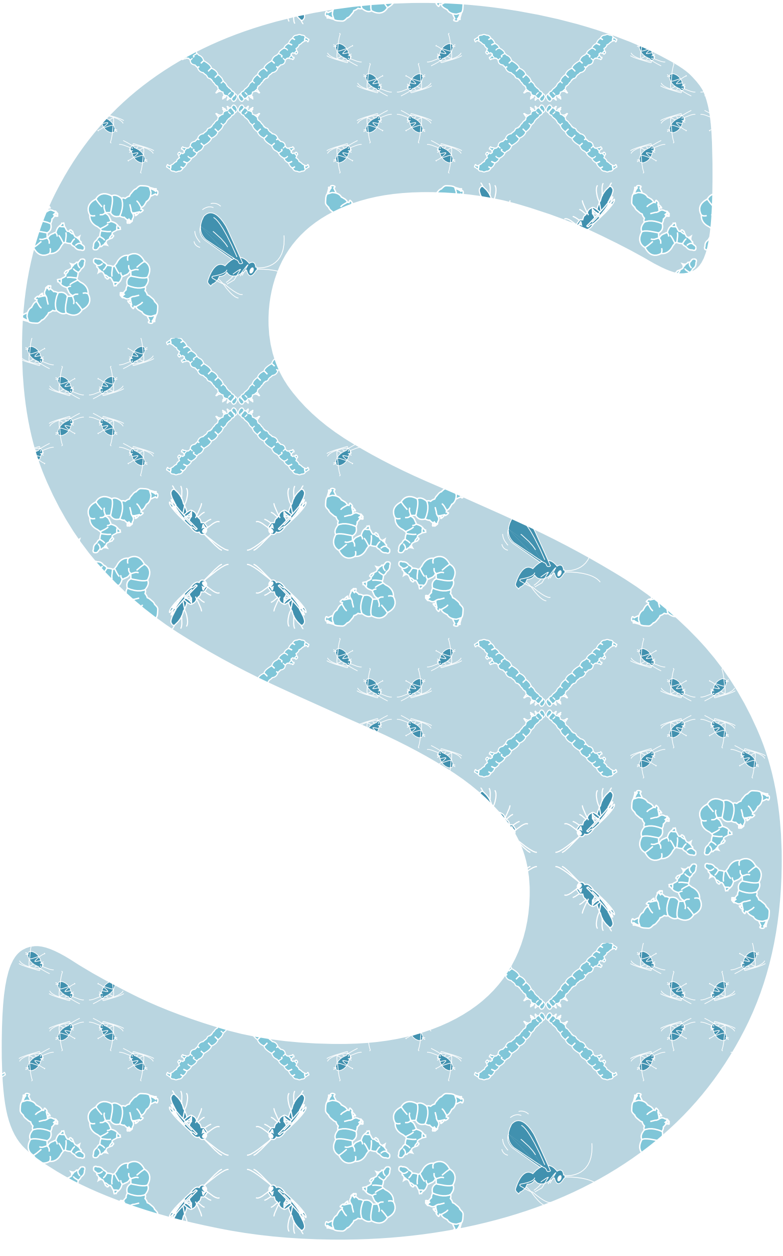


Summary

Parasitic wasps, or parasitoids, lay their eggs in or on other organisms. These organisms function as a host for the parasitoid's offspring, eventually resulting in the death of the host. The host-foraging process of parasitoids that search for herbivorous hosts can be complex when disturbing factors are present in the environment. One of these factors is the presence of herbivores that are not suitable for the development of parasitoid offspring, so-called non-host herbivores. Since individual plants are generally attacked by multiple herbivore species, parasitoids are confronted with the presence of non-hosts in the vicinity of their hosts. Non-hosts can interfere with the interaction between parasitoid and host during the foraging process at two levels. First, parasitoids forage for host-infested plants by making use of plant volatiles induced by the attacking host as infochemicals. Non-hosts also induce the production of plant volatiles, which makes the environment chemically complex. Second, after landing on a plant, parasitoids utilize host-produced infochemicals to precisely locate the host on the plant (e.g. chemicals in frass and silk). Also on this small scale, the presence of non-hosts increases the complexity of the environment by the production of (other) infochemicals.

Aim and set-up

The aim of this thesis was to study the impact of non-host presence on the parasitoid-host-food plant complex of the parasitoid *Cotesia glomerata* with its host caterpillar *Pieris brassicae* and a monoculture of the cultivated plant *Brassica oleracea*. To study the influence of non-hosts on the plant-volatile-based searching behaviour of the parasitoid, a wind-tunnel set-up in the laboratory was used. In this set-up, the parasitoids were given a choice between two plants or between the leaves of one plant. The plant/leaf on which the parasitoid landed was considered the preferred plant/leaf. A second laboratory set-up was used to study the influence of non-host herbivores on the host-infochemical-based searching behaviour of the parasitoid. In this on-plant experiment, the behaviour of the parasitoid was observed after landing on the plant. Using observational software, detailed observations were made for a maximum of one hour. The influence of non-hosts on the combination of plant-volatile-based and host-infochemical-based searching, i.e. the total foraging efficiency of the parasitoid, was investigated using an outdoor tent set-up in an agricultural field. In each tent (l x w x h: 3m x 4m x 2m, mesh size: 0.6 mm) 16 plants were infested with specific combinations of herbivores (depending on the treatment) and parasitoids were released. After one to three days (depending on research question) the number of parasitized hosts and the number of eggs per parasitized host were counted.

Effects of non-host traits on parasitoid foraging behaviour

Single non-host traits of several non-host species were systematically varied. First, a comparison was made between the influence of non-hosts with piercing-sucking and biting-chewing feeding habits. The results show that the feeding guild of non-host herbivores influenced the foraging behaviour of *C. glomerata*. Leaf-chewing non-hosts negatively impacted the plant-volatile-based searching behaviour of the parasitoid, whereas phloem-feeding non-hosts positively impacted the host-infochemical-based searching. The resulting host-finding efficiency was in general positively affected by phloem-feeding non-hosts.

Second, the position of host and non-host herbivores on the plant was varied. Host caterpillars were positioned on young leaves and non-host caterpillars on old leaves, which was a herbivore distribution as expected to be found in nature. Herbivores were also positioned in the reversed order or together on either the young or the old leaf. The different positions of hosts and non-hosts on the plant affected the plant-volatile-based and the host-infochemical-based foraging behaviour of the parasitoid, but not the host-finding efficiency. An unnatural distribution of herbivores over the plant (host feeding on old leaf, non-host feeding on young leaf), negatively affected the choice of the parasitoid for a leaf to land on, i.e. the parasitoid more often landed on the non-host infested leaf. Combined feeding by the host and non-host on one leaf, positively affected the number of hosts parasitized on that plant compared with the number of hosts parasitized when herbivores were separated. However, the parasitoid was able to compensate for these effects as a result of which the foraging efficiency was unaltered.

Third, the influence of non-host density on the behaviour of the parasitoid was studied by infesting the plants with either 10 or 50 non-host caterpillars. The density of non-hosts did influence the plant-volatile-based searching of the parasitoid. A high non-host density negatively affected parasitoid preference for host-infested plants. However, the host-infochemical-based foraging and the total foraging efficiency remained unaffected.

Fourth, it was investigated if an increasing non-host species diversity would have an effect on the foraging behaviour of the parasitoid and if species identity played a role in this context. Parasitoid behaviour was observed in laboratory environments with different levels of non-host diversity, where hosts were complemented with non-host herbivores from one to four species. Rather than the species diversity, the species identity of non-host herbivores had an influence on parasitoid host-infochemical-based searching. One of the tested non-host species, *Trichoplusia ni*, negatively affected the behaviour of the parasitoid when searching on the plant. However, neither non-host species identity nor diversity affected plant-volatile-based searching of the parasitoid.

Conditioning of parasitoids

Besides studying the influence of the feeding guild of non-hosts, their position on the plant, their density and species diversity and identity, this thesis investigated if the parasitoid *C. glomerata* could learn to associate non-host cues with the presence of hosts and if the parasitoid changed the parasitization preference accordingly. The parasitoids were given experiences with either hosts and non-host caterpillars, hosts and non-host aphids, or both combinations. After a day, the parasitoids were tested for their preference for a plant to land on in the wind-tunnel set-up or for their preference for plants to parasitize hosts on in the outdoor-tent set-up. *Cotesia glomerata* associated plant cues induced by simultaneous feeding of hosts and non-hosts with the presence of the host. In a wind-tunnel set-up, the parasitoid showed an altered landing preference for infested plants according to the learned cues. However, in an outdoor tent set-up, the parasitoid did not show an altered parasitization preference.

Discussion of experimental results

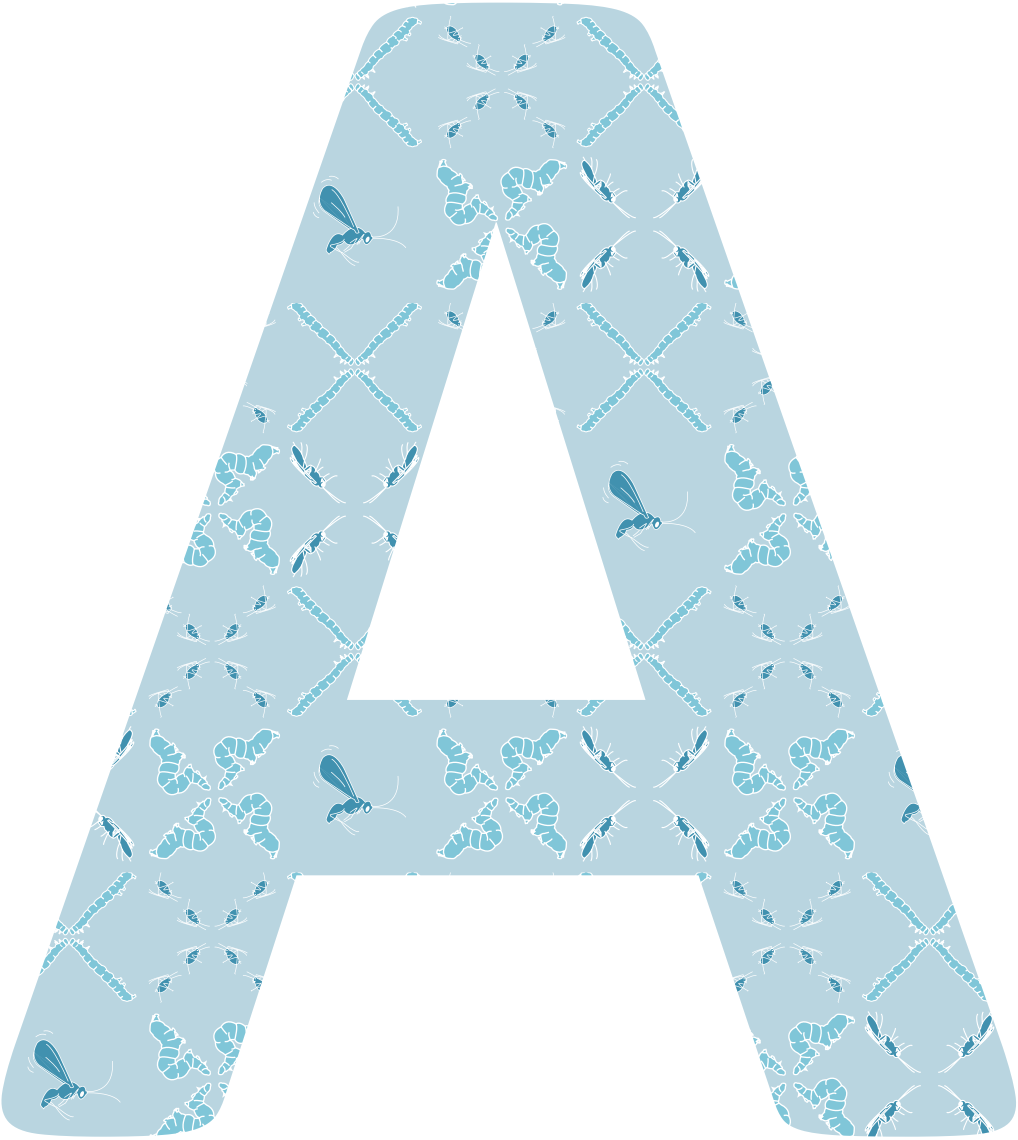
The results of this thesis show that non-host herbivore traits can affect the different phases of foraging by *C. glomerata* either positively, negatively or neutrally. The non-host effect on the total foraging efficiency is not necessarily a result of the sum of the effects on the first and the second foraging phase. In fact, the results of two out of three outdoor-tent experiments that investigated the foraging efficiency of the parasitoid showed no non-host effect, while the separate foraging phases were affected by non-host presence. The observed influence of non-host presence on parasitoid behaviour could have been caused by an altered performance of host and parasitoid on non-host infested plants. The presence of non-hosts could, via the plant, indirectly affect the performance of the host. The parasitoid larvae developing inside the host could consequently be affected. This could have led to an influence of non-host presence on parasitoid foraging behaviour. The behaviour of the parasitoid could also have been affected by the release of different volatiles by different leaves of an infested plant, or by the behaviour and infochemicals of particular non-host species.

At the level of parasitoid-host dynamics, in this thesis non-host presence could have created environments in which hosts became associational susceptible or associational resistant to *C. glomerata*. For example, parasitoids foraged more efficiently in environments with non-host aphids, which made the host caterpillar more susceptible to parasitism. The presence of specific non-host species might also indirectly affect parasitoid-host dynamics through their influences on the interaction web. The non-host *T. ni*, for example, directly affected the behaviour of the parasitoid and could thereby indirectly have affected the interaction between parasitoid and host. Based on the results from this thesis, two hypotheses on foraging behaviour at a landscape level, proposed in chapter 2, could be specified. First, non-hosts are expected to have a negative influence on plant-volatile-based searching of parasitoids in monocultures, whereas the host-infochemical-based searching in such landscapes could both be negatively and positively affected by non-host presence. Second, a hypothesis on parasitoid foraging in landscapes with host herbivores with a clumped distribution was specified. When parasitoids that are foraging for hosts with a clumped distribution have located hosts, they will find a large number of hosts at once; the parasitoids are not time-limited. However, a large number of hosts can be parasitized at one location: the parasitoids are egg-limited. In landscapes with host herbivores with a clumped distribution, non-host presence is not expected to affect parasitoid fitness regarding time-limitations. However, non-hosts could either negatively or neutrally affect parasitoid fitness regarding egg-limitations.

The results of this thesis further show that the study of single phases of parasitoid foraging behaviour cannot predict the outcome of the total foraging efficiency. Some possible arguments could explain the difference between the outcomes of the separate experiments; the experiments differed in temporal scales and therefore the relative value given to the experiments are different, the circumstances in laboratory and field are different, the opportunity to make use of previously gained experience was unequal between experiments. The thesis also shows that the foraging efficiency of *C. glomerata* on the plant is positively affected by the presence of non-host aphids. Combined with the ability of *C. glomerata* to associate host cues with the presence of non-host aphids, this could give new openings for optimization of biological pest control programs in environments were non-host presence correlates with the presence of hosts.

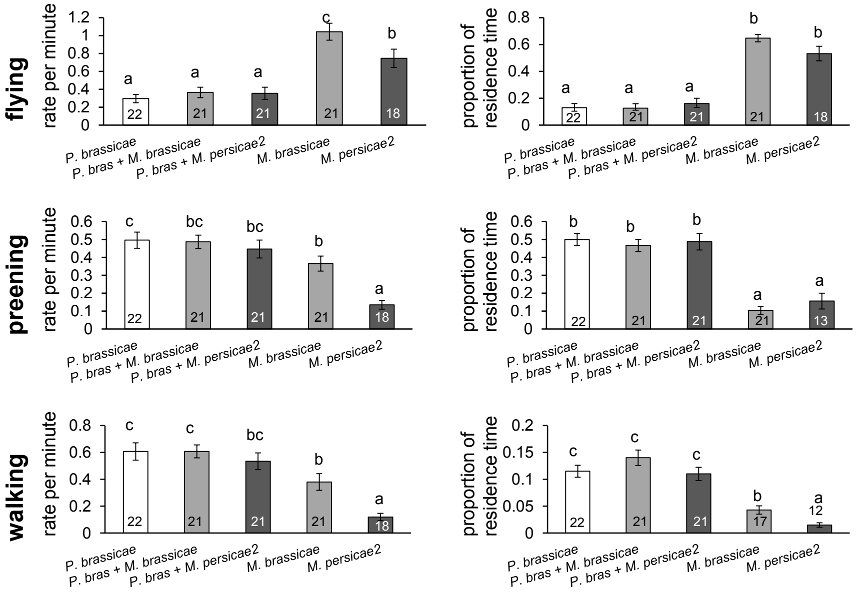
Conclusion

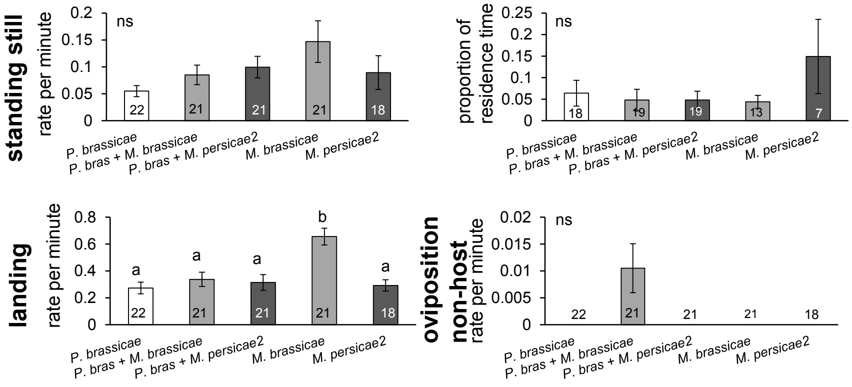
The foraging efficiency of the parasitoid *C. glomerata* when searching for its host *P. brassicae* is not strongly affected by non-host herbivore presence. The use of herbivore-induced plant volatiles by *C. glomerata* during this foraging process is not interrupted by non-host herbivores. It is advised to consider all phases of the foraging process in studies of parasitoid foraging behaviour, preferably in one experiment that covers the whole searching process. Altogether, this thesis gives a clear and comprehensive overview of the impact of non-host presence on a parasitoid-host-food plant complex and it thereby contributes to the fundamental knowledge of insect foraging in a multi-herbivore context.



Appendices

Chapter 3


flying
rate per minute
1.2
1
0.8
0.6
0.4
0.2
0
a a a c b
22 21 21 21 18
proportion of residence time
0.8
0.6
0.4
0.2
0
a a a b b
22 21 21 21 18
preening
rate per minute
0.6
0.5
0.4
0.3
0.2
0.1
0
c bc bc b a
22 21 21 21 18
proportion of residence time
b b b a a
22 21 21 21 13
walking
rate per minute
0.8
0.6
0.4
0.2
0
c c bc b a
22 21 21 21 18
proportion of residence time
0.2
0.15
0.1
0.05
0
c c c b a
22 21 21 17 12
P. brassicae
P. bras + M. brassicae
P. bras + M. persicae2
M. brassicae
M. persicae2


standing still
rate per minute
0.2
0.15
0.1
0.05
0
ns
22 21 21 21 18
proportion of residence time
0.25
0.2
0.15
0.1
0.05
0
ns
18 19 19 13 7
landing
rate per minute
0.8
0.6
0.4
0.2
0
a a a b a
22 21 21 21 18
oviposition non-host
rate per minute
0.02
0.015
0.01
0.005
0
ns
22 21 21 21 18
P. brassicae
P. bras + M. brassicae
P. bras + M. persicae2
M. brassicae
M. persicae2

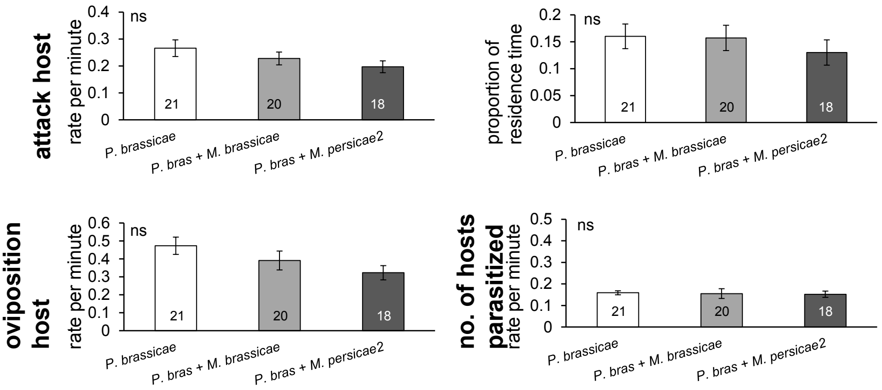
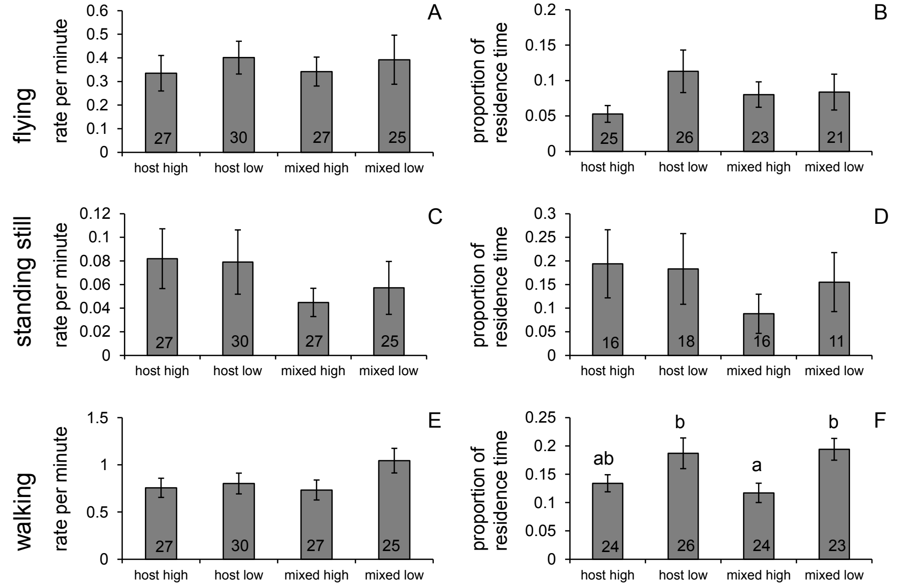


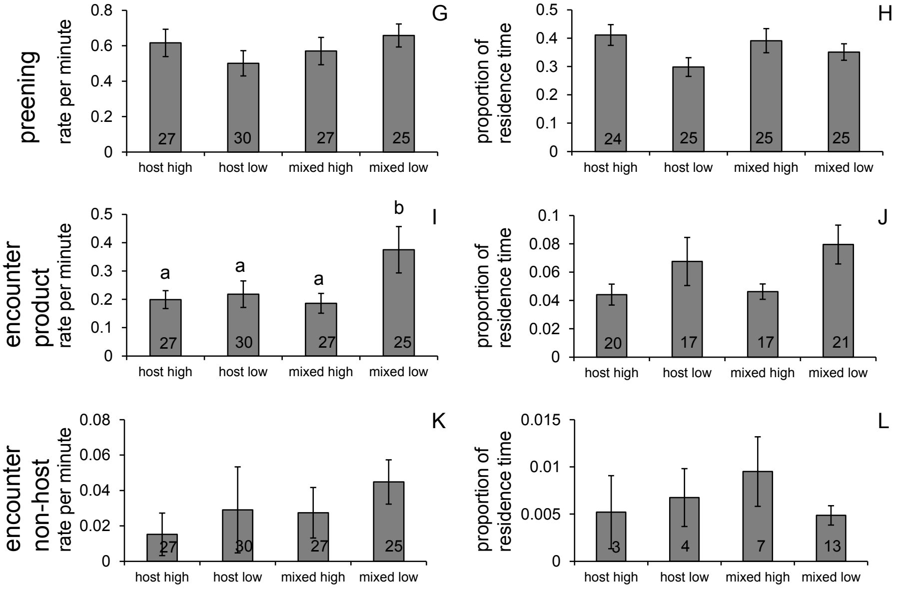
Figure S1. Rate per minute and proportion of residence time ±SE of *C. glomerata* in the flight chamber experiment for the behaviours flying, preening, walking, standing still and attack host. Rate per minute of *C. glomerata* for the behaviours landing, oviposition non-host, oviposition host and number of hosts parasitized. Numbers in/above bars indicate the number of observations analysed per treatment. Different letters indicate different means based on generalized linear models (P < 0.001) and least significant differences (α = 0.05), ns = no significant differences.

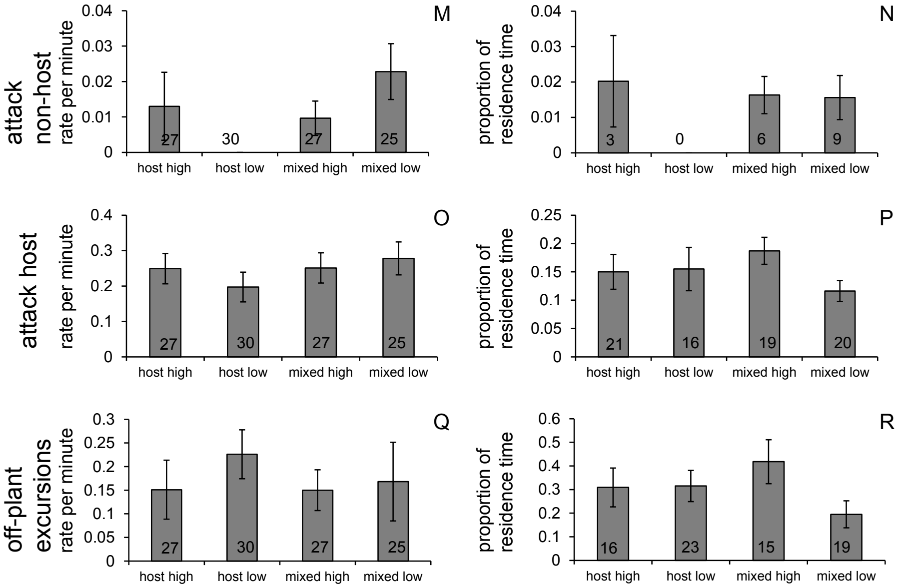
Dependent variable	**Factor**	**F**	**n.d.f.**	**d.d.f.**	**P**
% parasitized hosts	Herbivore identity	9.33	6	84.0	<0.001
	Feeding guild	9.70	6	88.0	<0.001
% plants with parasitized hosts	Herbivore identity	7.35	6	84.0	<0.001
	Feeding guild	8.00	6	88.0	<0.001
no. eggs found in parasitized hosts	Herbivore identity	15.52	6	74.8	<0.001
	Feeding guild	15.88	6	78.2	<0.001

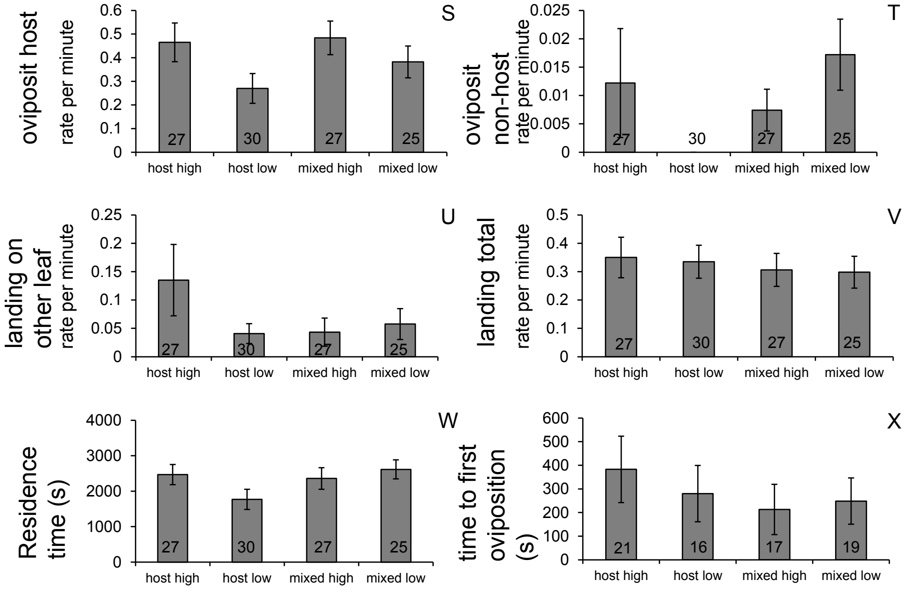
Table S1. Effect of series (the week in which experiments were conducted) on results of the outdoor tent experiment.

Chapter 4


flying
standing still
walking
rate per minute
proportion of residence time
A
0.6
0.5
0.4
0.3
0.2
0.1
0
27
30
27
25
B
0.2
0.15
0.1
0.05
0
25
26
23
21
C
0.12
0.1
0.08
0.06
0.04
0.02
0
27
30
27
25
D
0.3
0.25
0.2
0.15
0.1
0.05
0
16
18
16
11
E
1.5
1
0.5
0
27
30
27
25
F
0.25
0.2
0.15
0.1
0.05
0
ab
b
a
b
24
26
24
23
host high
host low
mixed high
mixed low


preening
encounter product
encounter non-host
rate per minute
proportion of residence time
G
0.8
0.6
0.4
0.2
0
27
30
27
25
H
0.5
0.4
0.3
0.2
0.1
0
24
25
25
25
I
0.5
0.4
0.3
0.2
0.1
0
a
a
a
b
27
30
27
25
J
0.1
0.08
0.06
0.04
0.02
0
20
17
17
21
K
0.08
0.06
0.04
0.02
0
27
30
27
25
L
0.015
0.01
0.005
0
3
4
7
13
host high
host low
mixed high
mixed low


M
attack non-host
rate per minute
0.04
0.03
0.02
0.01
0
27
30
27
25
host high
host low
mixed high
mixed low
N
proportion of residence time
0.04
0.03
0.02
0.01
0
3
0
6
9
host high
host low
mixed high
mixed low
O
attack host
rate per minute
0.4
0.3
0.2
0.1
0
27
30
27
25
host high
host low
mixed high
mixed low
P
proportion of residence time
0.25
0.2
0.15
0.1
0.05
0
21
16
19
20
host high
host low
mixed high
mixed low
Q
off-plant excursions
rate per minute
0.3
0.25
0.2
0.15
0.1
0.05
0
27
30
27
25
host high
host low
mixed high
mixed low
R
proportion of residence time
0.6
0.5
0.4
0.3
0.2
0.1
0
16
23
15
19
host high
host low
mixed high
mixed low


S
oviposit host
rate per minute
0.6
0.5
0.4
0.3
0.2
0.1
0
27
30
27
25
host high
host low
mixed high
mixed low
T
oviposit non-host
rate per minute
0.025
0.02
0.015
0.01
0.005
0
27
30
27
25
host high
host low
mixed high
mixed low
U
landing on other leaf
rate per minute
0.25
0.2
0.15
0.1
0.05
0
27
30
27
25
host high
host low
mixed high
mixed low
V
landing total
rate per minute
0.5
0.4
0.3
0.2
0.1
0
27
30
27
25
host high
host low
mixed high
mixed low
W
Residence time (s)
4000
3000
2000
1000
0
27
30
27
25
host high
host low
mixed high
mixed low
X
time to first oviposition (s)
600
500
400
300
200
100
0
21
16
17
19
host high
host low
mixed high
mixed low

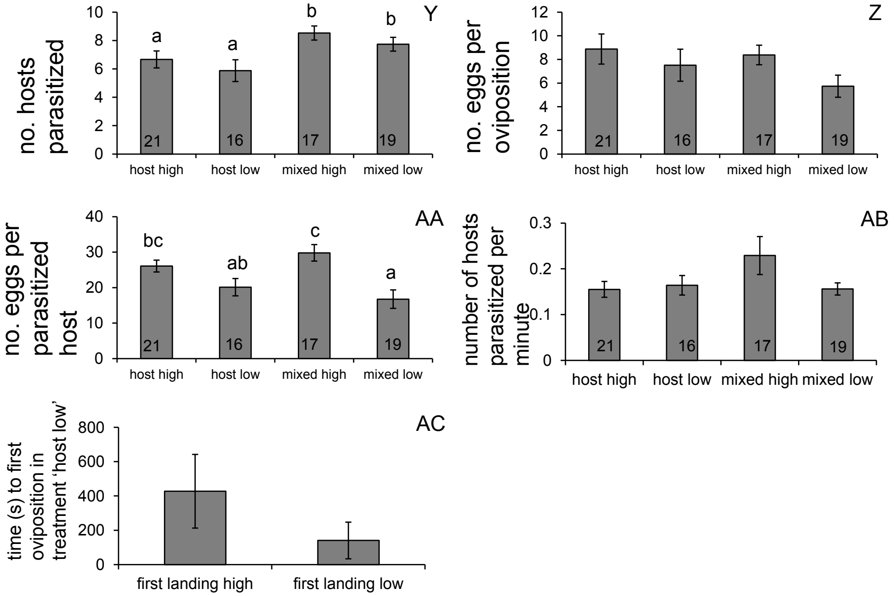


Figure S1. Behaviour of *Cotesia glomerata* parasitoids in the flight chamber experiment in which *Brassica oleracea* plants were infested with herbivores according to the treatments: 'host high' (ten first-instar *Pieris brassicae* caterpillars on the higher leaf, ten first-instar *Mamestra brassicae* larvae on the lower leaf), 'host low' (ten first-instar *P. brassicae* larvae on the lower leaf, ten first-instar *M. brassicae* larvae on the higher leaf), 'mixed high' (ten first-instar *P. brassicae* and ten first-instar *M. brassicae* larvae on the higher leaf) and 'mixed low' (ten first-instar *P. brassicae* and ten first-instar *M. brassicae* larvae on the lower leaf). Rate per minute and proportion of residence time ± SE of *C. glomerata* for the behaviour flying, standing still, walking, preening, encounter product, encounter non-host, attack non-host, attack host, off-plant excursions. Rate per minute of *C. glomerata* for the behaviour oviposit host, oviposit non-host, landing on other leaf, landing total. Residence time, time to first oviposition. Numbers of hosts parasitized, eggs per oviposition, eggs per parasitized host, numbers of hosts parasitized per minute and time to first oviposition in treatment 'host low'. Numbers in bars indicate the number of observations analysed per treatment. Similar letters indicate similar means based on generalized linear models and least significant differences (α = 0.05).

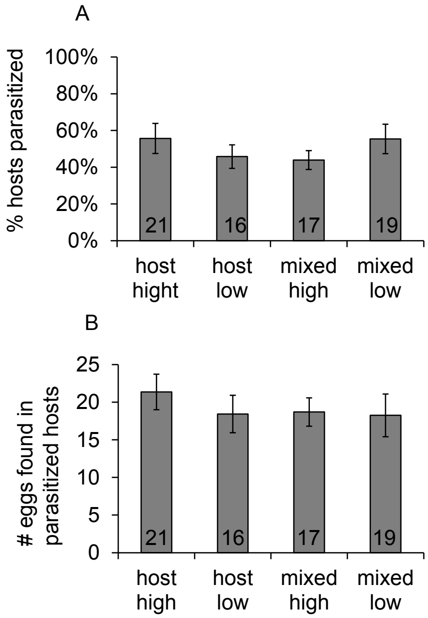


Figure S2. Percentage of *Pieris brassicae* hosts parasitized (A) and number of eggs found in parasitized hosts (B) by *Cotesia glomerata* parasitoids in the tent experiment. *Brassica oleracea* plants were infested with herbivores according to the treatments: 'host high' (ten first-instar *P. brassicae* caterpillars on the higher leaf, ten first-instar *Mamestra brassicae* larvae on the lower leaf), 'host low' (ten first-instar *P. brassicae* larvae on the lower leaf, ten first-instar *M. brassicae* larvae on the higher leaf), 'mixed high' (ten first-instar *P. brassicae* and ten first-instar *M. brassicae* larvae on the higher leaf) and 'mixed low' (ten first-instar *P. brassicae* and ten first-instar *M. brassicae* larvae on the lower leaf). No significant differences were found based on generalized linear mixed models and least significant differences (α = 0.05). Numbers in bars indicate the number of analysed parasitoids per treatment.

Chapter 5

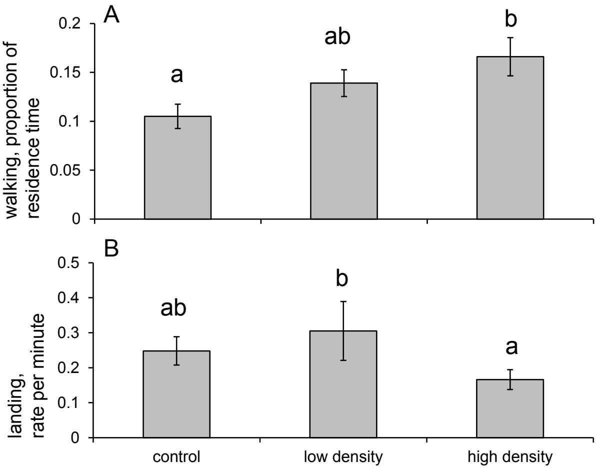
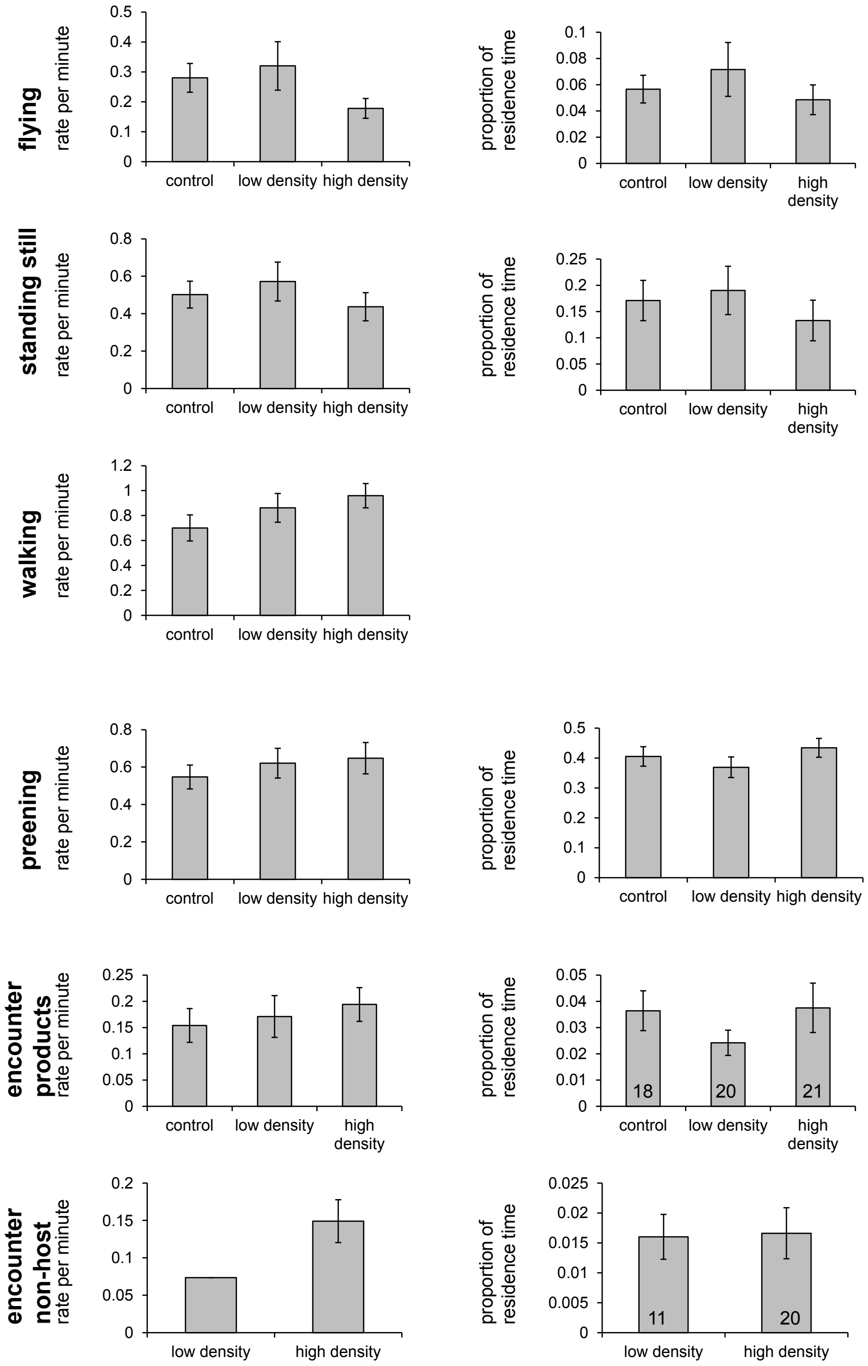


Figure S1. Behaviour of *C. glomerata* on plants infested with ten first-instar *P. brassicae* host caterpillars (control), with ten first-instar *P. brassicae* plus ten first-to-second-instar *M. brassicae* non-host caterpillars (low density) or with ten first-instar *P. brassicae* plus 50 first-to-second-instar *M. brassicae* caterpillars (high density) as observed in the flight chamber experiment. A) proportion of residence time ± SE spent on walking, B) rate per minute ± SE of landing on the plant. N = 22 per treatment, different letters indicate significant differences based on generalized linear models ($P < 0.05$).


flying
rate per minute
0.5
0.4
0.3
0.2
0.1
0
control
low density
high density
proportion of residence time
0.1
0.08
0.06
0.04
0.02
0
control
low density
high density
standing still
rate per minute
0.8
0.6
0.4
0.2
0
control
low density
high density
proportion of residence time
0.25
0.2
0.15
0.1
0.05
0
control
low density
high density
walking
rate per minute
1.2
1
0.8
0.6
0.4
0.2
0
control
low density
high density
preening
rate per minute
0.8
0.6
0.4
0.2
0
control
low density
high density
proportion of residence time
0.5
0.4
0.3
0.2
0.1
0
control
low density
high density
encounter products
rate per minute
0.25
0.2
0.15
0.1
0.05
0
control
low density
high density
proportion of residence time
0.05
0.04
0.03
0.02
0.01
0
18
20
21
control
low density
high density
encounter non-host
rate per minute
0.2
0.15
0.1
0.05
0
low density
high density
proportion of residence time
0.025
0.02
0.015
0.01
0.005
0
11
20
low density
high density

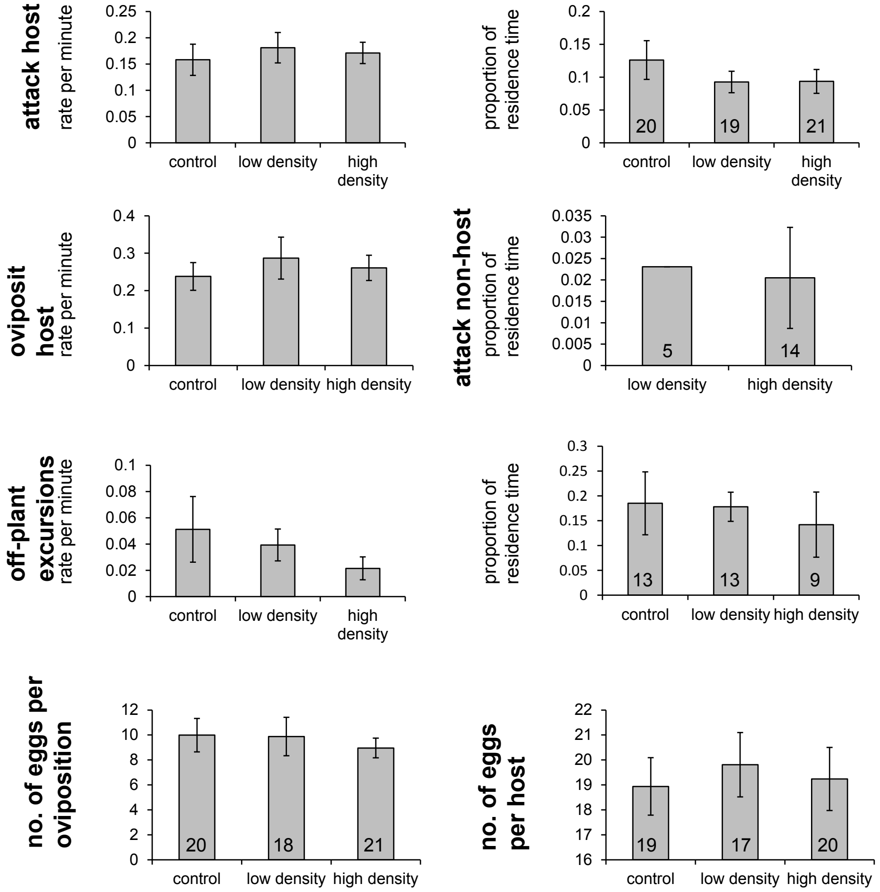
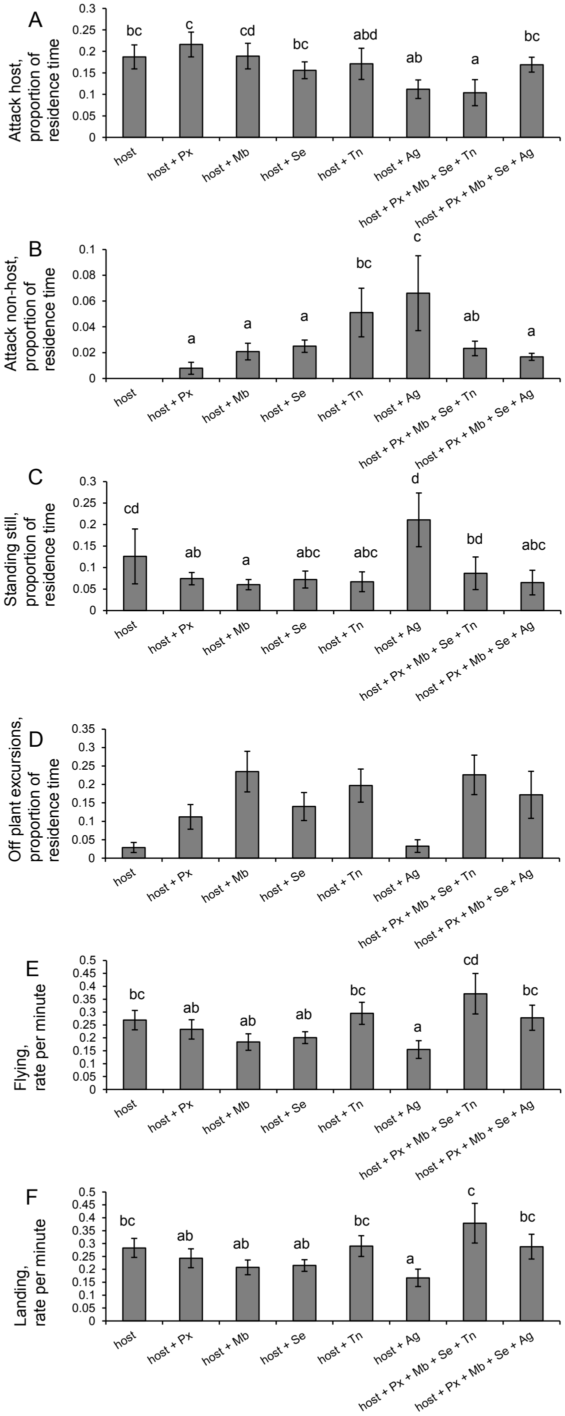


Figure S2. Behaviour of *C. glomerata* on plants infested with ten first-instar *P. brassicae* host caterpillars (control), with ten first-instar *P. brassicae* plus ten first-to-second-instar *M. brassicae* non-host caterpillars (low density) or with ten first-instar *P. brassicae* plus 50 first-to-second-instar *M. brassicae* caterpillars (high density) as observed in the flight chamber experiment. Rate per minute ± SE of flying, standing still, walking, preening, encounter products, encounter non-host, attack host, oviposit in host, off-plant excursions. Proportion of residence time ± SE spent on flying, standing still, preening, encounter products, encounter non-host, attack host, attack non-host, off-plant excursions. Number of eggs per oviposition in host, number of eggs per parasitized host. N = 22 per treatment, unless otherwise indicated by numbers in bars. No significant differences were found using generalized linear models (α = 0.05).


A
Attack host, proportion of residence time
0.3
0.25
0.2
0.15
0.1
0.05
0
bc
c
cd
bc
abd
ab
a
bc
host
host + Px
host + Mb
host + Se
host + Tn
host + Ag
host + Px + Mb + Se + Tn
host + Px + Mb + Se + Ag
B
Attack non-host, proportion of residence time
0.1
0.08
0.06
0.04
0.02
0
a
a
a
bc
c
ab
a
host
host + Px
host + Mb
host + Se
host + Tn
host + Ag
host + Px + Mb + Se + Tn
host + Px + Mb + Se + Ag
C
Standing still, proportion of residence time
0.3
0.25
0.2
0.15
0.1
0.05
0
cd
ab
a
abc
abc
d
bd
abc
host
host + Px
host + Mb
host + Se
host + Tn
host + Ag
host + Px + Mb + Se + Tn
host + Px + Mb + Se + Ag
D
Off plant excursions, proportion of residence time
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
host
host + Px
host + Mb
host + Se
host + Tn
host + Ag
host + Px + Mb + Se + Tn
host + Px + Mb + Se + Ag
E
Flying, rate per minute
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
bc
ab
ab
ab
bc
a
cd
bc
host
host + Px
host + Mb
host + Se
host + Tn
host + Ag
host + Px + Mb + Se + Tn
host + Px + Mb + Se + Ag
F
Landing, rate per minute
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
bc
ab
ab
ab
bc
a
c
bc
host
host + Px
host + Mb
host + Se
host + Tn
host + Ag
host + Px + Mb + Se + Tn
host + Px + Mb + Se + Ag

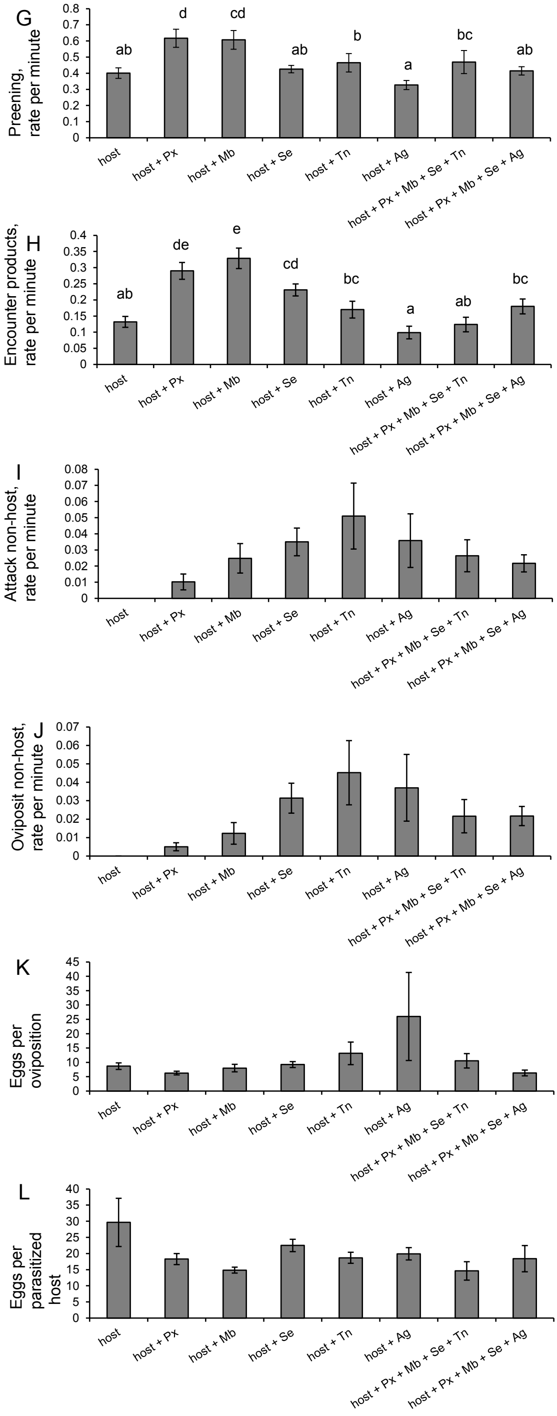

G
Preening, rate per minute
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
ab
d
cd
ab
b
a
bc
ab
host
host + Px
host + Mb
host + Se
host + Tn
host + Ag
host + Px + Mb + Se + Tn
host + Px + Mb + Se + Ag
H
Encounter products, rate per minute
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
ab
de
e
cd
bc
a
ab
bc
host
host + Px
host + Mb
host + Se
host + Tn
host + Ag
host + Px + Mb + Se + Tn
host + Px + Mb + Se + Ag
I
Attack non-host, rate per minute
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
host
host + Px
host + Mb
host + Se
host + Tn
host + Ag
host + Px + Mb + Se + Tn
host + Px + Mb + Se + Ag
J
Oviposit non-host, rate per minute J
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
host
host + Px
host + Mb
host + Se
host + Tn
host + Ag
host + Px + Mb + Se + Tn
host + Px + Mb + Se + Ag
K
Eggs per oviposition
45
40
35
30
25
20
15
10
5
0
host
host + Px
host + Mb
host + Se
host + Tn
host + Ag
host + Px + Mb + Se + Tn
host + Px + Mb + Se + Ag
L
Eggs per parasitized host
40
35
30
25
20
15
10
5
0
host
host + Px
host + Mb
host + Se
host + Tn
host + Ag
host + Px + Mb + Se + Tn
host + Px + Mb + Se + Ag

Figure S1. Behaviour of *C. glomerata* parasitoids on plants infested with only hosts, hosts + one species of non-hosts or hosts + four species of non-hosts as observed in the flight chamber experiment. Plants were infested with (combinations of): host: *P. brassicae*, Px: *P. xylostella*, Mb: *M. brassicae*, Se: *S. exigua*, Tn: *T. ni*, Ag: *A. gamma* in amounts as presented in table 2 (chapter 6). For the treatment 'host', ten *P. brassicae* caterpillars were used. Proportion of residence time ± SE spent on attack host (A), attack non-host (B), standing still (C), off-plant excursions (D), Rate per minute ± SE of flying (E), landing (F), preening (G), encounter products (H), attack non-host (I), oviposit in non-host (J). Number of eggs per oviposition in host (K), number of eggs per parasitized host (L). Different letters indicate significantly different means based on generalized linear models ($P < 0.05$) and least significant differences ($\alpha = 0.05$).

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x
Marjolein

Curriculum Vitae

Marjolein de Rijk was born in Leimuiden on November 9th, 1985. She went to secondary school in Alphen aan den Rijn, after which she started the BSc Biology at Wageningen University in 2004. During the BSc she specialized in Ecology. She continued with the MSc Biology in 2007. Her first master thesis was entitled: ‘Hitch-hiking behaviour of egg parasitoids on Heliconiini butterflies in a tropical lowland rainforest in Panama’. For this, she conducted field work in the tropical rainforest of Panama and she performed behavioural experiments and molecular analyses in the Laboratory of Entomology, Wageningen University. Her internship was entitled: ‘Collection methods, biting behaviour and cultivation of *Anopheles aquasalis* in Paramaribo, Suriname and collection methods and biting behaviour of *Anopheles darlingi* in Palumeu, Suriname’. The work was partly performed in the rainforest of Suriname and partly in its capital Paramaribo. The second thesis of her MSc was entitled: ‘Effects of herbivory by *Delia radicum* on the roots of *Brassica nigra* on the performance of the solitary parasitoid *Cotesia rubecula* and its host *Pieris rapae*’. This project was conducted at NIOO-KNAW in Heteren. Next to her study Biology, she gave whole-day workshops for high school students visiting Wageningen and she visited high schools herself to give workshops. For the BSc-course ‘Mens- en Dierkunde II’ she was teaching-assistant for four years. After finishing the MSc Biology in 2010, she worked at the department Management Studies of Wageningen University, to organize an international conference (9th WICaNeM) and she was a member of a field experiment team at Utrecht University. In 2010 she started working as a research assistant at the Laboratory of Entomology on the advancement of odor blends to effectively attract or repel malaria mosquitoes. One year later, in November 2011, she started her PhD project on the foraging behaviour of parasitoids in multi-herbivore environments. During the PhD project, she participated in the training and supervision programme of the national Graduate school Experimental Plant Sciences (EPS). She also organized the biweekly PhD-lunch meeting at the Laboratory of Entomology for one year, she organized together with five other PhD-candidates the 2nd Wageningen PhD Symposium and she was the representative of the PhD-candidates of the Laboratory of

Entomology in the monthly staff meeting of the department for over two years. In the final year of her PhD she took a course on project management (PRINCE2® Foundation) that she completed successfully.

Publications

De Rijk, M., D. Yang, B. Engel, M. Dicke, and E. H. Poelman. 2016. Feeding guild of non-host community members affects host-foraging efficiency of a parasitic wasp. Ecology (in press).

De Rijk, M., M. Krijn, W. Jenniskens, B. Engel, M. Dicke, and E. H. Poelman. 2016. Flexible parasitoid behaviour overcomes constraint resulting from position of host and non-host herbivores. Animal Behaviour (in press).

van Loon, J. J. A., R. C. Smallegange, G. Bukovinszkiné-Kiss, F. Jacobs, M. de Rijk, W. R. Mukabana, N. O. Verhulst, D. J. Menger, and W. Takken. 2015. Mosquito attraction: Crucial role of carbon dioxide in formulation of a five-component blend of human-derived volatiles. Journal of Chemical Ecology 41:567-573.

Menger, D. J., B. Otieno, M. De Rijk, W. R. Mukabana, J. J. Van Loon, and W. Takken. 2014. A push-pull system to reduce house entry of malaria mosquitoes. Malaria Journal 13:119.

De Rijk, M., M. Dicke, and E. H. Poelman. 2013. Foraging behaviour by parasitoids in multi-herbivore communities. Animal Behaviour 85:1517-1528.

Kruidhof, H. M., M. de Rijk, D. Hoffmann, J. A. Harvey, L. E. M. Vet, and R. Soler. 2012. Effect of belowground herbivory on parasitoid associative learning of plant odours. Oikos 122:1094-1100.

Hiwat, H., R. Andriessen, M. de Rijk, C. J. M. Koenraadt, and W. Takken. 2011. Carbon dioxide baited trap catches do not correlate with human landing collections of *Anopheles aquasalis* in Suriname. Memorias do Instituto Oswaldo Cruz 106:360-364.

Hiwat, H., M. De Rijk, R. Andriessen, C. J. M. Koenraadt, and W. Takken. 2011. Evaluation of methods for sampling the malaria vector *Anopheles darlingi* (Diptera, Culicidae) in Suriname and the relation with its biting behavior. Journal of Medical Entomology 48:1039-1046.

Guerrieri, E., M. E. Huigens, C. Estrada, J. B. Woelke, M. de Rijk, N. E. Fatouros, A. Aiello, and J. S. Noyes. 2010. *Ooencyrtus marcelloi* sp. nov. (Hymenoptera: Encyrtidae), an egg parasitoid of Heliconiini (Lepidoptera: Nymphalidae: Heliconiinae) on passion vines (Malpighiales: Passifloraceae) in Central America. Journal of Natural History 44:81-87.

Under review

De Rijk, M., X. Zhang, J. A. H. Van der Loo, B. Engel, M. Dicke, and E. H. Poelman. 2016. Density- and trait-mediated indirect interactions alter host foraging behaviour of parasitoids without altering foraging efficiency. Under review.

De Rijk, M., Q. Wang, E. Papagiannaki, M. Dicke, and E. H. Poelman. 2016. Herbivore species identity rather than diversity of the non-host community determines parasitoid foraging behaviour. Under review.

De Rijk, M., V. Cegarra Sánchez, H. M. Smid, B. Engel, L. E. M. Vet, and E. H. Poelman. 2016. Associative learning of host presence in non-host environments influences parasitoid foraging. Under review.

In preparation

Woelke, J.B., A.V. Gumovsky, M. De Rijk, M.E. Huigens & V.N.Fursov. Two new *Trichogramma* (Hymenoptera: Trichogrammatidae) species collected from Heliconiini (Lepidoptera: Nymphalidae: Heliconiinae) eggs on passion vines (Malpighiales: Passifloracea) in Central America. In preparation.

Woelke, J.B., M. De Rijk, M.E. Huigens & V.N. Fursov. *Trichogrammatoidea embera* sp. nov. (Hymenoptera: Trichogrammatidae): a new egg parasitoid species of Heliconiini (Lepidoptera: Nymphalidae: Heliconiinae) on passion vines (Malpighiales: Passifloracea) in Central America. In preparation.

Education Statement of the Graduate School

Experimental Plant Sciences

Issued to: **Marjolein de Rijk**
Date: **12 February 2016**
Group: **Laboratory of Entomology**
University: **Wageningen University & Research Centre**

1) Start-up phase	*date*
► **First presentation of your project**	
Parasitoid behaviour in multi-herbivore habitats	Jun 29, 2012
► **Writing or rewriting a project proposal**	
► **Writing a review or book chapter**	
review: Foraging behaviour by parasitoids in multi-herbivore communities, Animal Behaviour 85 (2013), 1517-1528	2012
► **MSc courses**	
► **Laboratory use of isotopes**	
Subtotal Start-up Phase	_7.5 credits*_

2) Scientific Exposure	*date*
► **EPS PhD student days**	
EPS PhD student day 2012, University of Amsterdam	Nov 30, 2012
EPS PhD student day 2013, Leiden University	Nov 29, 2013
► **EPS theme symposia**	
EPS theme 2 symposium 'Interactions between Plants and Biotic Agents' & Willie Commelin Scholten Day, 2012, Wageningen University	Feb 10, 2012

EPS theme 2 symposium 'Interactions between Plants and Biotic Agents' & Willie Commelin Scholten Day, 2014, University of Amsterdam	Feb 25, 2014
EPS theme 2 symposium 'Interactions between Plants and Biotic Agents' & Willie Commelin Scholten Day, 2015, Utrecht University	Feb 20, 2015
► **NWO Lunteren days and other National Platforms**	
NERN days 2012, Lunteren (NL)	Feb 07-08, 2012
NERN days 2013, Lunteren (NL)	Feb 05-06, 2013
NERN days 2014, Lunteren (NL)	Feb 11-12, 2014
Symposium Current Themes in Ecology	Nov 20, 2014
NERN days 2015, Lunteren (NL)	Feb 10, 2015
► **Seminars (series), workshops and symposia**	
Entomologendag 2011	Dec 16, 2011
YELREM 2012	May 30, 2012
Interactive workshop on presentation skills - Demie Moore	Nov 06, 2012
WEES seminar Ron Ydenburg - Are top predators important in Dutch ecology?	Nov 22, 2012
Workshop Plant Insect Interactions 2012	Nov 28, 2012
Entomologendag 2012	Dec 14, 2012
ExPectationS (EPS Career day) 2013	Feb 01, 2013
WEES seminar Ulrich Frey - Nature conservation, a view from human behavioral ecology	Apr 19, 2013
YELREM 2013	May 17, 2013
Mini-symposium: how to write a world-class paper?	Jul 17, 2013
WEES seminar Johan van de Koppel - the ecology of animal movement: can we learn from physics?	Sep 19, 2013
Workshop Plant Insect Interactions 2013	Sep 24, 2013
KLV training: public speaking, the power of story-telling	Dec 02, 2013
Wageningen PhD symposium	Dec 10, 2013
Entomologendag 2013	Dec 13, 2013
WPC lecture: the secret of a successful PhD: making the implicit explicit	Feb 06, 2014

	WPC lecture: translating your science to society and policy makers	Mar 06, 2014
	YELREM 2014	May 21, 2014
	Ento seminar: an editor’s perspective of the reviewing process	May 27, 2014
	PhD workshop carousel day	Jun 02, 2014
	Workshop Plant Insect Interactions 2014	Nov 03, 2014
	Entomologendag 2014	Dec 19, 2014
	WEES seminar Doug Landis - Redesigning agricultural landscapes for multiple ecosystem services	Feb 19, 2015
	YELREM 2015	Jun 10, 2015
►	**Seminar plus**	
►	**International symposia and congresses**	
	Symposium on Insect Plant Interactions 2014, Neuchâtel (Switzerland)	Aug 17-21, 2014
	Netherlands-Japan Seminar on Parasitoid Biology, Wageningen (NL)	Aug 28-29, 2014
	International Entomophageous Insect Conference, Málaga (Spain)	Oct 04-09, 2015
►	**Presentations**	
	SIP Neuchatel (Poster)	Aug 17-21, 2014
	NAEM days Lunteren (Talk)	Feb 12, 2014
	NAEM days Lunteren (Poster)	Feb 10, 2015
	International Entomophageous Insect Conference (Poster)	Oct 04-09, 2015
►	**IAB interview**	
►	**Excursions**	
	PhD Excursion 2013 to Switzerland	Oct 27-Nov 02, 2013
	Subtotal Scientific Exposure	*16.9 credits**

	3) In-Depth Studies	*date*
►	**EPS courses or other PhD courses**	
	Linear models	Jun 05-07, 2013
	Generalized linear models	Jun 13-14, 2013
	Mixed linear models	Jun 20-21, 2013
►	**Journal club**	

PhD lunch meeting, Lab. of Entomology	2011-2014
Insect-Plant interactions lunch meeting, Lab. of Entomology	2011-2015
Brains & Behaviour meeting, Lab. of Entomology	2011-2013
► **Individual research training**	
Subtotal In-Depth Studies	*5.1 credits**

4) Personal development	*date*
► **Skill training courses**	
Competence assessment	Jan 24 & Mar 01, 2012
Voice matters, voice and presentation skills training	Feb 12 & 26, 2013
Techniques for Writing and Presenting a Scientific Paper	Apr 23-26, 2013
Mobilising your scientific network	Mar 19 & 27, 2014
Reviewing a scientific paper	Jun 10, 2014
Career orientation	Oct 08, 15, 22 & 29, 2014
Last stretch of the PhD program	May 22, 2015
Career assessment	Aug 11, 2015
► **Organisation of PhD students day, course or conference**	
Organisation of Wageningen PhD symposium	2014-2015
► **Membership of Board, Committee or PhD council**	
Chair of PhD lunch meeting Entomology	Aug 2013- Aug 2014
PhD representative in staff meeting Entomology	Jun 2013-Oct 2015
Subtotal Personal Development	*7.6 credits**

TOTAL NUMBER OF CREDIT POINTS*	**37.1**

Herewith the Graduate School declares that the PhD candidate has complied with the educational requirements set by the Educational Committee of EPS which comprises of a minimum total of 30 ECTS credits

** A credit represents a normative study load of 28 hours of study.*

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