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

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Abstract. 1. Foraging decisions of parasitoids are influenced by host density via density-mediated indirect interactions. However, in the parasitoid’s environment, non-suitable herbivores are also present. These non-hosts also occur in different densities, which can affect a parasitoid’s foraging behaviour.

2. The influence of non-host 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, recognise and accept the host.

3. By using laboratory and field setups, it was 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.

4. The findings show that a high non-host density, regardless of the species used, negatively affected parasitoid preference for host-infested plants, but that the behaviour on the plant and the total host-finding efficiency of the parasitoids were not influenced by non-host density.

5. These results are discussed in the context of density-mediated indirect interactions.

Key words. Density-mediated indirect interactions (DMIIs), field experiment, foraging efficiency, multi-herbivory, non-host.

Introduction

Arthropod herbivores in both natural and (agri)cultural environments occur in different densities, depending on, for example, climate conditions (Pramanik & 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 (DMIIs) (Abrams, 1995; Werner & Peacock, 2003). In general, as a consequence of changing plant traits (Maeda & 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 & Takabayashi, 2001; Gols et al., 2003; Horiuchi et al., 2003; Shiojiri et al., 2010; Girling et al., 2011). One group of carnivores that is thoroughly studied for response to herbivore communities 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 & 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 commonly 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 & Finke, 2014; Hammill et al., 2015).
The influence of non-hosts can be expressed during the foraging process of parasitoids that can be divided into two phases: (i) finding the host-infested plant from a distance and (ii) finding, recognising 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, so-called herbivore-induced plant volatiles (HIPVs), to locate host-infested plants (Vet & 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; Armur 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, recognise and accept the host (Van Alphen et al., 2003; Colazza et al., 2014). The physical presence of non-hosts, e.g. as an obstacle on a leaf (Hauzy et al., 2010), as well as their infochemicals (Takabayashi & Takahashi, 1990) can make parasitoids less efficient at host finding and even cause them to mistakenly attack non-hosts (Bukovinszky et al., 2012; Chabaane et al., 2015).

Several non-host herbivore features can play a role in affecting the interaction between parasitoid and host, e.g. feeding guild (Rodriguez-Saona et al., 2005; Soler et al., 2012a; Zhang et al., 2013; De Rijk et al., 2016b), origin (Desumont 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; Ponzie et al., 2014). An increase in non-host herbivore density may mask host presence in the two phases of parasitoid foraging. First, a larger proportion of non-hosts compared with hosts may dilute the HIPVs that are induced by hosts and reduce the potential that parasitoids will reliably use volatiles to locate host-infested plants (Ponzie et al., 2014). Second, when arriving on the plant, an increase in non-host density may increase the likelihood of encountering non-host herbivores before hosts and thereby potentially increase tendencies to leave the plant without parasitising a host (Vos et al., 2001). These effects may be non-host species-specific, because of the variation in HIPVs they may induce or the variation in their traits that affect direct recognition of their identity in parasitoid handling of potential hosts (De Rijk et al., 2013).

Here we tested the hypothesis that an increase in non-host density would affect parasitoid host location in the two phases of foraging and that these effects are specific for non-host species. We studied foraging behaviour of the gregarious endoparasitoid Cotesia glomerata (Hymenoptera: Braconidae) for its most common host, the gregarious caterpillars of Pieris brassicae (Lepidoptera: Pieridae), in the presence of non-host herbivores. To test for non-host density effects on parasitoid foraging, the non-hosts were infested in two different densities and we compared the density effect caused by four different species of non-host [Spodoptera exigua (Lepidoptera: Noctuidae), Autographa gamma (Lepidoptera: Noctuidae), Plutella xylostella (Lepidoptera: Plutellidae) and Mamestra brassicae (Lepidoptera: Noctuidae)]. Because in nature the host caterpillars of P. brassicae are found in a patchy distribution (Vos et al., 1998) and non-hosts are typically present on every plant, we tested host location in a background of either low or high non-host density. This implies that our results can answer the question of how parasitoids find their hosts when all plants in the environment are infested with a low or high non-host density. We combined three experiments that each assessed the effect of non-host densities on different phases of parasitoid foraging. We first studied the parasitoids during the initial phase of foraging in which parasitoids use HIPVs to locate host-infested plants, by using a two-choice wind tunnel experiment. In this setup, the flight response of the parasitoids towards plants infested with both hosts and non-hosts versus plants infested with only non-hosts was observed to address whether parasitoids are affected in locating plants with hosts by a background of different non-host densities. Second, the parasitoids were observed during the subsequent phase of foraging when searching for hosts after arrival on the chosen plant. In a non-choice on-plant experiment, distinct behavioural elements of the searching parasitoids were recorded when foraging for hosts in different densities of non-hosts. In the on-plant experiment, only the subset of treatments that included hosts in the presence or absence of non-hosts, omitting treatments with only non-hosts, were offered to the parasitoids due to the time-consuming character of the experiment and because it is well characterised that C. glomerata spends only a short time residing on plants infested with only non-host herbivores (Bukovinszky et al., 2012). Finally, the combination of both phases of foraging was studied to evaluate the total host-finding efficiency. This was tested by using a multiple-choice outdoor tent experiment. In this field tent setup, environments were created in which every plant was infested with non-host herbivores, while only half of the plants were infested with the host, i.e. similar to the wind tunnel tests. We discuss the foraging phase in which non-host densities affect host location, whether density effects are contingent upon non-host species identity, and whether these effects are reflected in total host-finding efficiency.

**Materials and methods**

**Plants**

*Brassicae oleracea var. gemmiferum* Cyrous plants (Brassiccales: Brassicaceae) were grown in a greenhouse (20 ± 2 °C, 60 ± 10% RH, LD 16:8 h photoperiod) in standard potting soil (0.7 litre Lentse potgrond, no. 4; Lent, the Netherlands) and fertilised two to three times a week using a liquid fertiliser (EC 2.1 mS cm⁻¹, pH 5.8; Unifarm, Wageningen, the Netherlands). Five-week-old plants were used in all experiments.

**Insects**

*Pieris brassicae*, *A. gamma*, *P. xylostella* and *M. brassicae* caterpillars were cultured on *B. oleracea var. gemmifera* Cyrous plants in a greenhouse (20 ± 2 °C, 60 ± 10% RH, LD 16:8 h). *Spodoptera exigua* caterpillars were cultured on an artificial...
diet consisting mainly of polenta, beer yeast and wheat-germs in a climate cell (25.5 °C, 50% RH, LD 16:8 h) 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 C. glomerata was cultured on P. brassicae caterpillars in a greenhouse (20 ± 2 °C, 60 ± 10% RH, LD 16:8 h). Parasitoid cocoons were collected daily from the rearing cages and kept in a climate cabinet (21 °C, LD 16:8). Emerged parasitoids were provided with honey and water ad libitum and were allowed to mate. Naive parasitoids, 1–7 days-old, were tested in the experiments.

Setup

Wind tunnel experiment. A wind tunnel as described by Geervliet et al. (1994) (wind tunnel conditions: 0.1 m s⁻¹ air speed, 22–24 °C, 60–72% RH) was used to test the flight response of C. glomerata towards plants with hosts in a non-host-infested environment. Two plants were positioned upwind in the wind tunnel; both of them were infested with the same number of first- to second-instar caterpillars of one of the non-host species, while only one of these plants was additionally infested with 10 first-instar hosts. In this way, two densities (low = 10 non-host caterpillars; high = 50 non-host caterpillars) of four non-host herbivore species (S. exigua, A. gamma, P. xylostella, M. brassicae) were tested. 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 10 hosts (see fig. 1 in De Rijk et al., 2016b for a similar experimental setup). To test if the difference in total herbivore numbers feeding on the two plants in each treatment affected the preference of parasitoids, two extra treatments were performed. In these choice tests, both plants received equal numbers of herbivores: for low density both plants were infested with 20 caterpillars (10 hosts + 10 M. brassicae caterpillars vs. 20 M. brassicae caterpillars), and for a high density both plants were infested with 60 caterpillars (10 hosts + 50 M. brassicae caterpillars vs. 60 M. brassicae caterpillars). All 11 treatments were tested using eight plant pairs, divided over 36 days in a random order (Table 1). For each plant pair, 10 female parasitoids were individually tested. The first plant the parasitoid landed on was considered the plant of choice. Parasitoids that did not land on a plant within 5 min 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.

Preparation of plants was done 1 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. In the high-density treatments, herbivores still had an ample amount of leaf to feed from.

On-plant experiment. An on-plant experiment was used to observe parasitoid behavior after landing on a plant that contained either only hosts or both hosts and non-hosts. The on-plant experiment was conducted in a gauze tent (L × W × H, 2.9 m × 2.0 m × 2.3 m) positioned inside a greenhouse compartment (25 ± 2 °C, 60 ± 10% RH) (Bukovinszky et al., 2012). Due to the time-consuming nature of the experiment, only one species of non-host was tested: M. brassicae, for which we previously identified, for a single density, non-host effects in on-plant foraging (Bukovinszky et al., 2012). Three treatments were used: plants infested with 10 first-instar M. brassicae plus 10 first-instar P. brassicae caterpillars (low density); plants infested with 50 first-instar M. brassicae plus 10 first-instar P. brassicae caterpillars (high density); and plants infested with 10 first-instar P. brassicae caterpillars (control) (see fig. 1 in De Rijk et al., 2016b for a similar experimental setup). Treatments of plants infested with only non-hosts were not included, because this experiment is time-consuming and from previous

| Table 1. Overview of treatments and replicates of the wind tunnel experiment, the on-plant experiment and the outdoor tent experiment. |
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| Experiments | Treatments | Low density | High density | Control |
| | | Plant A | Plant B | Plant A | Plant B | Plant A | Plant B | Replicates |
| Wind tunnel | 10 × Sc + 10 × Pb | 10 × Sc | 50 × Sc + 10 × Pb | 50 × Sc | 10 × Pb | – | Per treatment, 8 plant pairs (11 treatments × 8 plant pairs) |
| | 10 × Ag + 10 × Pb | 10 × Ag | 50 × Ag + 10 × Pb | 50 × Ag | | | |
| | 10 × Px + 10 × Pb | 10 × Px | 50 × Px + 10 × Pb | 50 × Px | | | |
| | 10 × Mb + 10 × Pb | 10 × Mb | 50 × Mb + 10 × Pb | 50 × Mb | | | |
| | 10 × Mb + 10 × Pb | 20 × Mb | 50 × Mb + 10 × Pb | 60 × Mb | | | |
| On-plant | 10 × Mb + 10 × Pb | – | 50 × Mb + 10 × Pb | – | 10 × Pb | – | Per treatment 22 parasitoids (3 treatments × 22 parasitoids) |
| | 10 × Sc + 10 × Pb | 10 × Sc | 50 × Sc + 10 × Pb | 50 × Sc | 10 × Pb | – | Per treatment 12 series* (7 treatments × 12 series) |
| | 10 × Px + 10 × Pb | 10 × Px | 50 × Px + 10 × Pb | 50 × Px | | | |
| | 10 × Mb + 10 × Pb | 10 × Mb | 50 × Mb + 10 × Pb | 50 × Mb | | | |

*The treatment ‘50 × Px + 10 × Pb (plants A), 50 ×Px (plants B)’ was replicated 11 times due to problems in the culture of Plutella xylostella. Insect species used – host herbivore: Pb, Pieris brassicae; non-host herbivores: Sc, Spodoptera exigua; Ag, Autographa gamma; Px, P. xylostella; Mb, Mamestra brassicae.
experience it is known that *C. glomerata* has a short residence time on such plants (Bukovinszky et al., 2012; De Rijk et al., 2016b). For each treatment, 22 parasitoids were observed (Table 1). On a table inside the gauze tent, three plants from the same treatment were placed, forming a triangle with approximately 50 cm in between. Three plants were used to increase the amount of plant volatiles in the tent, in order to stimulate attraction of the parasitoid towards the plants. 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 sideways while walking around on frass or feeding damage), encounter non-host, attack host, attack non-host, oviposit in host, oviposit in non-host, and 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 1 min. The observation stopped after the maximum observation time of 1 h, or when the parasitoid had spent more than 5 min 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. After the observations, host caterpillars were dissected under a stereomicroscope to count parasitoid eggs. Plants were prepared as described for the wind tunnel experiment.

**Outdoor tent experiment.** An outdoor tent experiment was used to test the efficiency of *C. glomerata* in finding its host in a non-host-infested environment. Tents (L × W × H, 3 m × 4 m × 2 m) made out of insect screen (0.6 mm mesh size) were positioned in an agricultural field in Wageningen, the Netherlands. For each series in time, tents were randomly divided over treatments, one tent per treatment. In total, seven treatments were used: hosts accompanied by one of three non-host species (*S. exigua*, *P. xylostella*, and *M. brassicae*) in two densities plus a control treatment in which only hosts were present. Except for one, every treatment was replicated 12 times. Due to the unexpected collapse of the *P. xylostella* culture, the treatment *P. xylostella* in high densities was replicated 11 times (Table 1).

The *B. oleracea* plants used in this experiment were grown in a greenhouse for 4 weeks and were transferred to gauze tents outside to acclimatise during the fifth week. After the fifth week, 16 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 at a low (10 caterpillars) or a high (50 caterpillars) density and every other plant was also simultaneously infested with 10 first-instar hosts (see fig. 1 in De Rijk et al., 2016b for a similar experimental setup). In the control treatment, only eight plants (every other plant) were infested with host caterpillars and the other plants were left uninfested. Plants were infested with herbivores as described for the wind tunnel experiment 1 day prior to parasitoid release. In the centre of each tent, three female and three male parasitoids were released 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 plants 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. Separate analyses for treatment, density, and species without interaction terms were conducted to be able to include the control treatment that lacked the different levels of non-host species or a density. Because there was no significant effect of parasitoid age, it was subsequently dropped from the model. The extra low- and high-density treatments, in which herbivore numbers of both plants in a pair were identical, were compared with the treatments ‘*M. brassicae* low density’ and ‘*M. brassicae* high density’ using a separate and similar logistic regression analysis as previously described. This comparison tested 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 the value of 1. Residence time and the number of hosts parasitised were analysed in the same way, with 60 min and 10 hosts as ‘binomial totals’. The rate min⁻¹ 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 generalised linear model (GLM) 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 analysed 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 parasitised. 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 generalised linear mixed models (GLMMs). The models to analyse the percentage of parasitised hosts per tent and the percentage of plants with parasitised hosts per tent comprised fixed effects for timing of the experiment 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 parasitised hosts per plant on which parasitised hosts were found per tent and the number of eggs per parasitised host per tent comprised fixed effects for timing of the experiment 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 responses were the number of parasitised hosts per tent with the log of the number of plants with parasitised hosts per tent as an offset, and the number of eggs per tent with the log of the number of parasitised hosts per tent as an offset, respectively. Inference was based on penalised quasi-likelihood (Breslow & Clayton, 1993). Quasi-Wald tests and approximate F-tests (Kenward & Roger, 1997) applied to the approximate linear mixed model from the last step of the iterative reweighted maximum likelihood algorithm were used. Calculations were performed using GenStat, 17th edition.

Results

Wind tunnel experiment

In the different treatments, 402 out of 640 (63%) 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 rather than 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), did the parasitoids have 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 at a high density compared with a situation without non-hosts [GLM, \( P = 0.05 \) (overall); least significant difference (LSD), \( P = 0.043 \) (high versus 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 (binomial tests, all \( P \leq 0.001 \)). We found that correcting treatments for equal numbers of caterpillars, i.e. by having the same numbers of non-hosts on one plant as the total number of hosts plus non-hosts on the other, did not yield different results. In the additional high-density treatment with equal numbers of caterpillars on both plants in a pair, 49 out of 67 (73%) responding parasitoids preferred the plants infested with hosts and non-hosts over non-host infestations (binomial test, \( P < 0.001 \)). Similarly, in the low-density treatment, 39 out of 56 (70%) responding parasitoids preferred the plants infested with hosts and non-hosts (binomial test, \( P = 0.005 \)). Comparing parasitoid responses with these treatments in which we applied equal herbivore numbers with responses to our treatments that had received hosts in addition to non-hosts showed 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 solely infested by non-hosts. This conclusion is, however, drawn from data for a single non-host herbivore species and cannot rule out the possibility that these effects may be different for other non-host herbivores.

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 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 min. This was not affected by the different herbivore infestations (GLM, \( P = 0.198 \)) (Figure S1a, Supporting Information), and neither was the number of hosts parasitised by each parasitoid (GLM, \( P = 0.63 \)) (Figure S1b, Supporting Information) nor the number of hosts parasitised min\(^{-1}\) (GLM, \( P = 0.497 \)) (Figure S1c, Supporting Information). Parasitoids more often attacked and oviposited in non-hosts on plants with a high non-host density 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. 2). The duration of walking was also affected by non-host density; the parasitoids walked for a longer time on plants with a high non-host density than on host-only-infested control plants (GLM, \( P = 0.028 \)) (Figure S2a, Supporting Information). The rate of landing, on the other hand, was negatively affected by a high non-host density compared with a low non-host density (GLM, \( P = 0.05 \)) (Figure S2b, Supporting Information). All other observed behaviours were unaffected by non-host presence or density (Figure S3, Supporting Information).

Outdoor tent experiment

In total, 4303 (64.8%) host caterpillars were recovered from the plants, of which 2244 (52.1%) were parasitised. The timing of the experiment influenced parasitism rates, probably because of weather conditions (percentage of parasitised hosts, series effect, GLMM, \( P < 0.001 \); percentage of plants with parasitised hosts, series effect, GLMM, \( P < 0.001 \); number of hosts per parasitised plant, series effect, GLMM, \( P < 0.001 \); number of eggs...
per plant, series effect, GLMM, $P < 0.001$). The percentage of parasitised hosts was not affected by non-host density (GLMM, $P = 0.224$), nor was the percentage of plants with parasitised hosts (GLMM, $P = 0.418$), or the number of hosts per parasitised plant (GLMM, $P = 0.190$) (Fig. 3a–c). However, non-host density did have an influence on the number of eggs found per parasitised host; more eggs were found in an environment with a high non-host density than one with a low non-host density [GLMM $= 0.047$ (overall), LSD, $P = 0.0138$ (high versus low)]. Nevertheless, neither of these treatments differed from the control treatment with only hosts (Fig. 3d).

**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 *C. glomerata* in locating plants on which their host was feeding, in particular with the non-host *S. exigua*, while the behaviour on the plant was not affected by non-host density. The hampered volatile-based searching but neutral effect of non-host herbivore densities on plant foraging decisions did not result in a reduction in overall parasitism efficiency of the parasitoids in field tent tests. This shows that the parasitoids compensated for the time lost when flying to plants on which a high density of non-host herbivores was present and not the hosts they were searching for, suggesting that the parasitoids were not time-limited in their foraging decisions.

The non-host density-mediated effects on parasitoid flight response towards plants could originate from both quantitatively and qualitatively altered HIPVs. Quantitatively, volatiles (HIPVs) 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 & 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, as has been shown for high host/prey herbivore densities (Maeda & Takabayashi, 2001; Gols *et al.*, 2014).
the rate of attacking non-hosts and ovipositing in them was higher for situations with a high density of non-hosts than for low-density situations. That numbers of parasitised hosts per time unit for host-only and host plus non-host-infested plants were similar is in contrast to previous findings by Bukovinszky et al. (2012), who presented data showing that C. glomerata parasitised more hosts per unit time on plants infested by hosts only than on plants infested by both hosts and non-hosts (Bukovinszky et al., 2012). 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 parasitise hosts also differs (Vos et al., 1998; Vos & 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 attacking them and ovipositing in them (Takabayashi & Takahashi, 1990; Vos et al., 2001; Bukovinszky et al., 2012; 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., 2012; 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 parasitised 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, which 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 not, however, translate into lower parasitism rates in tents with high non-host densities in the outdoor experiment. Our study shows that the 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 and those infested by non-host caterpillars (Geervliet et al., 1996; Shiojiri et al., 2000; Vos et al., 2001; Bukovinszky et al., 2012, but see Chabaane et al., 2015).

2003; Horiuchi et al., 2003; Shiojiri et al., 2010; Girling et al., 2011), resulting in a weaker preference for the host-infested plants. 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., 2016). On plants infested by host and non-host herbivores in the high-density treatments, the ratio of hosts:non-hosts 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, DMIs may additionally interact with trait-mediated indirect interactions, as 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 parasitised per time unit. However, in the presence of a high density of non-hosts, parasitoids spent more time walking compared with the time spent walking on host-only-infested plants, while a low non-host density showed an intermediate effect. Moreover,
Preferences are shown, however, when one of the plants is attacked by both host and non-host herbivores, which is common in nature (Shiojiri et al., 2000; Vos et al., 2001; Soler et al., 2007; Bukovinszky et al., 2012; Kruidhof et al., 2012; Soler et al., 2012b; Ponzio et al., 2014; Chabaane et al., 2015). We show that parasitoid preferences for dually attacked plants are affected by the density of non-host herbivores (Zhang et al., 2009; Yamamoto et al., 2011). However, this contrasts with Ponzio et al. (2014), who showed that parasitoid flight preferences are not influenced by increasing non-host densities when host and non-host were feeding from separate leaves. Increasing densities of these non-hosts when feeding together with the host from the same leaf, on the other hand, did affect parasitoid preferences, in the same way that it also (non-linearly) affected plant responses (Ponzio et al., 2016). 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.

Our study shows the importance of evaluating the outcome of the full foraging process of parasitoids to elucidate the importance of effects that impact on single steps of parasitoid foraging. Moreover, the complexity of agricultural or ecological communities in which parasitoids search for hosts should be considered, in order to further understand optimal foraging by parasitoids (Hunter, 2002). We show that densities and traits of non-host herbivores as part of these communities may play an important role in parasitoid host-finding strategies. It remains to be identified how density- and trait-mediated interactions caused by non-hosts are intertwined and collectively affect interaction webs such as those between parasitoids and herbivores, as studied here (Werner & Peacor, 2003; Utsumi et al., 2010; Eubanks & Finke, 2014; Hammill et al., 2015). Future studies should expand the knowledge of non-trophic interactions that form the basis of complex interaction webs that may largely determine the structure of host–parasitoid communities (Poelman, 2015).
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MdR and EHP conceived and designed the experiments. MdR, XZ, and JAHvdL collected data. MdR and BE analysed the data. MdR, EHP, and MD wrote the manuscript. BE, XZ, and JAHvdL provided editorial advice.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/een.12325

Figure S1. 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 (α = 0.05).

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. (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).

Figure S3. 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, 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).

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