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

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Abstract

Extensive research has been conducted 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. Arthropod communities typically are species-rich, and their species interactions, such as those between herbivores and their predators or parasitoids, may be particularly sensitive to changes in community composition. 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 present in a parasitoid’s environment which may complicate the foraging process for the parasitoid. Therefore, ecosystem changes in the diversity of herbivores may affect the foraging efficiency of parasitoids. The effect of herbivore diversity may be mediated by either species numbers per se, 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. On individual plants in these environments, we complemented host herbivores with 1–4 non-host herbivore species. We subsequently studied the behaviour of the gregarious endoparasitoid *Cotesia glomerata* L. (Hymenoptera: Braconidae) while foraging for its gregarious host *Pieris brassicae* L. (Lepidoptera: Pieridae). Neither non-host species diversity nor non-host identity influenced the preference of the parasitoid for herbivore-infested plants. However, after landing on the plant, non-host species identity did affect parasitoid behaviour, whereas non-host diversity did not. One of the non-host species, *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae), reduced the time the parasitoid spent on the plant as well as 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 multitrophic interaction of plants, herbivores, and parasitoids.

Introduction

Global biodiversity is rapidly declining, which affects ecosystem functioning and services (Cardinale et al., 2012). Extensive research has been conducted to investigate the underlying factors explaining the stabilizing effects of species diversity on ecosystems (Cardinale et al., 2012). Unravelled factors are, e.g., the asynchrony in how different species respond to environmental changes and at what rate such responses occur, and the reduction in competition between species in diverse environments (Cardinale et al., 2012; Loreau & de Mazancourt, 2013). Additionally, key species have been shown to contribute to ecosystem functioning by their unique species or group identity (Mouillot et al., 2011; Cardinale et al., 2012; Harvey et al., 2013). One of the biggest challenges in ecology is to predict what properties of biodiversity, i.e., species number per se or particular species traits, most prominently determine the performance of individual community members and the stability of species interactions.
A suitable biological system to disentangle components of biodiversity in their effect on species interactions is that of arthropod herbivores and their predators and parasitoid wasps (e.g., Vet & Dicke, 1992; Takabayashi et al., 2006; Wajnberg, 2006; Ponzio et al., 2013; de Rijk 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 & Hoffmeister, 2009). 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 exploit changes in plant volatiles induced by host feeding, to reliably detect host-infested plants (Vet & Dicke, 1992; Arimura et al., 2011; Hare, 2011; McCormick et al., 2012; Stam et al., 2014). Second, parasitoids make use of infochemicals from sources such as host frass and feeding damage to locate, recognize, and accept the host when foraging on the plant (van Alphen et al., 2003; de Rijk et al., 2013; Colazza et al., 2014).

Plants in most ecosystems are 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. 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., 2012; 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 affect the efficiency of parasitoid host location while foraging on the plant (Takabayashi & Takahashi, 1990; Bukovinszky et al., 2012; Chabaane et al., 2015). Therefore, whenever the species diversity changes within the trophic level of herbivores, including both hosts and non-hosts, this could affect the behaviour of parasitoids one trophic level higher (Vos et al., 2001; Thébault & Loreau, 2006; Kratina et al., 2007; Knop et al., 2014; Hammill et al., 2015). These effects could be mediated either by species numbers per se, by specific species traits, or by both (Mouillot et al., 2011; Cardinale et al., 2012; Narwani & Mazumder, 2012; Harvey et al., 2013). 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 foraging behaviour, and thereby weakened the interaction between parasitoids and hosts, eventually resulting in a positive effect of non-host diversity on the persistence of parasitoid communities. 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, 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). However, these studies did not focus on determining which phase of parasitoid foraging was most affected by the presence of a diverse non-host community. Moreover, it remains to be determined whether specific species that are part of the diversity treatments, or diversity per se, affect parasitoid foraging behaviour most.

Here, we aim to disentangle the effect of non-host species identity and diversity per se on two phases of host location by parasitoids. To experimentally show how non-host herbivore diversity and identity influence the behaviour of parasitoids, we created environments with various levels of non-host diversity by using five species of non-host herbivores. In these environments, the gregarious endoparasitoid Cotesia glomerata L. (Hymenoptera: Braconidae) was introduced to forage for its gregarious host Pieris brassicaceae L. (Lepidoptera: Pieridae). In natural situations, P. brassicae is found in a clumped distribution on only a few individual plants in a stand (Vos et al., 1998), whereas non-host herbivores can be found on every single plant. Therefore, C. glomerata were offered 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 setup to observe the flight response towards plants, and an additional setup to observe the behaviour of the parasitoid on the plant. We hypothesized that low non-host diversity would simplify the environment, leading to increased efficiency of host-finding behaviour during both phases of foraging (Wäschke et al., 2013). Low herbivore diversity may allow parasitoids to still detect host presence by herbivore-induced plant volatiles (HIPVs), and would reduce the recognition time of non-hosts during searching on the food plant. Effects of non-host species identity on parasitoid behaviour were expected to be revealed irrespective of herbivore diversity (Mouillot et al., 2011; Narwani & Mazumder, 2012; Harvey et al., 2013).
Material and methods

Plants and insects

Brassica oleracea L. var. gemmifera cv. Cyrus plants (Brassicaceae) of 5–6 weeks old were used in all experiments (greenhouse conditions for plant rearing: 22 ± 2 °C, 60 ± 10% r.h., and L16:D8 photoperiod). Plutella xylostella L. (Lepidoptera: Plutellidae), Mamestra brassicae L., Autographa gamma L. (both Lepidoptera: Noctuidae), and P. brassicae caterpillars were reared on B. oleracea plants (greenhouse conditions: 20 ± 2 °C, 60 ± 10% r.h., and L16:D8 photoperiod). Spodoptera exigua Hübner and Trichoplasia ni Hübner (both Lepidoptera: Noctuidae) caterpillars were reared on artificial diet mainly composed of polenta, beer yeast, and wheat-germs (climate cell conditions: 25.5 °C, 50% r.h., and L16:D8 photoperiod), and were kindly provided by the Laboratory of Virology of Wageningen University.

Cotesia glomerata parasitoids were reared on P. brassicae caterpillars (greenhouse conditions: 20 ± 2 °C, 60 ± 10% r.h., and L16:D8 photoperiod). Parasitoid cocoons were collected daily from rearing cages, and kept in a climate cabinet (21 °C, L16:D8 photoperiod). Emerged parasitoids were given honey and water ad libitum; they were allowed to mate, and 2- to 8-day-old naive females were used in the experiments.

Experimental set-up

Wind-tunnel experiments. In a two-choice wind-tunnel setup as described by Geervliet et al. (1994), the preference of C. glomerata for plants infested with various herbivore combinations was tested. In the wind tunnel (23–25 °C, 55–70% r.h., 0.1 m s⁻¹ wind speed), two sets of experiments were performed. The first was conducted from October 2013 to February 2014, the second from October 2014 to February 2015. In each set, four species of non-host herbivores were used next to the host P. brassicae: (1) P. xylostella, M. brassicae, S. exigua, and T. ni, and (2) P. xylostella, M. brassicae, S. exigua, and A. gamma. Plants were prepared 1 day before conducting the wind-tunnel experiment by gently transferring first and second instars (all of equal size) 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 (de Rijk et al., 2016a), 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% r.h., and L16:D8 photoperiod), and the bag and cotton wool were removed just before testing on the next day. 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 5 min after release were considered unresponsive.

In the first set of experiments, 10 responding parasitoids were tested. In the second set of experiments, 10 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 vs. plants infested with only non-hosts, whereby the level of diversity of non-host species was manipulated. 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 diversity level, the numbers of caterpillars 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 4 × in random order within a set of experiments.

In the second wind-tunnel experiment (experiment B) we tested whether effects seen in experiment A were caused by non-host species diversity or species 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 8 × (first set of experiments) or 4 × (second set of experiments) in random order.

On-plant experiment. An on-plant experiment in a gauze tent (2.9 × 2.0 × 2.3 cm, mesh size 0.6 mm) inside a greenhouse compartment (25 ± 2 °C, 60 ± 10% r.h.) was used to observe the behaviour of C. glomerata after landing on a herbivore-infested plant (see also Bukovinszky et al., 2012). In this no-choice experiment, the response of the parasitoid to plants infested with hosts and non-hosts with a low (one species) or high (four species) diversity level was observed. The intermediate 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 1 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 +
Table 1: Treatments as used in wind-tunnel experiment A. Non-host species *Trichoplusia ni* (Tn) was only used in the first set of experiments, non-host species *Autographa gamma* (Ag) and diversity level 4 were only used in the second set of experiments. Host, *Pieris brassicae*; Px; *Plutella xylostella; Mb; Mamestra brassicae; Se*; *Spodoptera exigua*

| Diversity level | Set of experiments | Plant 1 | Plant 2 |
|-----------------|-------------------|---------|---------|
| 1               | First and second  | Host + non-host Px | Non-host Px |
|                 | First and second  | Host + non-host Mb | Non-host Mb |
|                 | First and second  | Host + non-host Se | Non-host Se |
|                 | First             | Host + non-host Tn | Non-host Tn |
|                 | Second            | Host + non-host Ag | Non-host Ag |
| No. individuals | 10 hosts + 20 non-hosts | 30 non-hosts |
| 2               | First and second  | Host + non-host Px + Mb | Non-host Px + Mb |
|                 | First and second  | Host + non-host Px + Se | Non-host Px + Se |
|                 | First             | Host + non-host Px + Tn | Non-host Px + Tn |
|                 | Second            | Host + non-host Px + Ag | Non-host Px + Ag |
|                 | First and second  | Host + non-host Mb + Se | Non-host Mb + Se |
|                 | Second            | Host + non-host Mb + Tn | Non-host Mb + Tn |
|                 | First             | Host + non-host Mb + Se | Non-host Mb + Se |
|                 | Second            | Host + non-host Mb + Ag | Non-host Mb + Ag |
| No. individuals | 10 hosts + 10 non-hosts A + 10 non-hosts B | 15 non-hosts A + 15 non-hosts B |
| 3               | First and second  | Host + non-host Px + Mb + Se | Non-host Px + Mb + Se |
|                 | First             | Host + non-host Px + Mb + Tn | Non-host Px + Mb + Tn |
|                 | Second            | Host + non-host Px + Mb + Ag | Non-host Px + Mb + Ag |
|                 | First             | Host + non-host Px + Se + Tn | Non-host Px + Se + Tn |
|                 | Second            | Host + non-host Px + Se + Ag | Non-host Px + Se + Ag |
| No. 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               | Second            | Host + non-host Px + Mb + Se + Ag | Non-host Px + Mb + Se + Ag |
| No. individuals | 8 hosts + 5 non-hosts A + 5 non-hosts B + 5 non-hosts C | 7 non-hosts A + 7 non-hosts B + 7 non-hosts C + 7 non-hosts D |

*M. brassicae + S. exigua + T. ni.* Experiment 2 was conducted from October 2014 to February 2015 and used the combinations: host, host + *S. exigua*, host + *A. gamma*, and host + *P. xylostella + M. brassicae + S. exigua + A. gamma*. Plants were prepared as 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 10 host caterpillars.

Before the experiment started, three plants of the same treatment were placed ca. 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, Schaumburg, IL, USA) and Observer XT 10 software (Noldus Information Technology, Wageningen, The Netherlands), the duration and frequency of all but two of the following behavioural components of the parasitoid were recorded: off-plant excursions (flight excursions outside the vicinity of the plant), flying (in the vicinity of the plant), landing (on the plant), 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, and oviposition in a non-host. Of ‘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 at most 1 h, and was terminated earlier when the parasitoid was on an off-plant excursion for over 5 min. Parasitoids that flew to the walls of the tent immediately after release and stayed there for more than 1 min were considered unresponsive. Before a new observation was started, the plant that had been visited by the parasitoid was replaced by a new plant.
Host caterpillars were dissected and parasitoid eggs that had been injected were counted using a stereomicroscope.

**Statistical analysis**

To combine the two sets of experiments conducted in wind-tunnel experiment A, we first tested whether the overlapping treatments (i.e., those that did not include *T. ni* or *A. gamma*) gave 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 treatment on the log scale. None of the seven overlapping treatments indicated significant differences ($\alpha = 0.05$) between sets of experiments, therefore 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, therefore subsequently dropped from the model. Preference of parasitoids for infested plants was analysed with binomial tests per treatment or diversity level, testing the null hypothesis of equal preference for host- and non-host-infested plants vs. non-host only infested plants. Analysis for the second wind-tunnel experiment was performed similarly; overlapping treatments were compared using logistic regression analysis and combined, because no effects of experimental set were found.

Subsequently, logistic regression was used to compare treatments, and binomial tests to analyse 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 experiments on the logit (duration) or log (rate) scale. For 14 of the 26 behaviours and derived findings, no significant differences ($\alpha = 0.05$) were found between the overlapping treatments, thus data of these from both experiments were combined. Subsequently, generalized linear models were used on these combined data. For the duration of each behaviour expressed as a proportion of residence time, only those observations were included in which 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 min or 10 hosts. The rate (times per min) 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 v.17 (VSN International, Hemel Hempstead, UK).

**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 (Figure 1). The combined results indicate 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: \(P < 0.05\)) (Figure 2A). The distribution of this preference was equal for all non-host diversity levels (GLM: \(P = 0.52\)) (Figure 2A).

The effect of non-host species identity on parasitoid preference for plants infested with hosts and low or high non-host diversity was tested in wind-tunnel experiment B for five non-host species. Parasitoids did not display preferences for either plants containing a single non-host species or four non-host species (all binomial tests: \(P > 0.07\)) (Figure 2B), and this was similar for each of the five non-host species identities (GLM: \(P = 0.84\)) (Figure 2B).

**On-plant experiment**

The behaviour of 187 individual *C. glomerata* parasitoids was observed on plants either infested with only hosts, with hosts and a single non-host species (five combinations), or with hosts and four non-host species (two 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 the three other non-host species, compared with all other treatments, except for the infestation by hosts and *M. brassicae* (GLM: \(P = 0.003\)) (Figure 3A). The number of hosts parasitized was lowest in the presence of *T. ni* alone or in combination with the 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: \(P = 0.004\)) (Figure 3B).

The non-host species showed diverging effects on ‘duration of attack host’ (Figure S1A), ‘duration of attack non-host’ (Figure S1B), ‘duration of standing still’ (Figure S1C), ‘rate of encounter products’ (Figure S1H), ‘rate of flying’ (Figure S1E), ‘rate of landing’ (Figure S1F), and ‘rate of preening’ (Figure S1G) (for each GLM: \(P > 0.05\)). Overall, none of the species, nor the combinations of four species, clearly stood out in affecting the behaviour of the parasitoid. 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’ (Figure S1D), ‘rate of attack non-host’ (Figure S1I), ‘rate of oviposition non-host’ (Figure S1J), ‘eggs per oviposition’ (Figure S1K), and ‘eggs per parasitized host’ (Figure S1L) (for each GLM: \(P > 0.05\)).
Discussion

The presence of non-host herbivores affects parasitoid foraging efficiency for hosts (Bukovinszky et al., 2012; de Rijk et al., 2016b). Here we found that, when multiple non-host herbivores share the food plant with the host herbivore, the identity of these non-host herbivore species, rather than their diversity per se, affects foraging behaviour of the parasitoid *C. glomerata*. Presence of the non-host caterpillar *T. ni*, either alone or in combination with other non-host species, reduced the time *C. glomerata* spent on a plant and the number of *P. brassicae* hosts it parasitized.

In contrast with the behaviour of the parasitoid on the plant, parasitoid flight response to HIPVs emitted by plants infested with host and non-hosts was neither influenced by host 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, e.g., 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 derived from a food plant induced by a diversity of herbivores and when responding to a wide range of HIPVs, parasitoids might rely more on visual or chemical cues directly derived from the host in optimizing their foraging efficiency (Wäschke et al., 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 some 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* + non-host-infested plants over plants infested with only non-hosts (de Rijk et al., 2016b), like in our current study. Parasitoid foraging strategies might therefore differ between chemically simple vs. complex environments (Wäschke et al., 2013). During foraging in chemically simple environments in which non-hosts are scarce, the parasitoids might mainly rely on plant volatiles, whereas in complex environments direct visual or chemical host-cues could be of increased value in optimizing foraging efficiency.

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 *T. ni*. 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, regardless of whether other non-host species were present or not. The presence of *T. ni* also increased the time the parasitoids spent on attacking non-hosts. The parasitoid also spent longer on attacking *A. gamma* when this was the only non-host present, but the residence time on plants with this infestation was not affected. During these non-host attacks, *C. glomerata* has been found to oviposit eggs inside the non-host, and thus wastes potential offspring as the eggs deposited in non-hosts do not develop (Bukovinszky et al., 2012). 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. glomerata* 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, although *C. glomerata* may encounter this species as a native in other regions of its geographic range. Exotic herbivores have the potential to affect parasitoid behaviour (Chabaane et al., 2015), and thereby interactions within the ecological community (Desurmont & Pearse, 2014; Desurmont et al., 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, *S. 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 have been the source of altered parasitoid behaviour. Different foraging strategies are employed by *C. glomerata*, dependent on the host species for which the parasitoid is foraging (Vos et al., 1998; Vos & Hemerik, 2003). Therefore, whenever *T. ni* would feed on a plant together with another host species, e.g., *Pieris rapae* (L.), a different behavioural response might be elicited from the parasitoid.

Figure 3 On-plant behaviour of parasitoids. Mean (± SE) (A) residence time (s) of *Cotesia glomerata* and (B) number of *Pieris brassicae* hosts parasitized on *Brassica oleracea* plants infested with only hosts, hosts + one species of non-host, or hosts + four species of non-hosts. Plants were infested with (combinations of) host, *P. brassicae; Px*, *Plutella xylostella; Mb*, *Mamestra brassicae; Se*, *Spodoptera exigua; Tn*, *Trichoplusia ni; Ag*, *Autographa gamma*, in numbers as presented in Table 2. For the treatment ‘host’, 10 *P. brassicae* caterpillars were used. Means within a panel capped with different letters are significantly different, based on a generalized linear model and least significant differences (P<0.05).
Modelling studies found that 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 (Kratica 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 is consistent with the importance of species or group identity (Mouillot et al., 2011; Harvey et al., 2013; Hammill et al., 2015), group composition (Narwani & Mazumder, 2012), or a combination of diversity and identity (Cardinale et al., 2012) for ecosystem functioning.

The level of species diversity within a trophic level can influence processes in other trophic levels, which is shown for, e.g., the diversity of plants (Cardinale et al., 2011), plant genotypes (Abdala-Roberts & Mooney, 2014), predatory arthropods (Knop et al., 2014), microbial decomposers, and detritivorous invertebrates (Jabiol et al., 2013). In diversity-dependent interactions between trophic levels, edibility of resources can play an important part (Thébault & 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 multitrophic 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 reveals non-host effects over 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., 2012; 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 multitrophic 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 & de Mazancourt, 2013). Also in this context, species traits may be more important than diversity per se.

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References

Abdala-Roberts L & Mooney KA (2014) Ecological and evolutionary consequences of plant genotype diversity in a tri-trophic system. Ecology 95: 2879–2893.

van Alphen JJM, Bernstein C & Driesen G (2003) Information acquisition and time allocation in insect parasitoids. Trends in Ecology and Evolution 18: 81–87.

Arimura GI, Ozawa R & Maffei ME (2011) Recent advances in plant early signaling in response to herbivory. International Journal of Molecular Sciences 12: 3723–3739.

Bascompte J (2009) Disentangling the web of life. Science 325: 416–419.

Bukovinszky T, Poelman EH, Kamp A, Hemerik L, Prekatsakis G & Dicke M (2012) Plants under multiple herbivory: consequences for parasitoid search behaviour and foraging efficiency. Animal Behaviour 83: 501–509.

Cardinale BJ, Matulich KL, Hooper DU, Byrnes JE, Duffy E et al. (2011) The functional role of producer diversity in ecosystems. American Journal of Botany 98: 572–592.

Cardinale BJ, Dicke JE, Gonzalez A, Hooper DU, Perrings C et al. (2012) Biodiversity loss and its impact on humanity. Nature 486: 59–67.

Chabaane Y, Laplanche D, Turlings TCJ & Desurmont GA (2015) Impact of exotic insect herbivores on native tritrophic interactions: a case study of the African cotton leafworm, Spodoptera littoralis and insects associated with the field mustard Brassica rapa. Journal of Ecology 103: 109–117.

Colazza S, Cusumano A, Lo Giudice D & Peri E (2014) Chemo-orientation responses in hymenopteran parasitoids induced by substrate-borne semiochemicals. BioControl 59: 1–17.

Desurmont GA & Pearse IS (2014) Alien plants versus alien herbivores: does it matter who is non-native in a novel trophic interaction? Current Opinion in Insect Science 2: 20–25.

Desurmont GA, Harvey J, van Dam NM, Cristescu SM, Schiestl FP et al. (2014) Alien interference: disruption of infochemical networks by invasive insect herbivores. Plant, Cell and Environment 37: 1854–1865.

Dicke M, van Loon JJA & Soler R (2009) Chemical complexity of volatiles from plants induced by multiple attack. Nature Chemical Biology 5: 317–324.

Duffy JE, Cardinale BJ, France KE, McIntyre PB, Thébault E & Loreau M (2007) The functional role of biodiversity in ecosystems: incorporating trophic complexity. Ecology Letters 10: 522–538.

Geervliet JBF, Vet LEM & Dicke M (1994) Volatiles from damaged plants as major cues in long-range host-searching by the specialist parasitoid Cotesia rubecula. Entomologia Experimentalis et Applicata 73: 289–297.

Geervliet JBF, Vet LEM & Dicke M (1996) Innate responses of the parasitoids Cotesia glomerata and C. rubecula (Hymenoptera: Braconidae) to volatiles from different plant-herbivore complexes. Journal of Insect Behavior 9: 525–538.
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Godfray HCJ (1994) Parasitoids: Behavioral and Evolutionary Ecology. Princeton University Press, Princeton, NJ, USA.

Hammel E, Kratina P, Vos M, Petchey OL & Anholt BR (2015) Food web persistence is enhanced by non-trophic interactions. Oecologia 178: 549–556.

Hare JD (2011) Ecological role of volatiles produced by plants in response to damage by herbivorous insects. Annual Review of Entomology 56: 161–180.

Harvey E, Seguin A, Nozais C, Archambault P & Gravel D (2013) Identity effects dominate the impacts of multiple species extinctions on the functioning of complex food webs. Ecology 94: 169–179.

Jabiol J, McKie BG, Bruder A, Bernadet C, Gesnner MO & Chauvet E (2013) Trophic complexity enhances ecosystem functioning in an aquatic detritus-based model system. Journal of Animal Ecology 82: 1042–1051.

Knoe E, Zünd J & Sanders D (2014) Interactive prey and predator diversity effects drive consumption rates. Oikos 123: 1244–1249.

Knop E, Z€ebst, McKie BG, Bruder A, Bernadet C, Gesnner MO & Chauvet E (2013) Flexible parasitoid behavior overcomes distraction by position of host and non-host herbivores. Animal Behaviour 85: 1517–1528.

Kratina P, Vos M & Anholt BR (2007) Species diversity modulates predation. Ecology 88: 1917–1923.

Loreau M & de Mazancourt C (2013) Biodiversity and ecosystem stability: a synthesis of underlying mechanisms. Ecology Letters 16: 106–115.

McCormick AC, Unsicker SB & Gershenzon J (2012) The specificity of herbivore-induced plant volatiles in attracting herbivore enemies. Trends in Plant Science 17: 303–310.

Mouillot D, Villéger S, Scherer-Lorenzen M & Mason NWH (2011) Functional structure of biological communities predicts ecosystem multifunctionality. PLoS ONE 6: e17476.

Narwani A & Mazumder A (2012) Bottom-up effects of species diversity on the functioning and stability of food webs. Journal of Animal Ecology 81: 701–713.

Ponzio C, Gols R, Pieterse CMJ & Dicke M (2013) Ecological and phytohormonal aspects of plant volatile emission in response to single and dual infestations with herbivores and phytopathogens. Functional Ecology 27: 587–598.

Ponzio C, Gols R, Weldegergis BT & Dicke M (2014) Caterpillar-induced plant volatiles remain a reliable signal for foraging wasps during dual attack with a plant pathogen or non-host insect herbivore. Plant, Cell and Environment 37: 1924–1935.

de Rijk M, Dicke M & Poelman EH (2013) Foraging behaviour by parasitoids in multiple herbivore communities. Animal Behaviour 85: 1517–1528.

de Rijk M, Krijn M, Jenniskens W, Engel B, Dicke M & Poelman EH (2016a) Flexible parasitoid behavior overcomes distraction by position of host and non-host herbivores. Animal Behaviour 113: 125–135.

de Rijk M, Yang D, Engel B, Dicke M & Poelman EH (2016b) Feeding guild of non-host community members affects host-forging efficiency of a parasitic wasp. Ecology 97: 1388–1399.

Soler R, Badenes-Pérez FR, Broekgaarden C, Zheng SJ, David A et al. (2012) Plant-mediated facilitation between a leaf-feeding and a phloem-feeding insect in a brassicaceous plant: from insect performance to gene transcription. Functional Ecology 26: 156–166.

Stam JM, Kroeze A, Li Y, Gols R, van Loon JJA et al. (2014) Plant interactions with multiple insect herbivores: from community to genes. Annual Review of Plant Biology 65: 689–713.

Takabayashi J & Takahashi S (1990) An allelochemical elicits arrestment in Apanites karayi in feces of nonhost larvae Acanthoscelides obtectus. Journal of Chemical Ecology 16: 2009–2017.

Takabayashi J, Sabelis MW, Janssen A, Shiojiri K & van Wijk M (2006) Can plants betray the presence of multiple herbivore species to predators and parasitoids? The role of learning in phytochemical information networks. Ecological Research 21: 3–8.

Thébault E & Loreau M (2006) The relationship between biodiversity and ecosystem functioning in food webs. Ecological Research 21: 17–25.

Thiel A & Hoffmeister TS (2009) Decision-making dynamics in parasitoids of Drosophila. Advances in Parasitology, Vol. 70 (ed. by G Prevost), pp. 45–66. Academic Press, London, UK.

Vet LEM & Dicke M (1992) Ecology of inchochemical use by natural enemies in a tritrophic context. Annual Review of Entomology 37: 141–172.

Vos M & Hemerik L (2003) Linking foraging behavior to lifetime reproductive success for an insect parasitoid: adaptation to host distributions. Behavioral Ecology 14: 236–245.

Vos M, Hemerik L & Vet LEM (1998) Patch exploitation by the parasitoids Cotesia rubecula and Cotesia glomerata in multi-patch environments with different host distributions. Journal of Animal Ecology 67: 774–783.

Vos M, Berrocal SM, Karamaouna F, Hemerik L & Vet LEM (2001) Plant-mediated indirect effects and the persistence of parasitoid-herbivore communities. Ecology Letters 4: 38–45.

Wajnberg E (2006) Time allocation strategies in insect parasitoids: from ultimate predictions to proximate behavioral mechanisms. Behavioral Ecology and Sociobiology 60: 589–611.

Wäschke N, Meiners T & Rostás M (2013) Foraging strategies of parasitoids in complex chemical environments. Chemical Ecology of Insect Parasitoids (ed. by E Wajnberg & S Colazza), pp. 37–63. Wiley-Blackwell, Oxford, UK.

Zhang PJ, Broekgaarden C, Zheng SJ, Snoeren TAL, van Loon JJA et al. (2013) Jasmonate and ethylene signaling mediate whitefly-induced interference with indirect plant defense in Arabidopsis thaliana. New Phytologist 197: 1291–1299.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Behaviour of Cotesia glomerata parasitoids on plants infested with only hosts, hosts + one species of non-host, or hosts + four species of non-hosts as observed in the flight chamber experiment. Plants were infested with (combinations of) host, Pieris brassicae; Px, Plutella xylostella; Mb, Mamestra brassicae; Se, Spodopters exigua; Tn, Trichoplusia ni; Ag, Autographa gamma, in numbers as
presented in Table 2. For the treatment 'host', 10 *P. brassicae* caterpillars were used. Mean (± SE) proportion of residence time spent on (A) attack host, (B) attack non-host, (C) standing still, (D) off-plant excursions. Mean (± SE) rate per min of (E) flying, (F) landing, (G) preening, (H) encounter products, (I) attack non-host, (J) oviposit in non-host. Mean (± SE) number of eggs (K) per oviposition in host and (L) per parasitized host. Means within a panel capped with different letters are significantly different based on generalized linear models (P<0.05) and least significant differences (α = 0.05).