

Olfactory response of four aphidophagous insects to aphid- and caterpillar-induced plant volatiles

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Abstract Plants damaged by herbivores emit blends of volatile organic compounds (VOCs) that attract the herbivore's natural enemies. Most work has focussed on systems involving one plant, one herbivore and one natural enemy, though, in nature, plants support multiple herbivores and multiple natural enemies of these herbivores. Our study aimed to understand how different aphid natural enemies respond to aphid-induced VOCs, and whether attraction of the natural enemies that responded to aphid-induced VOCs was altered by simultaneous damage by a chewing herbivore. We used a model system based on *Brassica juncea* (Brassicaceae), *Myzus persicae* (Hemiptera: Aphididae) and *Plutella xylostella* (Lepidoptera: Plutellidae). *Ceraeochrysa cubana* (Neuroptera: Chrysopidae) did not show preferences for any plant odour, while *Cycloneda sanguinea* (Coleoptera: Coccinellidae) responded to undamaged plants over air but not to aphid-damaged plants over undamaged plants. Therefore, no further tests were carried out with these two species. *Chrysoperla externa* (Neuroptera: Chrysopidae) preferred aphid-damaged plants, but not caterpillar-damaged plants, over undamaged plants,

and preferred plants damaged by both herbivores over both undamaged plants and aphid-damaged plants. When tested for responses against undamaged plants, *Aphidius colemani* (Hymenoptera: Braconidae) preferred aphid-damaged plants but not plants damaged by caterpillars. Plants damaged by both herbivores attracted more parasitoids than undamaged plants, but not more than aphid-damaged plants. Thus, multiply damaged plants were equally attractive to *A. colemani* and more attractive to *C. externa* than aphid-damaged plants, while *C. cubana* and *C. sanguinea* did not respond to aphid-induced VOCs, highlighting how different natural enemies can have different responses to herbivore-damaged plants.

Keywords Tritrophic interactions · Multitrophic interactions · Induced plant defence · Volatile organic compounds · Indirect resistance · Semiochemicals

Introduction

In plant–insect communities, phenotypic changes in plants can affect the preference and performance of herbivores, as well as the foraging of herbivore natural enemies. When plants are damaged by herbivores, they can activate induced responses that increase resistance against the attacking herbivore (Karban 2011). These induced changes, however, can have cascading effects throughout the community through webs of indirect, trait-mediated interactions (Hare 2011). Changes in plant traits resulting from induced plant responses can modify traits of other organisms that interact with the plant, such as other herbivores that use the plant as a resource (Denno et al. 1995; Soler et al. 2012; Van Zandt and Agrawal 2004; Viswanathan et al. 2005, 2007), pollinators that visit the flowers (Kessler

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et al. 2011) and defences of neighbouring plants (Karban et al. 2000). Upon damage, plants change their profile of volatile organic compounds (VOCs) and natural enemies of the herbivores detect these changes and use these cues for host location, in what is known as “indirect defence” (Heil 2008). This has been widely demonstrated for different groups of insect natural enemies such as parasitoids (De Moraes et al. 1998; Guerrieri et al. 1993; Turlings et al. 1990), invertebrate predators (Hatano et al. 2008; Lins et al. 2014; Reddy 2002), entomopathogenic nematodes (Hiltpold et al. 2010; Rasmann et al. 2005, 2011) and insectivorous birds (Mäntylä et al. 2008).

Most effects of induced plant VOCs on herbivore natural enemies have been detected in systems consisting of a single plant, a single herbivore and a single natural enemy. However, in nature, plants are attacked by a suite of herbivores, either simultaneously or sequentially, and each of these herbivores can be attacked by several natural enemies. Often, plants have specific responses to each herbivore, and natural enemies also can respond selectively to plant VOCs induced by suitable hosts or prey and not to those induced by unsuitable hosts or prey (De Moraes et al. 1998; Du et al. 1996). This specificity is due to the activation of different biochemical pathways that are involved in VOC production. Damage by chewing herbivores has been related to the activation of jasmonic acid (JA)-dependent responses, while damage caused by phloem-feeding insects has been related to salicylic acid (SA)-dependent responses in the plant (Kant et al. 2015; Moran and Thompson 2001; Walling 2000). However, this picture is more complex, with extensive interactions between signalling pathways and herbivore manipulation of plant responses (Kant et al. 2015; Thaler et al. 2012; Walling 2008). These pathways can show negative crosstalk, and activation of one response can interfere with the other (Bostock 2005; Zhang et al. 2013b), and this can affect natural enemies that use plant volatiles to locate their hosts or prey (Zhang et al. 2009). Some herbivores can use this crosstalk to their advantage, inducing biochemical processes that act antagonistically to those involved in defence, and therefore shutting off the effective defences (Walling 2008; Zarate et al. 2007). However, parasitoids may have evolved to detect these interferences and can still find host plants (Zhang et al. 2013a).

Interactions between induced responses to different herbivores raise two important questions we are only beginning to answer. The first is how different natural enemies foraging in the same tritrophic environment respond to plant VOCs and how these responses depend on community context and influence community structure (Stam et al. 2014). As seen above, induced plant VOCs can mediate many interactions of an individual plant with associated organisms. Since the presence and abundance of

herbivore natural enemies is variable both spatially and temporally, we need to understand the responses of multiple natural enemies to induced plant defences, since changes in community composition might change the benefits of induced defences to the plant. The second important question is how much multiple herbivory affects these multitrophic interactions. It can be hypothesised that the negative interactions between induced defences described above should lead to interference in the attraction of herbivore natural enemies, in particular when the herbivores belong to different feeding guilds. It is known that aphids can interfere with VOCs induced by caterpillars (Schwartzberg et al. 2011) and that aphids can shut down VOC production in distal parts of the plant (Pareja et al. 2012), so this hypothesis is appealing. Studies have addressed several systems comprising different combinations of caterpillars, phytophagous mites and phloem-feeders, such as aphids and whiteflies. So far, multiple damage to plants has been shown to reduce attraction of natural enemies (Shiojiri et al. 2000, 2001; Zhang et al. 2009), enhance attraction (de Boer et al. 2008; Lins et al. 2014; Moayeri et al. 2007; Oliveira and Pareja 2014; Shiojiri et al. 2000, 2001) or have demonstrated no effect, with multiply damaged plants being as attractive as those suffering single-species damage (Agbogba and Powell 2007; Erb et al. 2010; Oliveira and Pareja 2014; Ponzio et al. 2014). Different natural enemies can differ in their degree of specialisation, and this could greatly affect the response to plant VOC blends. It could be expected that multiple damage would interfere more acutely with responses by specialised natural enemies, since these need to detect the highly specific cues that signal presence of their prey or host.

The aim of this study was to understand how four aphidophagous species respond to VOCs emitted by plants damaged by an aphid species. We also investigated whether attraction of the natural enemies that responded to aphid-induced VOCs was altered by simultaneous damage by a chewing herbivore. We studied *Brassica juncea* L. (Brassicaceae) damaged by the aphid *Myzus persicae* Sulzer (Hemiptera: Aphididae) and the caterpillar *Plutella xylostella* L. (Lepidoptera: Plutellidae). The responses of the aphid natural enemies to *B. juncea* VOCs were studied in a Y-tube olfactometer. We used three species of aphid predators—*Ceraeochrysa cubana* Hagen, *Chrysoperla externa* Hagen (Neuroptera: Chrysopidae) and *Cycloneda sanguinea* (L.) (Coleoptera: Coccinellidae) and the parasitoid *Aphidius colemani* Viereck (Hymenoptera: Braconidae). These species provide a contrast in terms of specialisation and foraging strategies. The chrysopids have predatory larvae that feed preferentially on aphids, but can also prey on other insects, in particular on eggs. Since the adults do not prey on aphids, female responses are purely

for oviposition. The ladybird *C. sanguinea* is a specialised aphid predator in both the adult and larval stages. The parasitoid *A. colemani* has, for a parasitoid, a broad host range, and attacks several aphid species. These species are all important aphid natural enemies in agricultural systems. All these species are increasingly regarded as important predators in agroecosystems, and *A. colemani* is also reared commercially in several countries.

Materials and methods

Plant growth and preparation

Brassica juncea seeds were germinated in garden compost (Topseed Garden—Agristar do Brasil Ltd. *Pinus* bark-based, supplemented with superphosphate and potassium nitrate, pH 5.8) and allowed to grow for 15 days in a greenhouse. At this age they were transplanted into 300 mL pots containing a 1:1 mixture of local oxisol and garden compost (described above). When plants reached 5–7 weeks of age (5–6 fully expanded leaves), they were used in the experiments.

Insect rearing

Myzus persicae and *P. xylostella* were collected from cabbage growing in the experimental fields of the Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil. *Myzus persicae* was reared in cages on live *Nicotiana glauca* (Solanaceae) plants and in 15 cm Petri dishes containing *N. glauca* leaves embedded in a 1 cm layer of 1 % agar. *Plutella xylostella* was reared by placing the larvae on *Brassica oleracea* var. *acephala* leaves in ventilated 30 × 15 × 7 cm plastic boxes. When the larvae pupated, the pupae were separated and placed in an acrylic rearing cage containing *B. oleracea* leaves for the emerging adults to lay eggs upon. The leaves with the eggs were placed in the rearing boxes for the larvae to emerge. Both insects were reared at 22 ± 2 °C, 70 ± 10 % relative humidity and 12:12 h photoperiod. During the experimental period, *M. persicae* colonies had been reared in the laboratory for 10–15 months, while *P. xylostella* had been reared for 18–23 months.

Four different aphid natural enemy species were reared in the laboratory. *Cycloneda sanguinea* adults were collected on the campus of the Universidade Federal de Lavras (UFLA) and were reared in 10 × 10 cm (height × diameter) PVC cylinders lined with filter paper and covered with transparent PVC cling film. The adult ladybirds were fed daily with a mixture of field-collected *Lipaphis erysimi* and *Uroleucon ambrosiae* (Hemiptera: Aphididae), in the absence of host plants. Their diet was

complemented with *Ephestia* (= *Anagasta*) *kuehniella* (Lepidoptera: Pyralidae) eggs. Ladybird eggs were removed daily and placed in Petri dishes covered with PVC cling film containing *E. kuehniella* eggs. After eclosion each larva was placed individually in an 8.5 × 2.5 cm (height × diameter) test tube and fed with *E. kuehniella* eggs and aphids during the first instar, with aphids only from the second instar onwards. All *C. sanguinea* rearing was carried out at 25 ± 2 °C, 70 ± 10 % relative humidity and 12:12 h photoperiod. Adults were used in experiments 2–5 days after emergence. During the experimental period, *C. sanguinea* had been reared in the laboratory for a period of 10–15 months.

The chrysopids *C. cubana* and *C. externa* were obtained from established rearing colonies at the Universidade Federal de Lavras (UFLA) that were approximately 3 years old, but that undergo yearly re-stocking with field-collected individuals. Adults were kept in 45 × 90 × 50 cm acrylic cages and fed with a mixture of honey and yeast. Eggs were removed weekly and placed individually in 8.5 × 2.5 cm (height × diameter) test tubes. Once the larvae emerged, they were fed with *E. kuehniella* eggs. Pupae were transferred to cages containing cotton wool soaked in a mixture of honey and yeast. Adult females were used in experiments 10–12 days after emergence. Rearing was carried out at 25 ± 2 °C, 70 ± 10 % relative humidity and 12:12 h photoperiod.

For rearing *A. colemani*, *M. persicae* mummies were collected on the campus of the Universidade Federal de Lavras (UFLA). In order to guarantee colony purity, single adult females were placed in a Petri dish with approx. 50 *M. persicae* and allowed to oviposit. When the adults emerged from the mummies, a few were identified and, if confirmed as *A. colemani*, the remainder were placed in a rearing cage (45 × 90 × 50 cm) containing *M. persicae* feeding on *N. glauca* plants. This was carried out regularly in order to maintain genetic diversity in the colony. Rearing was carried out at 19 ± 2 °C, 70 ± 10 % relative humidity and 12:12 h photoperiod. *A. colemani* colonies were reared in the laboratory for a period of 10–15 months when used in experiments. For use in experiments, adult females were removed directly from the rearing colony and left in the bioassay room for 2 h to acclimatise.

Olfactory responses

To assess the olfactory responses of aphid natural enemies, we used glass Y-tube olfactometers as a bioassay arena. The Y-tube used for the lacewings had a 2 cm internal diameter, a 15 cm trunk and 12 cm arms, with a 120° angle between the arms. The tube used for the ladybird and the parasitoid had a 1 cm internal diameter, a 12 cm trunk and

9 cm arms and a 90° angle between the two arms. For the lacewings and the parasitoid, the Y-tube was placed horizontally on a white bench, while for the ladybird, the Y-tube was placed with the two arms angled upwards at 45° to allow for the negative geotaxis common in ladybirds (Almeida and Ribeiro-Costa 2009) and that we also observed for this species (Mayara Oliveira and Martín Pareja, personal observation). The plants were placed in glass vessels, into which charcoal-filtered air was pumped. The vessels were connected to the olfactometer with Teflon (PTFE) tubes that carried the odour into the bioassay arena at 750 mL/min/arm. The insects were tested individually, introducing them into the base of the olfactometer and observing them for 10 min. If the insect entered 2 cm into one of the arms and remained for at least 15 s, it was considered to have made a choice, since dry ice tests indicated that this distance was beyond the mixture area in the olfactometer junction. If the insect did not make a choice after 10 min, it was considered a non-responder. After each insect tested, the Y-tube was swapped for a clean tube and the side the odours were presented was swapped every three insects tested. Each insect was used only once. Before setting up the bioassay system, all glassware and Teflon tubing was cleaned with ethanol and placed in an oven at 120 °C for at least 3 h.

For the initial experiments, we compared the olfactory response of the four aphid natural enemies to the following odour comparisons: (1) undamaged plant versus clean air; (2) undamaged plant versus plant damaged by 100 *M. persicae*. For the natural enemies that showed a preference for the aphid-damaged plant, we carried out further bioassays; (3) undamaged plant versus plant damaged by three third-instar *P. xylostella* larvae; (4) undamaged plant versus plant damaged simultaneously by 100 *M. persicae* and three *P. xylostella* larvae; (5) plant damaged by 100 *M. persicae* versus plant damaged simultaneously by 100 *M. persicae* and 3 *P. xylostella* larvae. A bioassay was also carried out to test the bioassay setup without any odour in the olfactometer to confirm that the insects displayed positive anemotaxis in the arena, presenting clean air in both arms. After ten insects were tested, the plants were swapped for new plants in order to replicate the odour sources. The total number of insects tested was 100 for *C. cubana*, 110 for *C. sanguinea*, 80 for *C. sanguinea* and 60 for *A. colemani* for each test combination. For the lacewings *C. cubana* and *C. externa*, as well as for the parasitoid *A. colemani*, only mated females were used, since male adults are not aphidophagous. For the ladybird *C. sanguinea* both males and females were used, and the individuals were sexed after each individual responded. All insects used were naive.

To carry out the damage treatments, plants were infested with 100 mixed instars of *M. persicae* or three-third instar *P.*

xylostella larvae. All plants, including control plants, were placed in porous plastic bags and left for 3 days at 22 ± 2 °C, 70 ± 10 % relative humidity and 12:12 h photoperiod. After 3 days of damage, and just before beginning the bioassays, all herbivores were removed and the plants were carefully swabbed with cotton tips dipped in warm water in order to remove traces of honeydew and faeces. The same procedure was carried out on the undamaged plants in order to control for any effect of the manipulation.

Statistical analyses

The choices made by each insect were modelled using a binomial generalised linear model (logistic regression), fitting “plant combination” as a blocking factor. For *C. sanguinea* “sex” was also fitted to control for differences in male and female responses. The significance of the response was carried out using a Wald χ^2 test on the model intercept. Effects of plant combination and sex (only for *C. sanguinea*) were tested by model simplification and a χ^2 test on the change in model residual deviance.

Results

None of the predators showed a directional response (no preference for a given side independent of odour stimulus) when only clean air was presented through both arms (*C. cubana*— $\chi^2 = 0.267$, $P = 0.606$; *C. externa*— $\chi^2 = 0.016$, $P = 0.898$; *C. sanguinea*— $\chi^2 = 0.089$, $P = 0.765$; *A. colemani*— $\chi^2 = 0.133$, $P = 0.715$; Figs. 1, 2, 3), confirming that the bioassay arena was unbiased. When an undamaged *B. juncea* plant was presented against clean air, neither *C. cubana* ($\chi^2 = 3.419$, $P = 0.064$; Fig. 1) nor *C. externa* ($\chi^2 = 0.441$, $P = 0.506$; Fig. 2) showed a preference for the plant odour. *Cycloneda sanguinea*, on the other hand, showed a clear preference for the odour of the plant ($\chi^2 = 5.040$, $P = 0.024$; Fig. 1), as did *A. colemani* ($\chi^2 = 14.892$, $P < 0.001$; Fig. 3).

When aphid-damaged plants were presented against undamaged plants to the different aphid natural enemies, *C. cubana* showed no response to aphid-induced odours ($\chi^2 = 0.307$, $P = 0.580$; Fig. 1), and neither did *C. sanguinea* ($\chi^2 = 0.996$, $P = 0.318$; Fig. 1). *Chrysoperla externa* did, however, show a preference for aphid-damaged plants ($\chi^2 = 8.697$, $P = 0.003$; Fig. 2), as did *A. colemani* ($\chi^2 = 16.459$, $P < 0.001$; Fig. 3). Because *C. cubana* and *C. sanguinea* did not show any responses to aphid-damaged plants, no further bioassays were carried out with these species. Plants damaged by *P. xylostella* were not more attractive than undamaged plants to *C. externa* ($\chi^2 = 0.471$, $P = 0.493$; Fig. 2), nor to *A. colemani* ($\chi^2 = 2.531$, $P = 0.112$; Fig. 3).

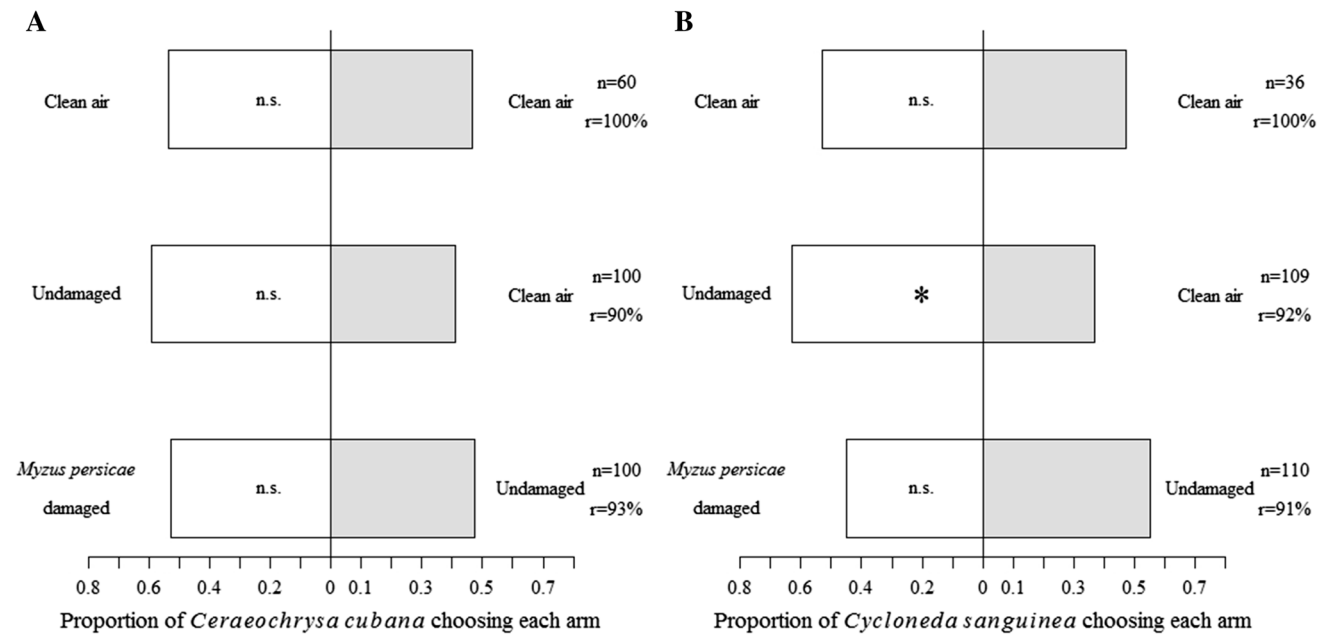
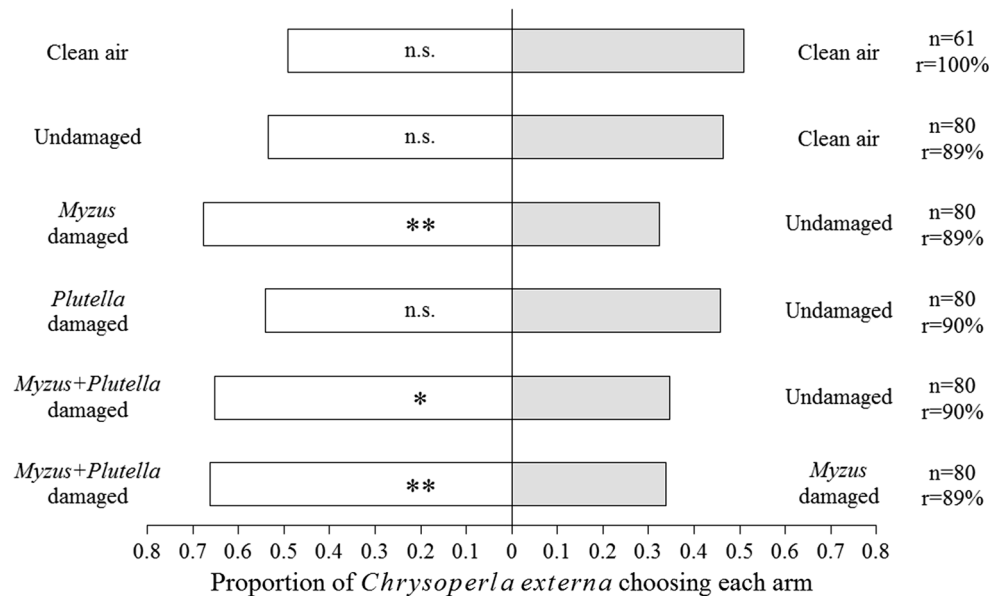


Fig. 1 Olfactory response of **a** *Ceraeochrysa cubana* and **b** *Cycloneda sanguinea* in Y-tube olfactometer experiments to odours from undamaged *Brassica juncea* and *B. juncea* damaged by *Myzus persicae* (proportion of responding insects that chose each odour). The clean air versus clean air experiment was carried out to test for

directional bias. *n* indicates the total number of insects tested, while *r* is the percentage that responded choosing one of the odours. Analyses were carried out with a Wald χ^2 test on the logistic regression intercept—*n.s.* not significant, $0.05 > P > 0.01$

Fig. 2 Olfactory response of *Chrysoperla externa* in Y-tube olfactometer experiments to odours from undamaged *Brassica juncea* and *B. juncea* suffering different combinations of single-species or two-species damage (proportion of responding insects that chose each odour). The clean air versus clean air experiment was carried out to test for directional bias. *n* indicates the total number of insects tested, while *r* is the percentage that responded choosing one of the odours. Analyses were carried out with a Wald χ^2 test on the logistic regression intercept—*n.s.* not significant, $0.05 > P > 0.01$

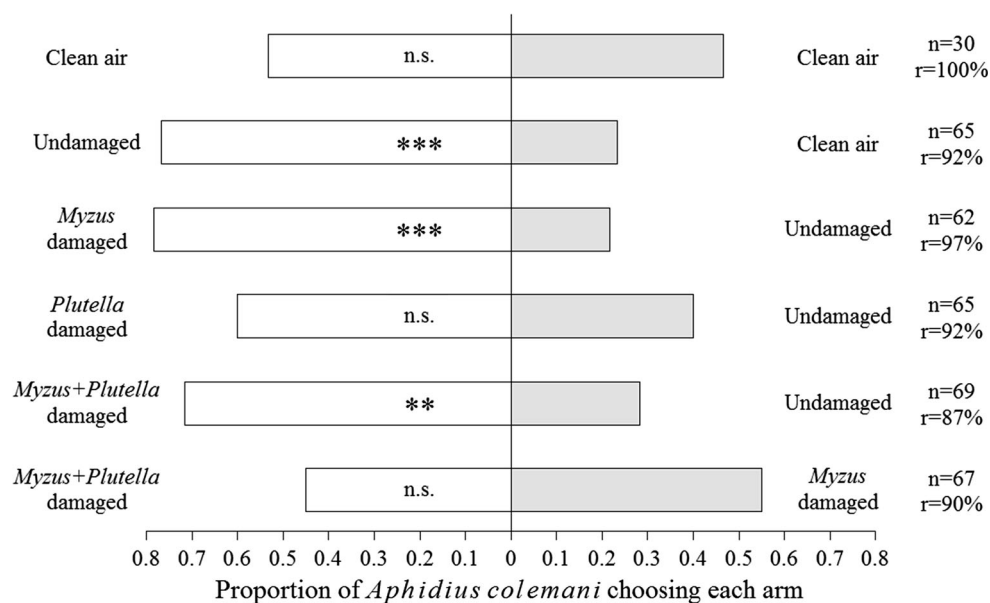


When multiple damage was carried out on *B. juncea*, *C. externa* were more attracted to multiply damaged plants than to undamaged plants ($\chi^2 = 6.513$, $P = 0.011$) or *M. persicae*-damaged plants ($\chi^2 = 7.161$, $P = 0.008$; Fig. 2). *Aphidius colemani* was also more attracted to plants damaged by both herbivores than to undamaged plants ($\chi^2 = 10.569$, $P = 0.001$), but did not discriminate

between multiply damaged plants and *M. persicae*-damaged plants ($\chi^2 = 0.621$, $P = 0.431$; Fig. 3).

Plant effects were non-significant in all but one of the analyses. For *C. sanguinea* response to undamaged versus *M. persicae*-damaged plants, there was significant variation between the different odour sources presented ($\chi^2 = 28.394$, $P < 0.001$). None of the analyses of *C.*

Fig. 3 Olfactory response of *Aphidius colemani* in Y-tube olfactometer experiments to odours from undamaged *Brassica juncea* and *B. juncea* suffering different combinations of single-species or two-species damage (proportion of responding insects that chose each odour). The clean air versus clean air experiment was carried out to test for directional bias. *n* indicates the total number of insects tested, while *r* is the percentage that responded choosing one of the odours. Analyses were carried out with a Wald χ^2 test on the logistic regression intercept—*n.s.* not significant, $*0.05 > P > 0.01$



sanguinea response revealed differences between males and females.

Discussion

The natural enemies of herbivores must locate their hosts in a complex habitat, consisting of multiple plant species, damaged by different herbivores. Different species of natural enemies will use different strategies to separate the signal from the noise contained in complex VOC mixtures. In this study, we showed that certain aphid natural enemies are able to distinguish the odours emitted by damaged plants, whereas others did not respond herbivore-induced odours. The lacewing *C. cubana* showed no response to constitutive or aphid-induced plant VOCs. *Cycloneda sanguinea* responded to constitutive but not to aphid-induced VOCs. *Chrysoperla externa* did not respond to the constitutive plant VOC blend, but showed a strong response to both aphid and caterpillar-induced VOCs, and an even stronger response to plants damaged by both herbivores. The parasitoid *A. colemani* showed more specific responses, preferring aphid-induced odours but not caterpillar-induced odours. It did not differentiate between multiply damaged plants and those damaged solely by the aphid. These aphid natural enemies have different degrees of specialisation, as well as very different foraging strategies. For example, the lacewings are foraging for oviposition sites, while the ladybird is foraging for both food and oviposition sites. Thus, the different responses could be due to differences in specialisation, different needs at different points in the life cycle and different reliance on olfactory cues and other sensory modalities. It is, however,

interesting to note that the two most similar species (the two lacewings) differed greatly in their use of olfactory cues in this system.

In a different plant-aphid system, *C. sanguinea* was shown to respond to constitutive and aphid-induced VOCs from sweet pepper, *Capsicum annuum* (Oliveira and Pareja 2014), so the response of individual predator and parasitoid species is proving to be system specific since the same methods were used in both studies. The same species did not respond to undamaged tomato, but showed a response to aphid-damaged and mite-damaged tomato (*Solanum lycopersicum*) plants (Sarmiento et al. 2007, 2008). There is no previous information for olfactory responses of *C. cubana* to plant volatiles, but this species did not appear to use VOCs for foraging in our experiments. Predators have been shown to rely on experience and learning (Drukker et al. 2000). Neither *C. sanguinea* nor *C. cubana* showed responses to induced VOCs when naïve, so it is possible that they use flexible foraging strategies that depend on experience and can be learnt during adult life (Steidle and van Loon 2003; Vet and Dicke 1992). Furthermore, evolutionary history is likely to affect the sensitivity of natural enemies to plant VOCs. It is possible that native insects, such as these, do not detect the induced VOCs from an introduced plant such as *B. juncea* (Desurmont et al. 2014). It is, however, interesting to note that Oliveira and Pareja (2014) used *C. sanguinea* reared in the same way and found a response to aphid-induced VOCs from sweet pepper. The lacewing *C. externa*, on the other hand, showed a distinct response to aphid-damaged plants, as did the parasitoid *A. colemani*. A previous study has demonstrated response of *C. externa* to induced plant VOCs, and methyl salicylate appeared to play an important role

(Salamanca et al. 2015). Response to plant VOCs has been documented with other Chrysopidae, including *Chrysoperla* species (Han and Chen 2002a, b, c; Reddy 2002; Reddy et al. 2002, 2004; Tóth et al. 2006, 2009; Zhu et al. 1999, 2005). Responses of *A. colemani* to aphid-induced cues are very well documented (Agbogba and Powell 2007; Kalule and Wright 2004; Lo Pinto et al. 2004; Storeck et al. 2000). As is common for parasitoids, we found that *A. colemani* was not attracted to non-host induced VOCs in this system, adding to the evidence that these insects are very efficient at detecting plants damaged by hosts and non-hosts (De Moraes et al. 1998; Du et al. 1996).

We hypothesised that simultaneous damage by *P. xylostella* on aphid-damaged plants would interfere with the production of aphid-induced VOCs by the plant, and therefore attraction of the aphid natural enemies would be compromised. This hypothesis was based on the evidence that suggests that biosynthetic pathways induced by chewing herbivores and those induced by phloem-feeders are different and they can interfere with each other via negative crosstalk (Bostock 2005; Koornneef and Pieterse 2008; Moran and Thompson 2001; Thaler et al. 2012; Zarate et al. 2007). Aphid feeding has been shown to interfere with caterpillar-induced VOC emissions (Schwartzberg et al. 2011) and can even affect VOC release by flowers (Pareja et al. 2012). Since insects are extremely sensitive to minor variations in ratios between compounds in VOC profiles (Bruce and Pickett 2011; Pareja et al. 2009), this crosstalk between signalling pathways could interfere with natural enemy attraction. We did not, however, observe any interference in the behaviour of aphid natural enemies when *P. xylostella* simultaneously damaged the plant. *Chrysoperla externa* is not known to prey on *P. xylostella*, though other species in the genus can prey on *P. xylostella* eggs and second instar larvae (Salas-Araiza et al. 2015), so it is possible that odour from plants damaged by this species represents a reliable chemical cue. The response by this lacewing to multiply damaged plants over aphid-damaged plants is more difficult to explain. It is possible that in this system, aphids and caterpillars are inducing pathways with positive crosstalk, resulting in enhanced responses by natural enemies. Responses by *A. colemani* are very similar to those presented by Ponzio et al. (2014) for the parasitoid *Cotesia glomerata*. These authors found that *C. glomerata* was more attracted to *Brassica nigra* with dual damage by its host *Pieris brassicae* and the aphid *Brevicoryne brassicae* than to undamaged plants. Also, dual damage was equally attractive to *C. glomerata* as plants damaged only by its host (Ponzio et al. 2014). Thus, it appears that parasitoids might be very fine-tuned to VOCs indicating host presence, and it is likely that parasitoid larvae could develop equally well, or even better, on multiply damaged

plants, as has been demonstrated for the aphid parasitoid *Diaeretiella rapae* (Soler et al. 2012).

For predators, enhancement of attraction to plants suffering multiple damage has been demonstrated in a few systems (de Boer et al. 2008; Lins et al. 2014; Moayeri et al. 2007), reduction of attraction in one system (Zhang et al. 2009) and a neutral effect in another (Lins et al. 2014). For parasitoids, we have information on a greater number of systems, but results are also variable (de Rijk et al. 2013). In the cabbage system, attraction of one parasitoid species can be enhanced by multiple damage, while attraction of another parasitoid is reduced (Shiojiri et al. 2000). Adding to this complexity, both parasitoid and predator experience can modulate responses through learning (Erb et al. 2010; Lins et al. 2014). A key factor that could explain these differential responses is how natural enemies perform on plants that are suffering multiple damage, since the defensive state of the plant can affect the quality of the herbivore as a host or prey item. Soler et al. (2012) showed that multiple damage to the plant does not have a negative effect on parasitoid performance. However, these effects could be highly system dependent, since plant-mediated interactions between herbivores can be asymmetrical (Soler et al. 2012; Uesugi et al. 2013; Van Zandt and Agrawal 2004; Viswanathan et al. 2005, 2007), and these asymmetries could potentially trickle through the community. If there is facilitation between herbivores, and natural enemies of herbivores perform better on multiply damaged plants (Soler et al. 2012), then the olfactory response of natural enemies could have evolved to prefer these plants, since higher quality hosts can lead to increased performance (Van Emden and Kifle 2002). On the other hand, if there is reduced host quality on multiply damaged plants, then the olfactory responses could have evolved to avoid these plants and, therefore, avoid low-quality hosts. Thus, olfactory responses by natural enemies could be highly dependent on the result of these plant-mediated interactions between herbivores. This link between herbivore performance on induced plants and natural enemy performance deserves more attention.

This study demonstrated how different members of an aphidophagous guild respond to plant VOCs when undamaged, damaged by the aphid *M. persicae* and multiply damaged by the aphid and *P. xylostella* caterpillars. Responses were clearly different between different aphid natural enemy species, highlighting how different aphid natural enemies can have different responses to the same chemical cues. Furthermore, olfactory responses by *C. externa* and *A. colemani* were not compromised by the presence of damage by *P. xylostella*. Thus, multiple herbivore damage on the plant did not, in this system, hinder the olfactory responses observed. These results suggest

that, if we are to understand the evolution of indirect plant defence, we must consider the community of natural enemies associated with the plant and the herbivores. Further studies, in particular incorporating field experiments and natural enemy performance on multiply damaged plants, will help to elucidate the community-wide effects of induced defences, as well as the evolutionary pressures shaping tritrophic interactions.

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