

Attraction of a ladybird to sweet pepper damaged by two aphid species simultaneously or sequentially

Mayara Silva Oliveira · Martín Pareja

Received: 15 August 2014 / Accepted: 14 October 2014 / Published online: 22 October 2014
© Springer Science+Business Media Dordrecht 2014

Abstract Natural enemies of insect herbivores use volatile organic compounds (VOCs) released by plants to locate suitable patches for foraging. When a plant is damaged by herbivores, it can modify its VOC profile, enhancing attraction of the herbivore's natural enemies. In both natural and agricultural ecosystems, plants are usually attacked by more than one species of herbivore, either simultaneously or sequentially. Different herbivores, even those with similar feeding habits, can activate different defensive responses in the same plant and this can alter the attraction of natural enemies. This study aimed to understand how simultaneous and sequential damage to sweet pepper, *Capsicum annuum*, by two aphid species, *Aphis gossypii* and *Myzus persicae*, affects attraction of the aphid predator *Cycloneda sanguinea* to plant odours. Undamaged plants did not attract *C. sanguinea*, but plants damaged by either aphid were attractive to this predator. When plants were damaged simultaneously, the predator did not distinguish between the odours of plants damaged by both species from those damaged by a single species. When damage was sequential, a certain combination of damage (*A. gossypii* followed by *M. persicae*) made the plants more attractive than plants damaged by *A. gossypii* only.

Odour from plants from all other combinations of sequential damage proved equally attractive to *C. sanguinea* as plants damaged by a single species. The study allows us to conclude that, unlike for simultaneous damage, a specific sequence of sequential damage enhances attraction of the predator. This could potentially create enemy-dense space on previously damaged plants.

Keywords Tritrophic interactions · Multitrophic interactions · Induced defence · Indirect defence · Indirect interactions · Volatile organic compounds

Introduction

Upon damage by herbivores, plants change the profile of volatile organic compounds (VOCs) they release as part of an induced defensive response. These changes play important ecological roles such as repellence of herbivores (Bernasconi et al. 1998; De Moraes et al. 2001) and especially attraction of natural enemies of the herbivores, who use these VOCs to locate profitable patches for foraging (reviewed by Hare 2011; Heil 2008). Natural enemies of herbivores can respond to VOCs characteristic of general damage released after herbivory (Hare and Sun 2011; Hoballah and Turlings 2005; Kessler and Baldwin 2001), but generally they show very strong responses to plants damaged by herbivores that are suitable hosts or prey (Du et al. 1996, 1998; Turlings et al. 1990). Parasitoids in particular have been shown to distinguish between plants damaged by hosts and those damaged by non-host species, even when non-host species cause similar feeding damage to the plant (De Moraes et al. 1998; Du et al. 1996; Moraes et al. 2005). Predators have also been shown to have strong responses to induced plant VOCs (Gencer et al.

Handling Editor: Robert Glinwood.

M. S. Oliveira
Programa de Pós-Graduação em Entomologia, Universidade Federal de Lavras—UFLA, Campus Universitário, Caixa Postal 3037, Lavras, MG CEP 37200-000, Brazil

M. Pareja (✉)
Departamento de Biologia Animal, Universidade Estadual de Campinas—UNICAMP, Rua Monteiro Lobato 255, Caixa Postal 6109, Campinas CEP 13083-862, Brazil
e-mail: martinpareja@gmail.com

2009; Han and Chen 2002; Ninkovic et al. 2001; Reddy 2002) and can distinguish between plant VOCs induced by prey from those induced by non-prey (de Boer et al. 2004), but these responses can be modified by rearing and experience (de Boer et al. 2005; Lins Jr. et al. in press). The non-host species on the plant can also be important, since species that cause similar damage to the plant can also attract predators, while those that cause different damage may not always attract (van Poecke et al. 2003). These specific responses have been shown to be due to the intimate interaction between insect feeding and plant metabolic responses. Characteristics of herbivore saliva (Alborn et al. 1997; Mattiacci et al. 1995; Musser et al. 2002) combined with the type and timing of damage inflicted on the plant (Hoballah and Turlings 2005) will induce unique VOC profiles due to differential activation of plant metabolic pathways. In particular, chewing damage is related to activation of jasmonic acid (JA)-dependent responses, while damage by phloem-feeding insects is related to activation of salicylic acid (SA)-dependent responses (Moran and Thompson 2001), and these signalling pathways often show negative crosstalk (Bostock 2005; Zarate et al. 2007; Zhang et al. 2009, 2013), which can interfere with direct defence against herbivores (Soler et al. 2012, 2007a). Some studies, however, have shown that some species of parasitoids and predators do not have specific responses and are attracted to plants damaged by non-host herbivores (van Poecke et al. 2003). This implies that the presence of non hosts can have negative effects on natural enemy foraging efficiency.

Most of this knowledge has been obtained studying systems consisting of a plant damaged by a single herbivore. However, in both natural and agricultural ecosystems, plants are usually attacked by several herbivore species either simultaneously or in sequence. In order to gain understanding of the evolutionary pressures structuring multitrophic communities, we need more information on how herbivore natural enemies deal with information from plants damaged by more than one species of herbivore. When two herbivores feed on the same plant, the effects on induced VOCs are likely to be highly influenced by the feeding guild of the herbivores. Therefore, it is expected that multiple herbivory on the same plant should influence attraction of carnivores, and this effect will necessarily depend on the identity of the herbivores. Herbivores of the same feeding guild might amplify the induction of attractive VOCs, while those of different feeding guilds could inhibit this induction. Though appealing, evidence so far shows these interactions are more complicated.

As for single-species damage, most studies to date have focussed on parasitoids, and damage by multiple species can enhance, reduce or have no effect on attraction of these natural enemies. Studies by Shiojiri and co-workers have shown that

C. plutellae attraction to cabbage was reduced when damaged simultaneously by its host and by a non-host caterpillar, while attraction of *C. glomerata* was enhanced (Shiojiri et al. 2000, 2001). When damaged by herbivores from different feeding guilds, we would expect greater interference in the production of attractive VOCs. It has been demonstrated that phloem feeders can inhibit plant VOCs induced by caterpillars (Schwartzberg et al. 2011) and practically shut off volatile production in distal parts of the plant (Pareja et al. 2012), though we do not know if this interference affects parasitoid behaviour. Studies by Agbogba and Powell (2007) and Erb et al. (2010) showed that plants damaged by herbivores from different feeding guilds can be equally attractive to parasitoids when compared to host-damaged plants, while Soler et al. (2007b) showed that some parasitoids of foliar caterpillars may avoid plants with root herbivores, probably because there can be a negative effect on offspring development (Soler et al. 2007a, b). Fewer studies have been carried out for predators, despite being important source of herbivore mortality. Multiple species damage can enhance predator attraction (de Boer et al. 2008; Lins Jr. et al. in press; Moayeri et al. 2007) but, in one case, damage by a phloem feeder has clearly been shown to inhibit induced VOCs attractive to predatory mites (Zhang et al. 2009). Increased attraction to plants with multiple damage can result in a reduction of foraging efficiency, since within-patch behaviour can be altered upon contact with non hosts (Bukovinszky et al. 2012). Thus, information on the effect of non hosts during all stages of foraging behaviour is crucial in order to understand the evolution of foraging behaviours, and how they can be manipulated in agricultural systems for increased efficiency of pest control.

The aim of this study was to understand how damage by herbivores of the same feeding guild affects attraction of a predator to plant VOCs. We caused damage to sweet pepper, *Capsicum annuum* (Solanaceae), with either *Aphis gossypii*, *Myzus persicae* (Hemiptera: Aphididae) or both aphids. We then tested the response of an aphid predator, the ladybird *Cycloneda sanguinea* (Coleoptera: Coccinellidae), to the odours of the damaged plant. Since aphids can damage a plant simultaneously or in sequence, we tested the response of *C. sanguinea* to plants damaged simultaneously or sequentially by the two aphid species. Since both herbivores are from the same feeding guild and *C. sanguinea* can prey on both species, we hypothesised that attraction would not be affected by multiple damage.

Materials and methods

Aphid rearing

Aphis gossypii and *M. persicae* were reared in cages on live plants and in Petri dishes containing leaves embedded in a

1 cm layer of 1 % agar at 22 ± 2 °C, 70 ± 10 % relative humidity and 12:12 h photoperiod. *Aphis gossypii* were reared on cucumber plants (*Cucumis sativus*) (Cucurbitaceae), while *M. persicae* were reared on *Nicandra physaloides* (Solanaceae).

Cycloneda sanguinea rearing

Cycloneda sanguinea adults were collected on the campus of the Universidade Federal de Lavras (UFLA). The ladybirds were reared in 10×10 cm (height \times diameter) PVC cylinders lined with filter paper and covered with transparent PVC cling film. The adult ladybirds were fed daily with a mixture of *A. gossypii* and *M. persicae*. Eggs were removed daily and placed in Petri dishes covered with PVC cling film containing *Ephestia* (= *Anagasta*) *kuehniella* eggs. After eclosion, each larva was placed individually in a 8.5×2.5 cm (height \times diameter) test tube and fed with *E. kuehniella* eggs and aphids during the first instar, and 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.

Plant growth and preparation

We planted sweet pepper of the Cascadura Ikeda cultivar (Topseed Garden—Agristar do Brasil Ltda.) in compost (Plantimax®) in a seedbed. When the seedlings had two developing leaves, they were transplanted to 300 mL plastic pots containing a 1:1 mixture of soil and compost. The plants were kept in a greenhouse and watered daily. Plants were used when they had five fully expanded leaves.

Olfactory response

In order to determine *C. sanguinea* responses to odours of sweet pepper, we used a 1 cm internal diameter Y-tube olfactometer, which had a 12 cm trunk and 9 cm arms and a 90° angle between the two arms. The Y-tube was placed with the two arms angled upwards at a 45° to allow for the negative geotaxis common in ladybirds (Almeida and Ribeiro-Costa 2009) and that we observed for this species (M. Oliveira and M. Pareja, personal observation). The odour sources were placed in glass jars into which charcoal-filtered air was pumped. From these jars, Teflon (PTFE) tubes carried the odours to each arm of the Y-tube at 750 mL/min. *Cycloneda sanguinea* adults were introduced individually into the base of the Y-tube. If the individual entered 2 cm into one of the arms and remained for at least 30 s, it was considered to have made a choice, and the bioassay was terminated. If the individual did not

make a choice after 10 min, it was considered a non responder. After every insect tested, the Y-tube was replaced with a clean one. After three insects tested, the side each odour was presented was swapped. Each insect was used only once. For each experiment, a total of 100 *C. sanguinea* were tested (apart from one experiment where 101 were tested). Since there can be variation between odour sources (plants), after ten *C. sanguinea* tested, the plants that served as an odour source were changed for a new plant or combination of plants. Before each experiment, all glassware and Teflon tubing was washed with soapy water and ethanol and dried at 100 °C. The bioassay room was maintained at 25 °C. In order to test the olfactometer setup, a preliminary bioassay with no odours was carried out. In this bioassay, charcoal-filtered air was presented through both arms of the olfactometer.

Plants used in experiments were submitted to one of the following damage treatments: (1) undamaged controls; (2) damage by *A. gossypii* for 3 days; (3) damage by *M. persicae* for 3 days; (4) simultaneous damage by *A. gossypii* and *M. persicae* for 3 days; (5) sequential damage by *A. gossypii* for 3 days followed by *M. persicae* for 3 days; (6) sequential damage by *M. persicae* for 3 days followed by *A. gossypii* for 3 days. The aphids used to cause the damage were mixed second-fifth instars and adult apterae. During the damage period, the plants were covered with porous plastic bags. Prior to bioassays, all aphids were removed with a paint brush, and the plant was gently wiped with cotton tips dipped in warm water in order to remove traces of honeydew. The same procedure was carried out on the undamaged plants in order to control for any effect of the manipulation. The following pairwise experiments were carried out to test the effects of damage on attraction of the *C. sanguinea*:

1. Undamaged plant against moist soil and compost.
2. Plant damaged by 50 *A. gossypii* against moist soil and compost.
3. Plant damaged by 50 *M. persicae* against moist soil and compost.
4. Plant damaged by 50 *A. gossypii* against undamaged plant.
5. Plant damaged by 50 *M. persicae* against undamaged plant.
6. Plant damaged by 50 *A. gossypii* against plant damaged by 50 *M. persicae*.
7. Plant damaged by 100 *A. gossypii* against plant with simultaneous damage by 50 *A. gossypii* and 50 *M. persicae*.
8. Plant damaged by 100 *M. persicae* against plant with simultaneous damage by 50 *A. gossypii* and 50 *M. persicae*.

9. Plant damaged by 50 *A. gossypii* against plant with sequential damage by 50 *A. gossypii* followed by 50 *M. persicae*.
10. Plant damaged by 50 *A. gossypii* against plant with sequential damage by 50 *M. persicae* followed by 50 *A. gossypii*.
11. Plant damaged by 50 *M. persicae* against plant with sequential damage by 50 *A. gossypii* followed by 50 *M. persicae*.
12. Plant damaged by 50 *M. persicae* against plant with sequential damage by *M. persicae* followed by 50 *A. gossypii*.

Soil and moist compost were used as controls when only one odour source contained a plant in order to control for effects of soil odour and moisture that can influence bioassay results (Martínez and Hardie 2009). For the sequential damage treatments (9–12 above), after 3 days the first aphid was removed and the second aphid was placed on the plant. In order to control for this manipulation, we carried out a similar procedure on the single-species damage: after 3 days, all aphids were removed and 50 individuals of the same species were placed on the plant. Thus, the sequentially damaged and single-species damaged plants were submitted to identical procedures, apart from the species causing the damage.

Statistical analyses

The choice made by *C. sanguinea* were compared by χ^2 analysis, with an expected frequency of 50 % of responders choosing each odour. Non responders were excluded from the analysis.

Results

When *C. sanguinea* was tested in an odourless olfactometer (air against air), there was no directional bias ($\chi^2 = 0.014$; $P = 0.907$; Fig. 1). Undamaged plants were no more attractive than the odour of soil ($\chi^2 = 1.673$; $P = 0.196$; Fig. 1). When aphid-damaged plants were tested against soil, *C. sanguinea* showed a preference for *A. gossypii*—damaged plants ($\chi^2 = 4.878$; $P = 0.027$), but did not show a preference for *M. persicae* – damaged plants ($\chi^2 = 2.086$; $P = 0.149$; Fig. 1). However, when tested against undamaged plants *C. sanguinea* preferred plants damaged by *A. gossypii* ($\chi^2 = 5.261$; $P = 0.022$) and showed a similar preference when plants were damaged by *M. persicae* ($\chi^2 = 7.024$; $P = 0.008$; Fig. 1). Plants damaged by *A. gossypii* and plants damaged by *M. persicae* were equally attractive to *C. sanguinea* ($\chi^2 = 2.848$; $P = 0.092$; Fig. 2).

Simultaneous damage by *A. gossypii* and *M. persicae* had no influence on the attractiveness of pepper plants to *C. sanguinea*, with responses being similar when tested against plants damaged only by *A. gossypii* ($\chi^2 = 3.658$; $P = 0.056$) or against plants damaged only by *M. persicae* ($\chi^2 = 3.200$; $P = 0.074$; Fig. 2). Sequential damage had different effects depending on the order of damage and the comparison carried out. Plants damaged by *A. gossypii* followed by *M. persicae* were more attractive than plants damaged by *A. gossypii* only ($\chi^2 = 4.560$; $P = 0.033$), but were no more attractive than plants damaged only by *M. persicae* ($\chi^2 = 1.800$; $P = 0.170$; Fig. 3). Plants damaged by *M. persicae* followed by *A. gossypii* were no more attractive than plants damaged only by *A. gossypii* ($\chi^2 = 3.048$; $P = 0.081$) or plants damaged only by *M. persicae* ($\chi^2 = 1.494$; $P = 0.222$; Fig. 3).

Discussion

In both natural and agricultural ecosystems, plants are usually attacked by several herbivores, each of which has a unique interaction with the plant. Plant responses to attack by multiple herbivores can therefore result in VOC blends that are qualitatively or quantitatively different to those induced by single-species damage. These differences can have effects on the third trophic level by altering predator foraging behaviour. We have demonstrated that damage by two aphid species on sweet pepper does not interfere with *C. sanguinea* responses to induced plant odours and, depending on the sequence of infestation, damage by two aphid species can enhance attraction of this ladybird. This study was carried out with two aphid species, thus both species were of the same feeding guild, but studies with herbivores with different feeding guilds are returning similar results.

Cycloneda sanguinea responded more strongly to aphid-damaged plants than to undamaged plants, independent of whether the damage was caused by *A. gossypii* or *M. persicae*. Ladybirds are known to respond to many semiochemicals associated with aphids, including induced plant VOCs (Hatano et al. 2008; Pettersson et al. 2008), so the response we observed was expected. The weaker response to undamaged plants than to damaged plants is also consistent with what has been reported in the literature for other Coccinellidae (Hatano et al. 2008). In particular, *C. sanguinea* has been shown to respond to volatile cues in complex ways. Sarmento et al. (2008) showed that this species was not attracted to undamaged tomato plants, but showed a strong response to plants damaged by the aphid *Macrosiphum euphorbiae* and to plants damaged by the mite *Tetranychus evansi*. When directly contrasted, *C. sanguinea* preferred the odour of plants damaged by the

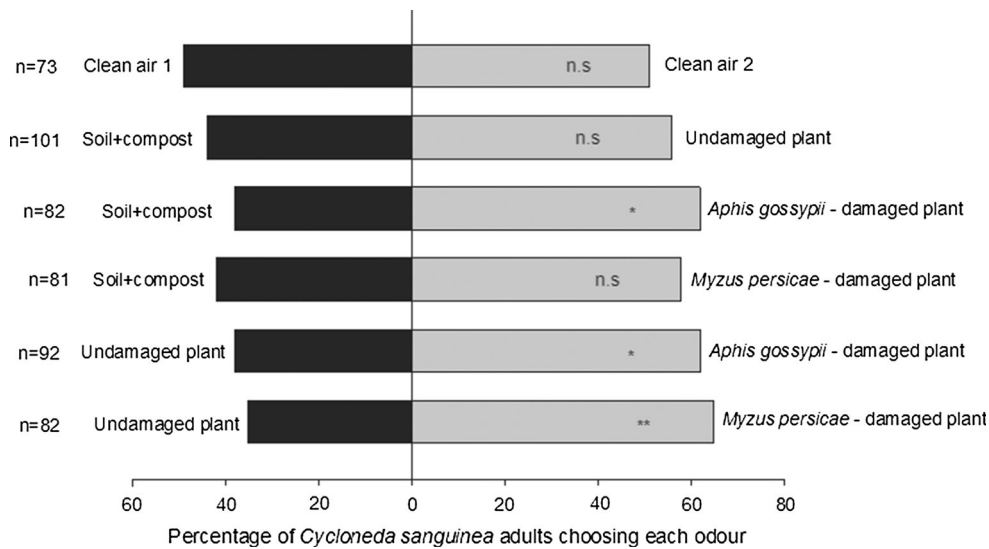
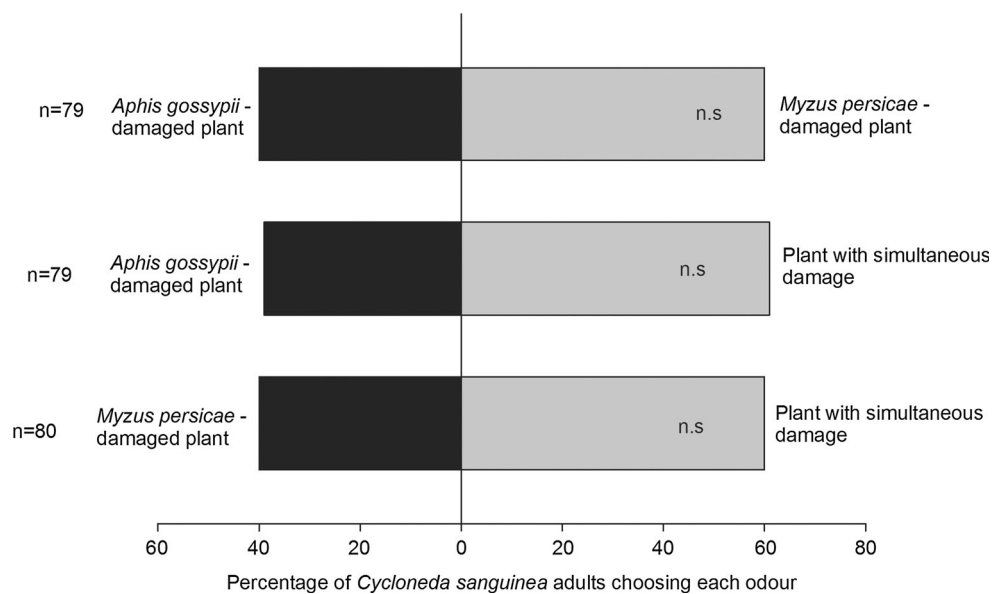


Fig. 1 Response of *Cycloneda sanguinea* in Y-tube olfactometer experiments (percentage of responding insects that chose each odour) to different odours from undamaged sweet pepper and sweet pepper damaged by a single aphid species. The clean air versus clean air experiment was carried out to test for directional bias. Soil and compost were used as controls for experiments where only one odour

source contained a plant. A total of 100 insects were tested (apart from one experiment where 101 were tested) and *n* indicates the number of those insects that responded choosing one of the odours. Analyses were carried out with χ^2 test with a 50 % expected response—n.s.: not significant; *: 0.05 > *P* > 0.01; **: 0.01 > *P* > 0.001

Fig. 2 Response of *Cycloneda sanguinea* in Y-tube olfactometer experiments (percentage of responding insects that chose each odour) to different odours from sweet pepper damaged by the two aphid species *Aphis gossypii* and *Myzus persicae* individually or simultaneously. A total of 100 insects were tested and *n* indicates the number of those insects that responded choosing one of the odours. Analyses were carried out with χ^2 test with a 50 % expected response—n.s.: not significant

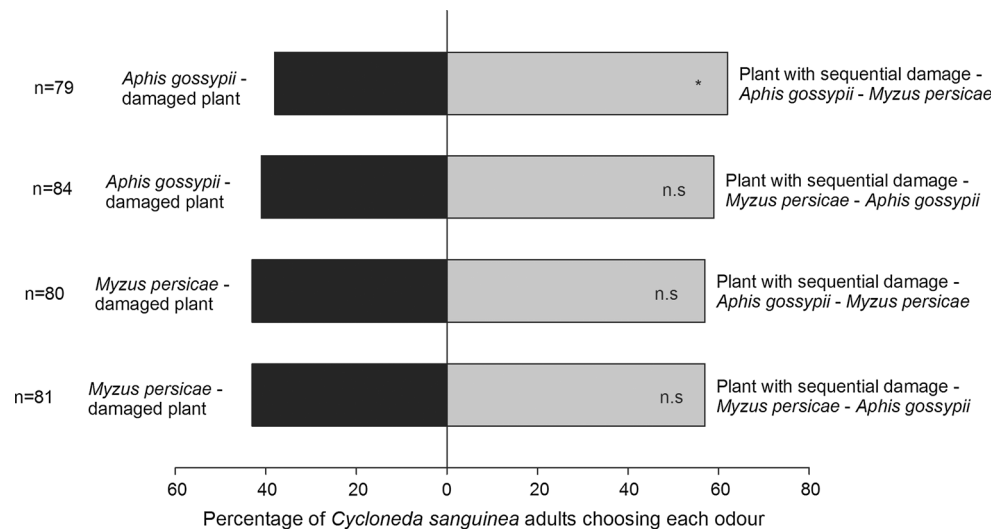


superior prey *M. euphorbiae* over that from plants damaged by *T. evansi* (Sarmiento et al. 2007). In our study, both aphids are suitable prey, though we do not know if there is a difference in the quality of both aphid species as prey for *C. sanguinea*. This could explain the lack of preference for plant volatiles damaged by the different aphid species. However, in a different system based on the plant *Brassica juncea*, *C. sanguinea* did not show responses to plant volatiles induced by a suitable prey species, the aphid *M. persicae*, nor to those induced by *Plutella xylostella*, a

sub-optimal prey species (J. F. França and M. Pareja, unpublished results). Therefore, recognition of volatiles induced by suitable and unsuitable prey species by this predator is highly dependent on the system being studied.

We did not expect simultaneous or sequential damage by two herbivore species from the same feeding guild to affect the attractiveness of plants to *C. sanguinea*. However, induced plant responses to different species can present subtle differences, and herbivores within the same feeding guild can manipulate plant defences in different

Fig. 3 Response of *Cycloneda sanguinea* in Y-tube olfactometer experiments (percentage of responding insects that chose each odour) to different odours from sweet pepper damaged by the two aphid species *Aphis gossypii* and *Myzus persicae* individually or sequentially. A total of 100 insects were tested and *n* indicates the number of those insects that responded choosing one of the odours. Analyses were carried out with χ^2 test with a 50 % expected response—n.s.: not significant; *: $0.05 > P > 0.01$



ways in order to overcome plant defences. However, natural enemies of the herbivores can often perceive these subtle differences in induced responses since they respond to very small changes in VOC ratios (Bruce and Pickett 2011; Pareja et al. 2009). For example, Du et al. (1996) showed that the parasitoid *A. ervi* can distinguish between induced plant responses induced by two different aphid species. Crosstalk between plant defence pathways (Bostock 2005) can alter the biosynthesis of secondary metabolites, altering the responses of insects to plant VOCs. This crosstalk can be used by herbivores to overcome plant defences (Zarate et al. 2007), and even herbivores with very similar feeding strategies can manipulate the plant in different ways to their own benefit (Sarmiento et al. 2011). However, *C. sanguinea* did not respond differently to plants damaged by both aphid species when compared to those damaged by a single species. When sequential damage was carried out, we observed a greater attractiveness of plants damaged by *A. gossypii* followed by *M. persicae* but only when compared to plants damaged only by *A. gossypii*.

Studies on multiple damage have returned mixed results in relation to tritrophic effects, but only a minority of studies have demonstrated that parasitoids and predators can distinguish host-infested plants from those infested by more than one herbivore (de Rijk et al. 2013). There are two factors that are likely to be the key to understanding the tritrophic effects of multiple herbivore damage. One that has been highlighted by several authors is how plants respond to different types of damage from herbivores. Insects from different feeding guilds activate different defence responses and this is central to understanding how damage by more than one herbivore on the same plant will affect natural enemies. In this study, the herbivores are of the same feeding guild, therefore it is likely that they

activate similar defence responses in plants and the combined damage by both aphids could be amplifying plant defence responses. A similar result was obtained when cabbage was damaged by two different caterpillar species, a host and a non host: the parasitoid *Cotesia plutellae* preferred plants damaged by the host over plants with multiple damage, while *Cotesia glomerata* preferred plants with damage by both herbivores (Shiojiri et al. 2000). Results with herbivores from different feeding guilds also have demonstrated that they too can have neutral or positive effects on natural enemy attraction. Working with the same plant, sweet pepper, Moayeri et al. (2007) showed that attraction of the generalist predator *Macrolophus caliginosus* was enhanced when the plant was damaged by the aphid *M. persicae* and the mite *Tetranychus urticae* when compared to plants damaged by a single herbivore species. The authors attributed this to a benefit in obtaining a mixed diet. However, other studies have shown enhanced attraction to multiple damage where one species is a non-host or non-prey. For example, Rodríguez-Saona et al. (2005) demonstrated that the parasitoid *Cotesia marginiventris*, which attacks *Spodoptera* species, was more attracted to plants damaged by both *S. exigua* and the non-host *M. euphorbiae*. The same parasitoid species, *C. marginiventris*, was shown not to be affected by dual damage in a different system, since plants damaged by its host, *Spodoptera littoralis*, and the piercing-sucking herbivore *Euscelidius variegatus* were equally attractive (Erb et al. 2010). However, in some species these responses can be modified by experience, and natural enemies can shift their preference towards odours associated with feeding or oviposition (Erb et al. 2010; Lins Jr. et al. in press).

The second key factor for understanding these responses is natural enemy specificity, which has long been considered to be important in understanding the use of plant

VOCs by predators and parasitoids (Steidle and van Loon 2003; Vet and Dicke 1992). Specialist natural enemies, such as parasitoids, could be hypothesised to be more affected by multiple damage than generalist predators. Thus, in our study, since *C. sanguinea* can prey on both aphid species, it might be responding to general aphid-induced cues and not specific blends that indicate the presence of a particular species. Though this is an appealing hypothesis, more research is needed, since studies with parasitoids have also returned mixed results, with some showing that non hosts enhance attraction (Bukovinszky et al. 2012), reduce attraction or have neutral effects, as discussed above. Most studies in tritrophic interactions, in particular studies on multiple species damage, have focussed on parasitoids, and more information is required on other herbivore natural enemies in order to obtain a more complete picture of how multiple damage affects tritrophic interactions. Of the studies carried out with predators, there is evidence that multiple herbivory can reduce (Zhang et al. 2009), enhance (de Boer et al. 2008; Lins Jr. et al. in press; Moayeri et al. 2007) or have no effect on the attractiveness of damaged plants (Lins Jr. et al. in press). So far, most studies have focussed on a single species of predator or parasitoid, and studies that focus on a suite of natural enemies could help to elucidate how different natural enemies respond to multiple damage in the same system. In a study with a guild of aphid predators responding to *Brassica juncea* VOCs, different species showed very different behavioural responses, but multiple herbivore damage did not interfere with the attraction of a parasitoid, while attraction of a lacewing species was enhanced (S. E. Silva, J. F. França and M. Pareja, unpublished results). Thus, if we consider a guild of predators and parasitoids, the ecological effects of multiple herbivory could be very different depending on the dominant natural enemies present.

We showed that attraction of the aphid predator *C. sanguinea* to sweet pepper plants damaged by two aphid species is not affected when compared to those plants damaged by a single species. Furthermore, a given sequence of damage by the two species (*A. gossypii* followed by *M. persicae*) enhanced attraction. Therefore, plants previously damaged by *A. gossypii* could possibly constitute enemy-dense space for *M. persicae*. Further studies on predation rates and consequences on *M. persicae* population growth are needed to understand whether these behavioural effects have ecological consequences. It is also interesting that only a given sequence of sequential damage enhanced attraction of *C. sanguinea* and VOC profile and gene-expression studies on these forms of sequential damage could elucidate how these forms of damage are affecting the plant. These results demonstrate that herbivores of the same feeding guild damaging the same plant

can have behavioural consequences on the third trophic level. Therefore, timing of damage by different herbivores, induced plant responses and competition between herbivores can contribute towards modulating the activity of predators on plants and their impact on herbivore communities. Further studies are needed with natural enemies with different feeding and foraging strategies, combined with chemical and transcriptome analyses (Kessler and Halitschke 2007) in order to elucidate how these effects will trickle through a more complex community.

Acknowledgments We are grateful to Viviane Ribeiro for help with rearing and to Alberto Arab and two anonymous reviewers for comments that greatly improved the manuscript. This work was funded by Fundação de Amparo à Pesquisa do estado de Minas Gerais—FAPEMIG project APQ-04256-10 and Conselho Nacional de Desenvolvimento Científico e tecnológico—CNPq/Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—CAPES Repensa project 562307/2010-9.

References

- Agbogba BC, Powell W (2007) Effect of the presence of a non-host herbivore on the response of the aphid parasitoid *Diaeretiella rapae* to host-infested cabbage plants. *J Chem Ecol* 33:2229–2235. doi:10.1007/s10886-007-9379-x
- Alborn HT, Turlings TCJ, Jones TH, Stenhagen G, Loughrin JH, Tumlinson JH (1997) An elicitor of plant volatiles from beet armyworm oral secretion. *Science* 276:945–948
- Almeida LM, Ribeiro-Costa CS (2009) Coleopteros predadores (Coccinellidae). In: Panizzi AR, Parra JRP (eds) *Bioecologia e Nutrição de Insetos: Base para o Manejo Integrado de Pragas*. Embrapa Informação Tecnológica, Brasília, pp 931–968
- Bernasconi ML, Turlings TCJ, Ambrosetti L, Bassetti P, Dorn S (1998) Herbivore-induced emissions of maize volatiles repel the corn leaf aphid, *Rhopalosiphum maidis*. *Entomol Exp Appl* 87:133–142
- Bostock RM (2005) Signal crosstalk and induced resistance: straddling the line between cost and benefit. *Annu Rev Phytopathol* 43:545–580
- Bruce TJA, Pickett JA (2011) Perception of plant volatile blends by herbivorous insects—Finding the right mix. *Phytochemistry* 72:1605–1611. doi:10.1016/j.phytochem.2011.04.011
- 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. *Anim Behav* 83:501–509. doi:10.1016/j.anbehav.2011.11.027
- de Boer JG, Posthumus MA, Dicke M (2004) Identification of volatiles that are used in discrimination between plants infested with prey or non-prey herbivores by a predatory mite. *J Chem Ecol* 30:2215–2230
- de Boer JG, Snoeren TAL, Dicke M (2005) Predatory mites learn to discriminate between plant volatiles induced by prey and non-prey herbivores. *Anim Behav* 69:869–879
- de Boer JG, Hordijk CA, Posthumus MA, Dicke M (2008) Prey and non-prey arthropods sharing a host plant: effects on induced volatile emission and predator attraction. *J Chem Ecol* 34:281–290
- De Moraes CM, Lewis WJ, Paré PW, Alborn HT, Tumlinson JH (1998) Herbivore-infested plants selectively attract parasitoids. *Nature* 393:570–573

- De Moraes CM, Mescher MC, Tumlinson JH (2001) Caterpillar-induced nocturnal plant volatiles repel conspecific females. *Nature* 410:577–580
- de Rijk M, Dicke M, Poelman EH (2013) Foraging behaviour by parasitoids in multiherbivore communities. *Anim Behav* 85:1517–1528. doi:10.1016/j.anbehav.2013.03.034
- Du Y-J, Poppy GM, Powell W (1996) Relative importance of semiochemicals from first and second trophic levels in host foraging behavior of *Aphidius ervi*. *J Chem Ecol* 22:1591–1605
- Du Y-J, Poppy GM, Powell W, Pickett JA, Wadhams LJ, Woodcock CM (1998) Identification of semiochemicals released during aphid feeding that attract the parasitoid *Aphidius ervi*. *J Chem Ecol* 24:1355–1368
- Erb M, Foresti N, Turlings TCJ (2010) A tritrophic signal that attracts parasitoids to host-damaged plants withstands disruption by non-host herbivores. *BMC Plant Biology* 10. doi:10.1186/1471-2229-10-247
- Gencer NS, Kumral NA, Sivritepe HO, Seidi M, Susurluk H, Senturk B (2009) Olfactory response of the ladybird beetle *Stethorus gilvifrons* to two preys and herbivore-induced plant volatiles. *Phytoparasitica* 37:217–224. doi:10.1007/s12600-009-0032-9
- Han BY, Chen ZM (2002) Behavioral and electrophysiological responses of natural enemies to synomones from tea shoots and kairomones from tea aphids, *Toxoptera aurantii*. *J Chem Ecol* 28:2203–2219
- Hare JD (2011) Ecological role of volatiles produced by plants in response to damage by herbivorous insects. *Annu Rev Entomol* 56:161–180
- Hare JD, Sun JJ (2011) Production of induced volatiles by *Datura wrightii* in response to damage by insects: effect of herbivore species and time. *J Chem Ecol* 37:751–764. doi:10.1007/s10886-011-9985-5
- Hatano E, Kunert G, Michaud JP, Weisser WW (2008) Chemical cues mediating aphid location by natural enemies. *Eur J Entomol* 105:797–806
- Heil M (2008) Indirect defence via tritrophic interactions. *New Phytol* 178:41–61. doi:10.1111/j.1469-8137.2007.02330.x
- Hoballah ME, Turlings TCJ (2005) The role of fresh versus old leaf damage in the attraction of parasitic wasps to herbivore-induced maize volatiles. *J Chem Ecol* 31:2003–2018. doi:10.1007/s10886-005-6074-7
- Lins Jr. JC, Loon JJA, Bueno VHP, Lucas-Barbosa D, Dicke M, Lenteren JCv (In Press) Response of the zoophytophagous predators *Macrolophus pygmaeus* and *Nesidiocoris tenuis* to volatiles of uninfested plants and to plants infested by prey or conspecifics. *BioControl*. doi:10.1007/s10526-014-9602-y
- Kessler A, Baldwin IT (2001) Defensive function of herbivore-induced plant volatile emissions in nature. *Science* 291:2141–2144. doi:10.1126/science.291.5511.2141
- Kessler A, Halitschke R (2007) Specificity and complexity: the impact of herbivore-induced plant responses on arthropod community structure. *Curr Opin Plant Biol* 10:409–414. doi:10.1016/j.pbi.2007.06.001
- Martínez AS, Hardie J (2009) Hygroreception in olfactometer studies. *Physiol Entomol* 34:211–216. doi:10.1111/j.1365-3032.2009.00675.x
- Mattiacci L, Dicke M, Posthumus MA (1995) β -Glucosidase: an elicitor of herbivore-induced plant odor that attracts host-searching parasitic wasps. *Proc Natl Acad Sci USA* 92:2036–2040
- Moayeri HRS, Ashouri A, Poll L, Enkegaard A (2007) Olfactory response of a predatory mirid to herbivore induced plant volatiles: multiple herbivory vs. single herbivory. *J Appl Entomol* 131:326–332. doi:10.1111/j.1439-0418.2007.01177.x
- Moraes MCB, Laumann R, Sujii ER, Pires C, Borges M (2005) Induced volatiles in soybean and pigeon pea plants artificially infested with the neotropical brown stink bug, *Euschistus heros*, and their effect on the egg parasitoid, *Telenomus podisi*. *Entomol Exp Appl* 115:227–237
- Moran PJ, Thompson GA (2001) Molecular responses to aphid feeding in *Arabidopsis* in relation to plant defense pathways. *Plant Physiol* 125:1074–1085
- Musser RO, Mum-Musser SM, Eichenseer H, Peiffer M, Ervin G, Murphy JB, Felton GW (2002) Caterpillar saliva beats plant defences. *Nature* 416:599–600
- Ninkovic V, Al Abassi S, Pettersson J (2001) The influence of aphid-induced plant volatiles on ladybird beetle searching behavior. *Biol Control* 21:191–195. doi:10.1006/bcon.2001.0935
- Pareja M, Mohib A, Birkett MA, Dufour S, Glinwood RT (2009) Multivariate statistics coupled to generalized linear models reveal complex use of chemical cues by a parasitoid. *Anim Behav* 77:901–909. doi:10.1016/j.anbehav.2008.12.016
- Pareja M, Qvarfordt E, Webster B, Mayon P, Pickett J, Birkett M, Glinwood R (2012) Herbivory by a phloem-feeding insect inhibits floral volatile production. *PLoS One* 7:e31971. doi:10.1371/journal.pone.0031971
- Pettersson J, Ninkovic V, Glinwood R, Abassi S Al, Birkett M, Pickett J, Wadhams L (2008) Chemical stimuli supporting foraging behaviour of *Coccinella septempunctata* L. (Coleoptera: coccinellidae): volatiles and allelobiosis. *Appl Entomol Zool* 43:315–321. doi:10.1303/aez.2008.315
- Reddy GVP (2002) Plant volatiles mediate orientation and plant preference by the predator *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae). *Biol Control* 25:49–55
- Rodríguez-Saona C, Chalmers JA, Raj S, Thaler JS (2005) Induced plant responses to multiple damagers: differential effects on an herbivore and its parasitoid. *Oecologia* 143:566–577
- Sarmiento RA, Venzon M, Pallini A, Oliveira EE, Janssen A (2007) Use of odours by *Cycloneda sanguinea* to assess patch quality. *Entomol Exp Appl* 124:313–318. doi:10.1111/j.1570-7458.2007.00587.x
- Sarmiento RA, Fd Lemos, Dias CR, Pallini A, Venzon M (2008) Infoquímicos induzidos por herbivoria mediando a comunicação entre plantas de tomate e o predador *Cycloneda sanguinea* (Coleoptera: Coccinellidae). *Ceres* 55:439–444
- Sarmiento RA et al (2011) A herbivore that manipulates plant defence. *Ecol Lett* 14:229–236. doi:10.1111/j.1461-0248.2010.01575.x
- Schwartzberg EG, Boroczky K, Tumlinson JH (2011) Pea aphids, *Acyrtosiphon pisum*, suppress induced plant volatiles in broad bean, *Vicia faba*. *J Chem Ecol* 37:1055–1062. doi:10.1007/s10886-011-0006-5
- Shiojiri K, Takabayashi J, Yano S, Takafuji A (2000) Flight response of parasitoids toward plant-herbivore complexes: a comparative study of two parasitoid-herbivore systems on cabbage plants. *Appl Entomol Zool* 35:87–92. doi:10.1303/aez.2000.87
- Shiojiri K, Takabayashi J, Yano S, Takafuji A (2001) Infochemically mediated tritrophic interaction webs on cabbage plants. *Popul Ecol* 43:23–29
- Soler R, Bezemer TM, Cortesero AM, Van der Putten WH, Vet LEM, Harvey JA (2007a) Impact of foliar herbivory on the development of a root-feeding insect and its parasitoid. *Oecologia* 152:257–264. doi:10.1007/s00442-006-0649-z
- Soler R et al (2007b) Root herbivores influence the behaviour of an aboveground parasitoid through changes in plant-volatile signals. *Oikos* 116:367–376
- Soler R, Badenes-Perez FR, Broekgaarden C, Zheng SJ, David A, Boland W, Dicke M (2012) Plant-mediated facilitation between a leaf-feeding and a phloem-feeding insect in a brassicaceous plant: from insect performance to gene transcription. *Funct Ecol* 26:156–166. doi:10.1111/j.1365-2435.2011.01902.x
- Steidle JLM, van Loon JJA (2003) Dietary specialization and infochemical use in carnivorous arthropods: testing a concept. *Entomol Exp Appl* 108:133–148

- Turlings TCJ, Tumlinson JH, Lewis WJ (1990) Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* 250:1251–1253
- van Poecke RMP, Roosjen M, Pumarino L, Dicke M (2003) Attraction of the specialist parasitoid *Cotesia rubecula* to *Arabidopsis thaliana* infested by host or non-host herbivore species. *Entomol Exp Appl* 107:229–236
- Vet LEM, Dicke M (1992) Ecology of infochemical use by natural enemies in a tritrophic context. *Annu Rev Entomol* 37:141–172
- Zarate SI, Kempema LA, Walling LL (2007) Silverleaf whitefly induces salicylic acid defenses and suppresses effectual jasmonic acid defenses. *Plant Physiol* 143:866–875. doi:[10.1104/pp.106.090035](https://doi.org/10.1104/pp.106.090035)
- Zhang P-J, Zheng S-J, van Loon JJA, Boland W, David A, Mumm R, Dicke M (2009) Whiteflies interfere with indirect plant defense against spider mites in Lima bean. *Proc Natl Acad Sci USA* 106:21202–21207. doi:[10.1073/pnas.0907890106](https://doi.org/10.1073/pnas.0907890106)
- Zhang PJ, Broekgaarden C, Zheng SJ, Snoeren TAL, van Loon JJA, Gols R, Dicke M (2013) Jasmonate and ethylene signaling mediate whitefly-induced interference with indirect plant defense in *Arabidopsis thaliana*. *New Phytol* 197:1291–1299. doi:[10.1111/nph.12106](https://doi.org/10.1111/nph.12106)