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ORIGINAL ARTICLE

Honeydew volatile emission acts as a kairomonal message for the Asian lady beetle *Harmonia axyridis* (Coleoptera: Coccinellidae)

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Abstract The Asian lady beetle *Harmonia axyridis* Pallas is considered as an invasive species in most territories where it has been introduced. Because aphid honeydew acts as an attractant for many aphid predators and parasitoids, the objectives of this work were to collect and identify the volatile compounds released from the aphid excretory product to evaluate how these semiochemicals could affect the *H. axyridis* foraging behavior. Twelve volatile chemicals were identified from the *Megoura viciae* Buckton honeydew including four alcohols, three ketones, three aldehydes, a pyrazine and a monoterpene. The volatiles 3-methyl-1-butanol and 3-methyl-butanal were highlighted as the two most abundant semiochemicals released from the *M. viciae* honeydew. *Vicia faba* L. plants treated with crude honeydew attracted more than 80% of the tested individuals with 40% of attracted beetles located on the plant. Four volatile compounds (3-hydroxy-2-butanone, 3-methyl-butanal, 3-methyl-1-butanol and limonene) were also highlighted to attract more than 75% of the coccinellids toward the odor source and to locate about 35% of them on the plants. Limonene was the most efficient attractant since 89% of the *H. axyridis* responded to this odor. The use of the identified semiochemicals as well as the composition of an artificial honeydew could certainly be helpful to control the dispersal of the Asian lady beetle *H. axyridis*.

Key words aphid honeydew, attractant, *Harmonia axyridis*, kairomone, semiochemicals

Introduction

Homopteran insects, including aphids, excrete copious amounts of honeydew which comprises substances derived from the host plant and the insect itself (specific sugars and amino acids) (Heidari & Copland, 1993; Leroy *et al.*, 2011). Many studies have envisaged this excretory product as a "contact kairomone" and/or an arrestant for predators and parasitoids (Budenberg, 1990; Budenberg *et al.*, 1992; McEwen *et al.*, 1993; Du *et al.*, 1997; Sadeghi & Gilbert, 2000; Scholz & Poehling, 2000; Sutherland *et al.*, 2001; Petersen & Hunter, 2002; Leroy *et al.*, 2010). These studies highlighted that aphid honeydew induces egg laying, increases the searches of hosts or prey and finally reinforces predatory and parasitic rates. Also, many predators and parasitoids use this excretory product as a food complement rich in sugars (mono-, di- and trisaccharides), in essential amino acids and also containing minerals, vitamins and organic acids (Wäckers, 2000). Although several authors have reported that lady beetle

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adults use visual information and chemical cues from plants and aphids in their foraging (Obata, 1997; Harmon *et al*., 1998; Al Abassi *et al*., 2000; Verheggen *et al*., 2007), natural aphid honeydew has only been studied a few times as a contact kairomone and an arrestant for coccinellid larvae (Carter & Dixon, 1984; Ide *et al*., 2007). To our knowledge, only the use of artificial honeydews (sucrose and yeast product solutions) for the control of coccinellids has been studied, showing that sugary products could be helpful in managing the Coccinellidae dispersal in field crops (Ben Saad & Bishop, 1976; Evans & Swallow, 1993; Evans & Richards, 1997).

Because aphid honeydew is known to act as an efficient kairomone for many predators and parasitoids, the purpose of the work reported here was to investigate whether semiochemicals released from the aphid excretory product could affect the foraging behavior of the coccinellid *H. axyridis*. To do so, volatile compounds from aphid honeydew were identified and then tested on the Asian lady beetle.

Materials and methods

Insects

In a climate-controlled room (16 h light photoperiod; 60% ± 5% relative humidity [RH]; 20°C ± 2°C), host-plants – *Vicia faba* L. – were grown in 9 × 8 cm plastic pots containing a mixture of vermiculite and perlite (1/1) and were infested with the aphid *Megoura viciae* Buckton. This aphid species was collected in field crops in 1990 and were reared for years at the University of Liege, Gembloux Agro-Bio Tech (Department of Functional and Evolutionary Entomology), Belgium. Aphids were weekly transferred onto new *V. faba* host plants and maintained in the same climate-controlled room. In the same climatic conditions, but in a different room, *Harmonia axyridis* larvae collected in Gembloux (Belgium) in 2009 were reproduced on a massive scale. Adults were reared with sugar, pollen and water and oviposition was induced by the introduction of aphid-infested host-plants in the rearing-boxes for 3 h. The complete life cycle took place in the rearing-boxes daily supplied with aphids.

Honeydew collection

To collect aphid honeydew, several *V. faba* plants, heavily infested with *M. viciae*, were placed 10 cm above aluminium foil. Using microcapillaries of 10 μL volume, the honeydew droplets falling on the aluminium sheet were directly collected in order to have freshly produced honeydew.

Identification of the honeydew volatile compounds

The volatile collection was performed by solid-phase microextraction (SPME) on 20 μL of crude honeydew. The SPME volatile collection was conducted using an 85 μm carboxen-polydimethylosiloxane (Carboxen-PDMS, stableflex) (Supelco, State College, PA, USA) coating fiber. Before each use, the fiber was conditioned at 300°C for 1 h in a split-splitless gas chromatography (GC) injector. Volatile collections were performed at 20°C ± 1°C during 24 h (Incubator Jouan Maxi Artic, Saint-Herblain, France). After each volatile collection, the SPME fiber was withdrawn from the vial and analyzed by GC-mass spectrometry (GC-MS). GC-MS analyses were carried out on a Agilent 6890N Network GC System coupled with an Agilent 5973 Network mass selective detector equipped with an HP-5 (Agilent, Diegem, Belgium) capillary column (30 m × 0.25 mm internal diameter, 0.25 μm film thickness). The oven temperature program was initiated at 40°C, held for 7 min then raised first at 4°C/min to 250°C, held for 5 min and raised in the second ramp at 7°C/min to 280°C and held for 1 min. Other operating conditions were as follows: carrier gas, He; with a constant flow rate of 1 mL/min; injector temperature, 260°C; splitless mode. Mass spectra were taken at 70 eV. Mass range was from m/z (mass-to-charge ratio) 35 to 350 amu (atomic mass unit). The honeydew volatile components were identified by comparing their mass spectra fragmentation patterns with those stored in the Wiley275.L computer library. Furthermore, to validate the identification based on the mass spectral data, the GC retention times of the identified natural components were compared to the retention times of synthetic standards (more than 97% pure; Sigma-Aldrich, Steinheim, Germany). The relative proportion (%) of each identified chemical cue was calculated as follows: the area under the peak of the considered chemical cue was divided by the total peak area (corresponding to the sum of the areas of all sample-related peaks). Five replications were performed (samples and controls).

Behavioural experiments

Effect of *M. viciae* honeydew on *H. axyridis* Behavioral experiments were conducted in 30 × 15 × 15 cm plastic boxes pierced with two holes (recovered with metal screening) on each lateral side. To test whether honeydew

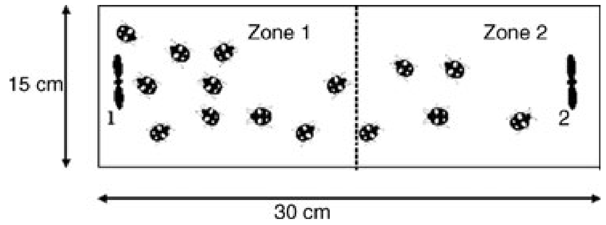

Fig. 1 Experimental device to record *Harmonia axyridis* behavior in response to semiochemicals or honeydew. 1, plant + rubber septum containing the tested semiochemical formulated in paraffin oil or plant treated with crude honeydew; 2, plant + rubber septum containing paraffin oil or clean plant without rubber septum.

could attract *H. axyridis*, experiments were performed using clean plants (without any aphid or honeydew) and plants treated with honeydew. A *V. faba* plant (10 cm high; 2 leaves) was treated with 50 μL of honeydew homogenously spread on leaves while another plant was not treated (clean plant). These two plants were respectively placed at one side of the box (distance between plants = 25 cm) before introduction of 15 *H. axyridis* (males and females combined) in the centre of the box (Fig. 1). Because male and female *H. axyridis* aggregate in the natural environment and because individuals are rarely observed alone, experiments were conducted on groups of 15 *H. axyridis* (rather than testing individuals one by one), males and females combined (random sex ratio). The side where they stood was recorded after 2 h as well as the numbers of individuals located on the plants at each side of the box. To validate the device used for this experiment, a test with clean plants at each side of the box was also conducted (negative control). The tested individuals were deprived of aphids for 24 h before the experiments. Three groups of 15 individuals each were tested. Individuals were different in each group. Average numbers of *H. axyridis* observed in each side of the box were compared by a one-way analysis of variance Anova (software Minitab® release 1.5).

Effect of *M. viciae* honeydew volatile compounds on *H. axyridis* Assays with semiochemicals were performed using the same device (boxes pierced with two holes) (Fig. 1). Semiochemicals identified from the *M. viciae* honeydew were purchased from Sigma-Aldrich (Steinheim, Germany), all had a chemical purity > 97% (GC analyses) and were formulated in paraffin oil at a concentration of 100 ng/μL (see Alhmedi *et al.*, 2010). As proposed by Verheggen *et al.* (2008), a rubber septum was used as a dispenser to release continuously the volatile chemicals. All semiochemicals were tested one by one: a dispenser filled with 100-μL of the paraffin oil solution was placed near the plant at one end of the box. Each semiochemical was tested at a dose of 10 μg, referring to the study of Alhmedi *et al.* (2010). At the other end of the box, a rubber septum filled with 100 μL of paraffin oil was placed on the plant. To validate the device used for this experiment, a test with rubber septa only containing paraffin oil at both sides of the box was also conducted (negative control). Fifteen *H. axyridis* adults (males and females combined) were then introduced into the box and the side where they stood was recorded after 2 h, distinguishing individuals located on plants. Tested individuals were deprived of aphids for 24 h before the experiments. Three groups of 15 individuals each were tested. Individuals were different in each group. Average numbers of *H. axyridis* observed on each side of the box were compared by a one-way analysis of variance Anova (software Minitab® release 1.5).

Results

Identification of the honeydew volatile compounds

The SPME volatile collections led to the identification of 12 volatiles specifically released from crude *M. viciae* honeydew, including alcohols (3-methyl-3-buten-1-ol; 3-methyl-1-butanol; 2-methyl-1-butanol, benzeneethanol), ketones (2-propanone; 2,3-butanedione, 3-hydroxy-2-butanone), aldehydes (3-methyl-butanal; 2-methyl-butanal, 2-methyl-2-butenal) a pyrazine (2,5-dimethylpyrazine) and a terpene (limonene) (Fig. 2A). Volatile relative proportions are presented in Table 1: 3-methyl-1-butanol (13.96% ± 4.49%) and 3-methyl-butanal (18.63% ± 2.26%) were highlighted as the two most abundant semiochemicals released from *M. viciae* honeydew. The other volatile proportions were between 4% and 10%.

Harmonia axyridis *behavioral responses*

When clean plants were located at both sides of the box, an equal number of individuals were noted in each zone ($F_{1,4} = 0.07$ and $P = 0.802$, one-way Anova) and *H. axyridis* was observed to move randomly during the 2 h of observation. The presence of honeydew on the *V. faba* leaves significantly influenced the distribution of *H. axyridis*: a significantly higher number of individuals (82% ± 7%) were attracted and observed in the area (<5 cm) comprising the plant treated with honeydew rather than in the area without honeydew on the plant ($F_{1,4} = 60.07$ and $P < 0.001$, one-way Anova) (Fig. 3).

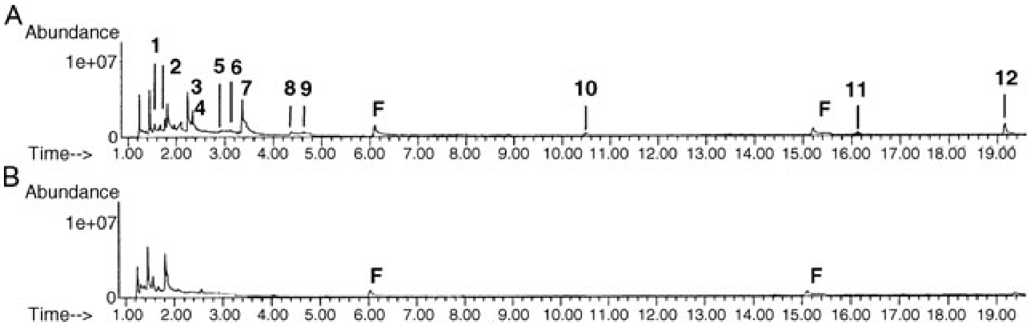

Fig. 2 Exemplary chromatogram of the volatile profile of *Megoura viciae* honeydew (solid-phase microextraction and gas chromatography-mass spectrometry analyses). A, crude *M. viciae* honeydew and B, empty vial (control). The labeled peaks are as follows: 1, 2-propanone; 2, 2,3-butanedione; 3, 3-methyl-butanal; 4, 2-methyl-butanal; 5, 3-hydroxy-2-butanone; 6, 3-methyl-3-buten-1-ol; 7, 3-methyl-1-butanol; 8, 2-methyl-1-butanol; 9, 2-methyl-2-butenal; 10, 2,5-dimethylpyrazine; 11, limonene; 12, benzeneethanol. The letter "F" indicates peaks related to the SPME fiber phases.

Furthermore, 40% ± 5.5% of individuals located in this area climbed and remained on the plants during the 2 h of observation.

Among the 12 identified and tested semiochemicals, four volatile compounds affected *H. axyridis* behavior and directed individuals toward the odor source. Indeed, after 2 h, a significantly higher number of lady beetles were localised at the odorant side of the box when using 3-methyl-butanal ($F_{1,4} = 91.12$ and $P < 0.001$, one-way Anova), 3-hydroxy-2-butanone ($F_{1,4} = 312.5$ and $P < 0.001$, one-way Anova), 3-methyl-1-butanol ($F_{1,4} = 13.5$ and $P = 0.021$, one-way Anova) and limonene ($F_{1,4} = 612.5$ and $P < 0.001$, one-way Anova) (Fig. 4). These semiochemicals respectively attracted 82% ± 7%, 78% ± 3%, 78% ± 9% and 89% ± 3% of the *H. axyridis* introduced in the boxes. In each case, about 35% ± 6% of individuals were observed to climb on the plants as well as on the rubber septum containing these odors. The other volatile compounds did not induce specific behaviors: coccinellids were observed to move randomly in the boxes without choosing one side rather than another and without climbing on the plants ($P > 0.05$, one-way Anova).

Table 1 Relative proportions (% ± SEM) of the volatile compounds collected by solid-phase microextraction (SPME) and identified by gas chromatography-mass spectrometry (GC-MS) from the *M. viciae* honeydew ($n = 5$).

Peak number	Reaction time(min)	Voatile compounds	Relative proportions
1	1.44	z-propanone	8.36 ± 2.69
2	1.76	2,3-butanaedione	6.71 ± 0.97
3	2.23	3-methyl-butanal	18.63 ± 2.26
4	2.32	2-methyl-butanal	8.56 ± 2.33
5	3.24	3-hydroxy-2-butanone	5.93 ± 0.37
6	3.29	3-methyl-3-buten-1-ol	4.76 ± 0.25
7	3.36	3-methyl-1-butanol	13.96 ± 4.49
8	4.38	2-methyl-1-butanol	6.56 ± 1.21
9	4.65	2-methyl-2-butenal	6.78 ± 3.21
10	10.54	2,5-dimethylpyrazine	4.21 ± 0.13
11	16.04	Limonene	5.81 ± 0.17
12	19.92	Benzeneethanol	9.73 ± 0.05

Discussion

Since the Asian lady beetle has rapidly become an invasive specie presenting negative impacts on native coccinellids and affecting the dynamics and composition of several guilds (Soares *et al.*, 2008), studies were performed

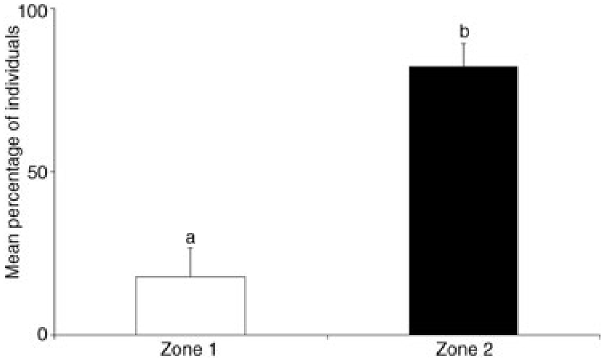

Fig. 3 *Harmonia axyridis* attraction in response to crude honeydew. Mean percentage of individuals (+SEM). Means with different letters are significantly different (one-way Anova, $P < 0.05$). Zone 1 = zone with a clean plant; Zone 2 = zone with a treated plant (50 μL of honeydew).

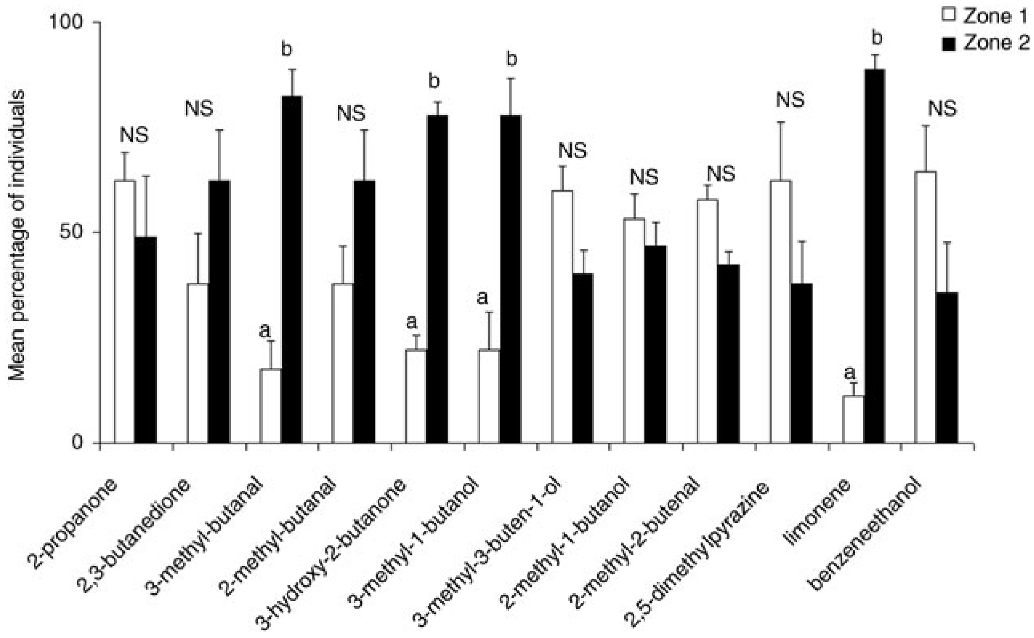

Fig. 4 *Harmonia axyridis* attraction in response to semiochemicals from the *Megoura viciae* honeydew. Mean percentage of individuals (+SEM). For one semiochemical, means with different letters are significantly different (one-way ANOVA, $P < 0.05$). N.S. indicates "no significant difference". Zone 1 = zone without semiochemicals; Zone 2 = zone with the tested semiochemical.

to control this harmful coccinellid. Uses of natural enemies including pathogens (Roy *et al*., 2007), parasitoids (Katsoyannos & Aliniazee, 1998), predators (Dutcher *et al*., 1999) and a parasitic mite (Webberley *et al*., 2004) were envisaged to regulate the *H. axyridis* invasion but none of these approaches have shown sufficient potential. Presently and according to Kenis *et al*. (2008), no biological control method is really available to lower the *H. axyridis* population densities in the natural environment and to limit the impact of this lady beetle on native species. Nowadays, attractant and deterrent semiochemicals seem to represent the best option to develop efficient trapping systems against this invasive coccinellid (Kenis *et al*., 2008).

The semiochemically mediated interactions between organisms are complex but have been specifically studied to identify cues for enhancing biological control (Ruther *et al*., 2002). That is why semiochemicals seem to be the most promising for the biological control of the Asian lady beetle *H. axyridis* and why works have already been done in this area. Olfactory cues are known to be used by adult lady beetles to direct their movements toward prey, visiting sites according to cues from aphids rather than in a random fashion (Nakamuta, 1991; Hodek, 1993; Schaller & Nentwig, 2000). In this sense, Verheggen and colleagues (2007) have proposed that *H. axyridis* could use two specific semiochemicals released from conspecifics (the (-)-β-caryophyllene) or from their aphid prey (the (*E*)-β-farnesene) to orientate their foraging behavior, and that these two semiochemicals could be considered to control *H. axyridis* dispersal (Verheggen *et al*., 2007). Semiochemicals released by plants, aphids and the coccinellids themselves were tested by Alhmedi and colleagues (2010), showing that (-)-β-caryophyllene and limonene acted as attractants and ovipositional stimulants for the Asian lady beetle. Concerning (-)-β-caryophyllene, the study by Brown *et al*. (2006), dealing with the semiochemicals involved in establishment and persistence of overwintering beetle aggregation, showed that this volatile compound is only emitted from *H. axyridis* females.

Because aphid honeydew is known for its kairomonal properties with respect to predators and parasitoids, volatile compounds released from *M. viciae* honeydew were collected and identified in this study. Some alcohols, ketones, aldehydes, a pyrazine and a monoterpene, were systematically detected by GC-MS analyses. Degradation and/or modifications of the large amounts of sugars and amino acids found in aphid honeydew could certainly explain the detection of these volatile compounds: for example, 3-methyl-butanal and 2-methyl-butanal as well as their corresponding alcohols, 3-methyl-1-butanol and 2-methyl-1-butanol, are known to be produced by direct modifications of amino acid-derived starter units, notably by bacteria (Schulz & Dickschat, 2007). Also, these semiochemicals, like diacethyl (2,3-butanedione), acetoin (3-hydroxy-2-butanone), 3-methyl-3-buten-1-ol and benzeneethanol are typical fermentation-associated substances (Nout & Barlet, 1998; Schulz & Dickschat, 2007).

Our behavioral experiments demonstrated that volatile cues associated with aphid honeydew are used as kairomonal substances by *H. axyridis*, orientating the large majority of individuals toward plants treated with honeydew. Previously, many studies have demonstrated that several coccinellid species respond to the presence of aphids, to the aphid alarm pheromone (the *E*-(β)-farnesene) and to volatiles released by plants (e.g. green leaf volatiles) (Nakamuta, 1991; Zhu *et al*., 1999; Hemptinne *et al*., 2000; Mondor & Roitberg, 2000; Al Abassi *et al*., 2000; Acar *et al*., 2001; Francis *et al*., 2004; Verheggen *et al*., 2007). To our knowledge, only the study by Carter and Dixon (1984) dealt with aphid honeydew tested on the coccinellid *Coccinella septempunctata* and no response to the aphid excretory product presented on broad bean leaves or to the odor of *Acyrthosiphon pisum* were shown. Attraction in response to honeydew has already been observed for other predators like Syrphidae and Chrysopidae that use this sugary substance to locate their prey (Budenberg, 1990; Budenberg *et al*., 1992; Du *et al*., 1997; Sadeghi & Gilbert, 2000; Petersen & Hunter, 2002; Sutherland *et al*., 2001; Leroy *et al*., 2010). In field crops, plants covered with honeydew droplets indicate close aphid presence which could explain that aphid natural enemies refer to honeydew to orientate their movements when searching for prey or selecting an ovipositional site. Our results validate this theory since *H. axyridis* was observed to move toward and to climb on plants treated with honeydew. In our study, the kairomonal effect of honeydew on *H. axyridis* was explained by the identification of four volatile compounds that significantly influenced the distribution of this coccinellid: 3-hydroxy-2-butanone, 3-methyl-1-butanol and 3-methyl-butanal all attracted and localised about 80% of the coccinellids near the odor source, while limonene guided 88% of the tested individuals toward the volatile compound release points. If *H. axyridis* is known to aggregate and to be attracted by conspecific cues (Verheggen *et al*., 2007), our experiments testing groups of 15 individuals demonstrated that honeydew and semiochemicals from honeydew influenced *H. axyridis* behaviors, even in presence of several lady beetles. This indicated that conspecific cues are not the only volatiles affecting the behaviors of this coccinellid, even if (-)-β-caryophyllene may contribute to the observed individual distributions. In our study, higher attraction was noted with pure compounds at a dose of 10 μg than with crude honeydew. This could be explained by the fact that only small quantities of volatiles are released from crude honeydew and because insect behavioral responses are known to vary according to the volatile concentrations applied (Zhu *et al*., 1999).

Acetoin (3-hydroxy-2-butanone) is already known to induce behavioral responses in some insect species. Indeed, it was identified as an attractant for the mosquito *Anopheles gambiae* (Diptera: Culicidae) (Meijerink *et al*., 2000), for the fruit chafer *Pachnoda marginata* (Coleoptera: Scarabaeidae) (Larsson *et al*., 2003) and for *Drosophila melanogaster* (Stensmyr *et al*., 2003a, b) in behavioral bioassays. Acetoin is also emitted by female *Rhizotrogus majalis* and detected by male antennae (Nojima *et al*., 2003) while males of *Scapanes australis* emit this odor, being sufficient to attract both sexes in field trapping experiments (Rochat *et al*., 2002). Acetoin has been also identified as a female-emitted sex pheromone in the summer chafer, *Amphimallon solstitiale* (Tolasch *et al*., 2003). Finally, in the palm weevil, *Rhynchophorus palmarum*, acetoin is a synergist to male-emitted volatile aggregation pheromones (Said *et al.*, 2005). The semiochemical 3-methyl-1-butanol strongly attracts the noctuid moths *Lacanobia subjuncta*, *Mamestra configurata* and *Xestia c-nigrum* (Lepidoptera: Noctuidae) (Landolt, 2000, 2001, 2002) as well as the fruit flies *Anastrepha suspensa*, *Anastrepha ludens* (Diptera: Tephritidae) (Nigg *et al*., 1994; Lee *et al*., 1995; Epsky *et al*., 1998; Kendra *et al*., 2005) and *Ceratitis capitata* (Diptera: Tephritidae) (Warthen *et al*., 1997). 3-methyl-1-butanal has been less well studied but is identified as a potential attractant for the Mediterranean fruit fly, *C. capitata* (Warthen *et al*., 1997).

Limonene is a very common plant volatile already reported as an attractant for *H. axyridis* by Alhmedi *et al*. (2010) but also for other biocontrol agents. Indeed, Hulcr *et al*. (2006) showed that limonene is attractive to the predator *Medetera setiventris* (Diptera: Dolichopodidae) and, in the same way, limonene has been identified as an attractant for the natural enemy *Dastarcus helophoroides* (Coleoptera: Bothrideridae) (Wei *et al*., 2008).

To our knowledge, if limonene has been already highlighted as a potential kairomone for *H. axyridis* in olfactory experiments and also in field studies (Alhmedi *et al*., 2010), 3-hydroxy-2-butanone, 3-methyl-1-butanol and 3-methyl-butanal have not been determined as chemical cues affecting the behaviors of the Asian lady beetle. Further field work has to be carried out to test the effectiveness of the identified attractive semiochemicals on *H. axyridis* coccinellids. Other points to be considered in further works are the possible synergic or antagonist effect of the tested pure compounds but also the distance of attraction of the identified attractants.

Our results constitute preliminaries for the potential use of these semiochemicals that could be helpful to regulate *H. axyridis* invasion and limit the impact of this lady beetle on native species. A promising approach to managing *H. axyridis* populations is the use of chemical repellents and attractants, using chemical repellents to push beetles away from specific crops or houses and chemical attractants to pull beetles into collecting traps.

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Disclosure

The authors declare that they have no conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject of their manuscript.

References

Acar, E.B., Medina, J.C., Lee, M.L. and Booth, G.M. (2001) Olfactory behaviour of convergent lady beetles (Coleoptera: Coccinellidae) to alarm pheromone of green peach aphid (Hemiptera: Aphididae). *Canadian Entomologist*, 133, 389–397.

Al Abassi, S., Birkett, M.A., Pettersson, J., Pickett, J.A., Wadhams, L.J. and Woodcock, C.M. (2000) Response of the seven-spot ladybird to an aphid alarm pheromone and an alarm pheromone inhibitor is mediated by paired olfactory cells. *Journal of Chemical Ecology*, 26, 1765–1771.

Alhmedi, A., Haubruge, E. and Francis, F. (2010) Identification of limonene as a potential kairomone for the harlequin ladybird *Harmonia axyridis* (Coleoptera: Coccinellidae). *European Journal of Entomology*, 107, 541–548.

Ben Saad, A.A. and Bishop, G.W. (1976) Attraction of insects to potato plants through use of artificial honeydews and aphid juice. *Entomophaga*, 21, 49–57.

Brown, A.E., Riddick, E.W., Aldrich, J.R. and Holmes, W.E. (2006) Identification of $(-)$-β- caryophyllene as a gender-specific terpene produced by the multicolored Asian lady beetle. *Journal of Chemical Ecology*, 32, 2489–2499.

Budenberg, W.J. (1990) Honeydew as a contact kairomone for aphid parasitoids. *Entomologia Experimentalis et Applicata*, 55, 139–148.

Budenberg, W.J., Powell, W. and Clark, S.J. (1992) The influence of aphids and honeydew on the leaving rate of searching aphid parasitoids from wheat plants. *Entomologia Experimentalis et Applicata*, 63, 259–264.

Carter, M.C. and Dixon, A.F.G. (1984) Honeydew: an arrestant stimulus for coccinellids. *Ecological Entomology*, 9, 383–387.

Du, Y., Guy, M.P., Powell, W. and Wadhams, L.J. (1997) Chemically mediated associative learning in the host foraging behaviour of the aphid parasitoid *Aphidius ervi* (Hymenoptera: Braconidae). *Journal of Insect Behavior*, 10, 509–521.

Dutcher, J.D., Estes, P.M. and Dutcher, M.J. (1999) Interactions in entomology: aphids, aphidophaga and ants in pecan orchards. *Journal of Entomological Science*, 34, 40–56.

Epsky, N.D., Heath, R.R., Dueben, B.D., Lauzon, C.R., Proveaux, A.T. and Maccollom, G.B. (1998) Attraction of 3-methylbutanol and ammonia identified from *Enterobacter agglomerans* to *Anastrepha suspensa*. *Journal of Chemical Ecology*, 24, 1867–1880.

Evans, E.W. and Richards, D.R. (1997) Managing the dispersal of ladybird beetles (Col.: Coccinellidae): Use of artificial honeydew manipulate spatial distributions. *Entomophaga*, 42, 93–102.

Evans, E.W. and Swallow, J.G. (1993) Numerical responses of natural enemies to artificial honeydew in Utah alfalfa. *Environmental Entomology*, 22, 1392–1401.

Francis, F., Lognay, G. and Haubruge, E. (2004) Olfactory responses to aphid and host plant volatile releases: *E*-β-farnesene an effective kairomone for the predator *Adalia bipunctata*. *Journal of Chemical Ecology*, 30, 741–755.

Harmon, J.P., Losey, J.E. and Ives, A.R. (1998) The role of vision and color in the close proximity foraging behavior of four coccinellid species. *Oecologia*, 115, 287–292.

Heidari, M. and Copland, M.J.W. (1993) Honeydew: A food or arrestant for the mealybug predator *Cryptolaemus montrouzieri*. *Entomophaga*, 38, 63–68.

Hemptinne, J.L., Gaudin, M., Dixon, A.F.G. and Lognay, G. (2000) Social feeding in ladybird beetles: adaptive significance and mechanism. *Chemoecology*, 10, 149–152.

Hodek, I. (1993) Habitat and food specificity in aphidophagous predators. *Biocontrol Science and Technology*, 3, 91–100.

Hulcr, J., Ubik, K. and Vrkoc, J. (2006) The role of semiochemicals in tritrophic interactions between the spruce bark beetle *Ips typographus*, its predators and infested spruce. *Journal of Applied Entomology*, 130, 275–283.

Ide, T., Suzuki, N. and Katayama, N. (2007) The use of honeydew in foraging for aphids by larvae of the ladybird beetle, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). *Ecological Entomology*, 32, 455–460.

Katsoyannos, P. and Aliniazee, M.T. (1998) First record of *Strongygaster triangulifera* (Loew) (Diptera: Tachinidae) as a parasitoid of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) in western North America. *Canadian Entomology*, 130, 905–906.

Kendra, P.E., Montgomery, W.S., Mateo, D.M., Puche, H., Epsky, N.D. and Heath, R.R. (2005) Effect of age on EAG response and attraction of female *Anastrepha suspensa* (Diptera: Tephritidae) to ammonia and carbon dioxide. *Environmental Entomology*, 34, 584–590.

Kenis, M., Roy, H.E., Zindel, R. and Majerus, M.E.N. (2008) Current and potential strategies against *Harmonia axyridis*. *BioControl*, 53, 235–252.

Landolt, P.J. (2000) New chemical attractants for trapping *Lacanobia subjuncta*, *Mamestra configurata*, and *Xestia c-nigrum* (Lepidoptera: Noctuidae). *Journal of Economical Entomology*, 93, 101–106.

Landolt, P.J. and Alfaro, J.F. (2001) Trapping *Lacanobia subjuncta*, *Xestia c-nigrum* and *Mamestra configurata* (Lepidoptera: Noctuidae) with acetic acid and 3-methyl-1-butanol in controlled release dispensers. *Environmental Entomology*, 30, 656–662.

Landolt, P.J. and Higbee, B.S. (2002) Both sexes of the true armyworm (Lepidoptera: Noctuidae) trapped with the feeding attractant composed of acetic acid and 3-methyl-1-butanol. *Florida Entomologist*, 85, 182–185.

Larsson, M.C., Stensmyr, M.C., Bice, S.B. and Hansson, B.S. (2003) Attractiveness of fruit and flower odorants detected by olfactory receptor neurons in the fruit chafer *Pachnoda marginata*. *Journal of Chemical Ecology*, 29, 1253–1268.

Lee, C.J., DeMilo, A.B., Moreno, D.S. and Martinez, A.J. (1995) Analyses of the volatile components of a bacterial fermentation that is attractive to the Mexican fruit fly, *Anastrepha ludens*. *Journal of Agricultural and Food Chemistry*, 43, 1348–1351.

Leroy, P.D., Verheggen, F.J., Capella, Q., Francis, F. and Haubruge, E. (2010) An introduction device for the aphidophagous hoverfly *Episyrphus balteatus* (De Geer) (Diptera: Syrphidae). *Biological Control*, 54, 181–188.

Leroy, P.D., Wathelet, B., Sabri, A., Francis, F., Verheggen, F.J., Capella, Q., Thonart, P. and Haubruge, E. (2011) Aphid-host plant interactions: Does aphid honeydew exactly reflect the host plant amino acid composition? *Arthropod-Plant Interactions*, 15, 1–7.

McEwen, P.K., Jervis, M.A. and Kidd, N.A.C. (1993) Influence of artificial honeydew on larval development and survival in *Chrysoperla carnea* (Neur., Chrysopidae). *Entomophaga*, 38, 241–244.

Meijerink, J., Braks, M.A.H., Brack, A.A., Adam, W., Dekker, T., Posthumus, M.A., van Beek, T.A. and van Loon, J.J.A. (2000) Identification of olfactory stimulants for *Anopheles gambiae* from human sweat samples. *Journal of Chemical Ecology*, 26, 1367–1382.

Mondor, E. and Roitberg, B. (2000) Has the attraction of predatory coccinellids to cornicle droplets constrained aphid alarm signaling behavior? *Journal of Insect Behavior*, 3, 321–329.

Nakamuta, K. (1991) Aphid alarm pheromone component, (*E*)-β-farnesene, and local search by a predatory lady beetle, *Coccinella septempunctata brukii* Mulsant (Coleoptera: Coccinellidae). *Applied Entomology and Zoology*, 20, 479–483.

Nigg, H.N., Mallory, L.L., Fraser, S., Simpson, S.E., Robertson, J.L., Attaway, J.A., Callaham, S.B. and Brown, R.E. (1994) Test protocols and toxicity of organophosphate insecticides to Caribbean fruit fly (Diptera: Tephritidae). *Journal of Economical Entomology*, 87, 589–595.

Nojima, S., Sakata, T., Yoshimura, K., Robbins, P.S., Morris, B.D. and Roelofs, W.L. (2003) Male-specific EAD active compounds produced by female European chafer *Rhizotrogus majalis* (Razoumowsky). *Journal of Chemical Ecology*, 29, 503–507

Nout, M.J.R. and Barlet, R.J. (1998). Attraction of a flying nitidulid (*Carpophilus humeralis*) to volatiles produced by yeasts grown on sweet corn and a corn-based medium. *Journal of Chemical Ecology*, 24, 1217–1239.

Obata, S. (1997) The influence of aphids on the behaviour of adults of the ladybird beetle, *Harmonia axyridis* (Col.: Coccinellidae). *BioControl*, 42, 103–106.

Petersen, M.K. and Hunter, M.S. (2002) Ovipositional preference and larval – early adult performance of two generalist lacewing predators of aphids in pecans. *Biological Control*, 25, 101–109.

Rochat, D., Morin, J.P., Kakul, T., Beaudoin-Ollivier, L., Prior, R., Renou, M., Malosse, I., Stathers, T., Embupa, S. and Laup, S. (2002) Activity of male pheromone of melanesian rhinoceros beetle *Scapanes australis*. *Journal of Chemical Ecology*, 28, 479–500.

Roy, H.E., Brown, P.M.J., Rothery, P., Ware, R.L. and Majerus, M.E.N. (2007) Interactions between the fungal pathogen *Beauveria bassiana* and three species of coccinellid: *Harmonia axyridis*, *Coccinella septempunctata* and *Adalia bipunctata*. *BioControl*, 53, 265–276.

Ruther, J., Meiners, T. and Steidle, J.L.M. (2002) "Rich in phenomena lacking in terms. A classification of kairomones". *Chemoecology*, 12, 161–167.

Sadeghi, H. and Gilbert, F. (2000) Oviposition preference of aphidophagous hoverflies. *Ecological Entomology*, 25, 91–100.

Said, I., Renou, M., Morin, J.P., Ferreira, J.M.S. and Rochat, D. (2005) Interactions between acetoin, a plantvolatile, and pheromone in *Rhynchophorus palmarum*: behavioral and olfactory neuron responses. *Journal of Chemical Ecology*, 31, 1789–1805.

Schaller, M. and Nentwig, W. (2000) Olfactory orientation of the seven-spot ladybird beetle, *Coccinella septempunctata* (Coleoptera: Coccinellidae): attraction of adults to plants and conspecific females. *European Journal of Entomology*, 97, 155–159.

Scholz, D. and Poehling, H.M. (2000) Oviposition site selection of *Episyrphus balteatus*. *Entomologia Experimentalis et Applicata*, 94, 149–158.

Schulz, S. and Dickschat, J.S. (2007) Bacterial volatiles: the smell of small organisms. *Natural Product Reports*, 24, 814–842.

Soares, A.O., Borges, I., Borges, P.A.V., Labrie, G. and Lucas, E. (2008) *Harmonia axyridis*: What will stop the invader? *BioControl*, 53, 127–145.

Stensmyr, M.C., Dekker, T. and Hansson, B.S. (2003a) Evolution of the olfactory code in the *Drosophila melanogaster* subgroup. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 270, 2333–2340.

Stensmyr, M.C., Giordano, E., Balloi, A., Angioy, A.M. and Hansson, B.S. (2003b) Novel natural ligands for *Drosophila olfactory* receptor neurons. *Journal of Experimental Biology*, 206, 715–724.

Sutherland, J.P., Sullivan, M.S. and Poppy, G.M. (2001) Oviposition behaviour and host colony size discrimination in *Episyrphus balteatus* (Diptera: Syrphidae). *Bulletin of Entomological Research*, 91, 411–417.

Tolasch, T., Solter, S., Toth, M., Ruther, J. and Francke, W. (2003) (R)-acetoin-female sex pheromone of the summer chafer *Amphimallon solstitiale* (L.). *Journal of Chemical Ecology*, 29, 1045–1050.

Verheggen, F.J., Fagel, Q., Heuskin, S., Lognay, G., Francis, F. and Haubruge, E. (2007) Electrophysiological and behavioral responses of the multicolored Asian lady beetle, *Harmonia axyridis* Pallas, to sesquiterpene semiochemicals. *Journal of Chemical Ecology*, 33, 2148–2155.

Verheggen, F.J., Arnaud, L., Bartram, S., Gohy, M. and Haubruge, E. (2008) Aphid and plant secondary metabolites induce oviposition in an aphidophagous hoverfly. *Journal of Chemical Ecology*, 34, 301–307.

Wäckers, F.L. (2000) Do oligosaccharides reduce the suitability of honeydew for predators and parasitoids? A further facet to the function of insect-synthesized honeydew sugars. *Oikos*, 90, 197–201.

Warthen, J.D., Lee, C.J., Jang, E.B., Lance, D.R. and Mclnnis, D.O. (1997) Volatile, potential attractants from ripe coffee fruit for female mediterranean fruit fly. *Journal of Chemical Ecology*, 23, 1891–1900.

Webberley, K.M., Hurst, G.D.D., Husband, R.W., Schulenburg, J.H.G.V.D., Sloggett, J.J., Isham, V., Buzcko, J. and Majerus, M.E.N. (2004) Host reproduction and a sexually transmitted disease: causes and consequences of *Coccipolipus hippodamiae* distribution on coccinellid beetles. *Journal of Animal Ecology*, 73, 1195–1200.

Wei, J.R., Yang, Z.Q., Hao, H.L. and Du, J.W. (2008) (R)-(+)-limonene, kairomone for *Dastarcus helophoroides*, a natural enemy of longhorned beetles. *Agricultural and Forest Entomology*, 10, 323–330.

Zhu, J., Cosse, A.A., Obrycki, J.J., Boo, K.S. and Baker, T.C. (1999) Olfactory reactions of the twelve-spotted lady beetle, *Coleomegilla maculata* and the green lacewing, *Chrysoperla carnea* to semiochemicals released from their prey and host plant: electroantennogram and behavioural responses. *Journal of Chemical Ecology*, 25, 1165–1177.

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