Effect of wheat plant volatiles on aphids and associated predator behavior: selection of efficient infochemicals for field study*

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Abstract [Objectives]  Semiochemicals are involved in tritrophic interactions and affect the behaviors of both herbivores and beneficial insects. Many volatile molecules from the wheat plant [Triticum aestivum L. (Grainaceae)] have previously been identified. To understand their effects on aphids and related auxiliaries. [Methods]  The impact of four of these volatiles; methyl-salycilate, cis-3-hexenyl acetate, hexenol, and 1-hexanol, was tested on the wheat aphid [Sitobion avenae (Fabricus)] (Homoptera: Aphididae) and two major predators of this pest encountered in field crops, the hoverfly (Episyrphus balteatus (DeGeer) (Diptera: Syrphidae)) and the multicoloured Asian ladybird (Harmonia axyridis (Pallas)) (Coleoptera: Coccinellidae). Different doses of these chemicals were tested by performing four arm-ofaflower and wind-tunnel assays. [Results]  The results show that methyl-salycilate seemed to be attractive only toward H. axyridis but 3-hexenyl acetate and hexenol were attractive to both S. avenae and H. axyridis. However, these positive responses decreased with increased dose of the tested compounds. 3-hexenyl acetate also induced positive response in E. balteatus but 1-hexanol was more attractive to aphids than to their natural enemies. [Conclusion]  In conclusion, these results highlight the potential benefit of combining methyl-salycilate, 3-hexenyl acetate and hexenol in push and pull traps aimed to control S. avenae. Contrary to the three aforementioned compounds, 1-hexanol did not seem to have much potential as a semiochemical for the biological control of aphids.

Key words  wheat, volatiles, infochemicals, aphid, predator, behaviour

1  Introduction

Some reports have indicated that the use of semiochemicals in traps could make them really efficient in controlling pests in fields (Martel et al., 2005; Ruther and Mayer, 2005). Therefore, great attention has been paid to the use of such compounds in integrated pest management strategies (IPM) for few years. Semiochemicals are involved in tritrophic interactions, affecting the behaviours of both phytophagous and beneficial insects. The potential use of such infochemicals in the biological control of aphids has then been considered (Agelopoulos et al., 1999). Compared to visual signals, chemical cues provide more reliable information in a long distance or when preys are hidden (Verkerk et al., 1998) and allows insects to gain more crucial information from their environment (Touhara and Vosshall, 2009). Olfaction in a variety of aphid species and enemies play an important role for both the pre- and post-alighting stages of host selection (Pickett

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Plants respond to herbivore damages by producing HIPVs that sometimes repel herbivores and attract natural enemies of pests that are responsible for the plant damages (Turlings et al., 1990; Bernasconi et al., 1998; Paul and Tumlinson, 1999; Francis et al., 2004; Liu et al., 2005; Mallinger et al., 2011). Such behaviours could be obtained in aphids and their natural enemies by using several molecules, such as aphid-induced plant volatiles, that can then be used in fields to promote a push-pull strategy (Hardie et al., 1994; Liu et al., 2001; James, 2005). The foraging behaviour of aphids and their enemies have also been reported to be influenced by the concentration of volatiles liberated by plants (Guerrieri et al., 1999; Han and Chen, 2002; Mustaparta, 2002).

The wheat aphid, *Sitobion avenae*, is a major wheat pest in the world. It pierces and sucks juice of wheat plants, and also acts as carrier of plant viruses. The control of their populations is difficult given their short generation time and high reproduction rate (Irwin and Thresh, 1988; Smith and Boyko, 2007). *Harmonia axyridis* and *Episyrphus balteatus* have been reported to be abundant and efficient enemies of *S. avenae*, playing then an important role in reducing the populations of this species in wheat fields (Li et al., 1990; Wang and Shen, 2002).

In this work, four important compounds of wheat, namely methylsalicylate, cis-3-hexenyl acetate, hexenol and 1-hexanol were selected to be tested on *S. avenae*, *H. axyridis* and *E. balteatus*. Methylsalicylate is a typical aphid-induced plant volatile (Pettersson et al., 1994; Liu et al., 2001). The three other compounds were both HIPVs and common Green Leaf Volatiles (GLVs) from wheat (Guo and Liu, 2005; Quiroz and Niemeyer, 1998; Pareja et al., 2007; Piesik et al., 2008). Hexenol is known as leaf alcohol that acts as an attractant to many predatory insects (Visser et al., 1996; Ruther and Kleier, 2005; Yin et al., 2005; Cheng et al., 2010) and elicits significantly oriented flight of aphid parasitoids (Du et al., 1998).

The aim of the experiments presented here was to compare the activity of GLVs to aphids and two related important natural enemies in order to identify semiochemicals which could be used efficiently in push-pull traps to attract beneficial insects and repel pest.

## 2 Materials and methods

### 2.1 Insect rearing

*Sitobion avenae* came from a long-term laboratory rearing on wheat in Entomology Department in Gembloux. Wheat plants were grown in conventional compost in 9 cm diameter plastic pots under (20 ± 2)°C and 16 hour photoperiod. Plants with 10 cm height were inoculated with aphids. The infested plants were isolated in separate conditioned rooms with identical environmental conditions. Adults were starved for 12 h before they were used to test the chemical cues in behavioural assays.

*Harmonia axyridis* individuals were obtained from a mass-production set up in a controlled environment incubator presenting the following conditions: (20 ± 2)°C, 45% ± 5% RH and 16 : 8 (L : D) photoperiod. The rearing was conducted in 36 cm×15 cm×8 cm aerated plastic boxes (±20 individuals per container). *Acyrtosiphon pisum* was daily supplied to larvae whereas adults were fed with sugar, a water-impregnated sponge and multiflower pollen. Oviposition was induced by introducing some *V. faba* stems infested of *A. pisum* in boxes containing adults. Initial adults were collected in infested dwellings situated in the vicinity of Gembloux and Verlaine during the winter of 2008. All the tested individuals owned to the fifth generation of this breeding. Male and female of ladybird adults were starved for 24 h before the beginning of the bioassays (only water was still provided).

*Episyrphus balteatus* was reared in wooden cages (60 cm × 100 cm × 100 cm) located in a climate-controlled room (L : D= 16 : 8; 60% ± 5% RH; (20 ± 2)°C). Larvae were obtained from a
mass-production: the hoverflies were supplied with sugar, multiflower pollen and water and the oviposition was induced by the introduction of host-plants infested with *A. pisum* in the rearing-cage during 3 h. The complete life cycle took place on the host-plants daily re-infested with aphids. *Syriphid pupae* were provided by Katz Biotech AG (Baruth, Germany). Female hoverflies (15-20 days old) were starved for 24 h, only supplied with water, before the experiment start.

### 2.2 Four-arm olfactometer assays

Four-arm olfactometers were used to test the behavioural responses of aphids and ladybirds towards the various chemical compounds. The olfactometer used to test ladybirds was constructed entirely of Teflon® and presented a 40 cm walking area (from centre to odour source) whereas the one used for aphids was made up of Plexiglas and possessed a 10.5 cm walking area. Both were similar to the one described by Vet *et al.* (1983). A pump provided an air stream that was successively passed through an activated charcoal filter, distilled water and the odour source. Airflow in each of the four arms was adjusted with a flow meter to 50 mL/min. Odour-emitting samples were placed in a 25 mL glass flask linked to one of the four olfactometer arms. A ventilation of the exposure chamber was ensured thanks to a pump pulling a 200 mL/min flow. The olfactometer assays were conducted in a controlled room presenting a temperature of (20 ± 2)°C and a relative humidity of 65% ± 5%. The four tested compounds, i.e. methyl salicylate (99%), cis-3-hexenyl acetate (≥ 98%), hexenol (98%), 1-hexanol (99%) (Sigma-Aldrich, Steinheim, Germany) were formulated in paraffin oil. Three amounts (1, 10 and 100 µg) of these semiochemicals, were placed on a 0.5 cm² piece of filter paper and offered to the tested insects.

*Sitobion avenae* and *H. axyridis* were individually tested in the olfactometer. Males and females of *H. axyridis* were differentiated during this assay according to the criteria described by McCornack *et al.* (2007). Each individual was placed at the centre of the exposure chamber. Each aphid was observed during 5 min and ladybirds were tested during 3 min, these two times being respectively sufficient for *S. avenae* and *H. axyridis* to explore all the set-up. The time spent by each insect in every olfactometer areas was recorded. Every 5 observations, the olfactometers were rotated by 90 degrees to avoid directional bias. Between each replicate, each system was cleaned with pure ethanol (≥ 99.5%, Sigma-Aldrich, Steinheim, Germany) and rinsed with distilled water. Twenty replicates were performed with aphids, *H. axyridis* males and *H. axyridis* females for each amount of the four tested volatiles. The exact numbers of repetitions performed for every bioassay are mentioned in Table 1.

### 2.3 Wind-tunnel assays

The same four volatile compounds were tested on *E. balteatus* in a wind-tunnel. Each semiochemical was also formulated in paraffin oil. Rubber septa were filled with 100 µg of each compounds and fixed on a stick in the wheat plant pot. Ten wheat pots were placed simultaneously at the end of the tunnel (at the fan side), in such a way that insects had to fly upwind to join them. Clean plants constituted the negative controls and the positive control consisted of wheat plants infested with twenty *S. avenae* aphids per pot.

The wind-tunnel was constructed of Plexiglas (2.4 m × 0.8 m × 0.6 m) and a fan was used to pull air through the tunnel with a velocity of 0.4 m/s. The air was filtered through activated charcoal and pushed through a series of baffles to create a laminar flow. The wind speed was then constant in the entire tunnel. Illumination was provided by neon tubes mounted 40 cm above the wind tunnel giving a light intensity of 2 300 lx. Temperature and relative humidity were respectively (20 ± 2) °C and 65% ± 5% RH.

Ten *E. balteatus* were simultaneously introduced into the tunnel, 2 m downwind from the odour source (each assay was done in three replicates). Individuals were given 2 h to respond in each set of
Table 1  Synthesis of tested volatile molecule effects and related statistical values on *Sitobion avenae* aphid and both sexes of *Harmonia axyridis* predator in four-arm olfactometer experiments

<table>
<thead>
<tr>
<th>Molecules and doses</th>
<th><em>Sitobion avenae</em></th>
<th><em>Harmonia axyridis</em> females</th>
<th><em>Harmonia axyridis</em> males</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>$P$ value</td>
<td>$F_{(3,40-4)}$</td>
</tr>
<tr>
<td>Methyl-salicylate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 µg</td>
<td>20</td>
<td>0.812</td>
<td>0.32</td>
</tr>
<tr>
<td>10 µg</td>
<td>20</td>
<td>0.479</td>
<td>2.41</td>
</tr>
<tr>
<td>100 µg</td>
<td>20</td>
<td>0.769</td>
<td>0.98</td>
</tr>
<tr>
<td>3-hexenyl acetate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 µg</td>
<td>20</td>
<td>0.818</td>
<td>9.3</td>
</tr>
<tr>
<td>10 µg</td>
<td>20</td>
<td>0.379</td>
<td>8.15</td>
</tr>
<tr>
<td>100 µg</td>
<td>20</td>
<td>0.612</td>
<td>3.09</td>
</tr>
<tr>
<td>Hexenol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 µg</td>
<td>20</td>
<td>0.532</td>
<td>7.29</td>
</tr>
<tr>
<td>10 µg</td>
<td>20</td>
<td>0.174</td>
<td>6.73</td>
</tr>
<tr>
<td>100 µg</td>
<td>20</td>
<td>0.004**</td>
<td>4.41</td>
</tr>
<tr>
<td>1-hexanol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 µg</td>
<td>20</td>
<td>0.379</td>
<td>1.03</td>
</tr>
<tr>
<td>10 µg</td>
<td>20</td>
<td>0.884</td>
<td>4.7</td>
</tr>
<tr>
<td>100 µg</td>
<td>20</td>
<td>0.295</td>
<td>8.81</td>
</tr>
</tbody>
</table>

** indicates extremely significant difference ($P < 0.01$); +, ++, +++ indicate the attract or repel effect are significant difference at the three level $P$<0.05, 0.01, 0.001, respectively.
experiments. The positions of individuals were observed every 20 min during 2 h and the percentage of individuals on the plant was determined.

2.4 Statistical analyses

One-way analyses of variance (ANOVA) were performed to compare the time spent by insects in the four areas (one containing the semiochemical compound and the three others constituting controls). To verify that the observed differences were well due to an effect of the chemical compounds and that the experimental bioassay did not present any bias, the between-group sum of squares were compared to the within-4 areas sum of squares. The result of these comparisons appears in Table 1 (P value (bias)). These statistical analyses were performed for each bioassay (the three amounts of each semiochemical and that for every tested insect) by using Minitab®15.1.1.0 (State College, Pennsylvania, USA).

For *E. balteatus* wind tunnel results, percentage of individuals was analyzed by mixed generalized linear models, with a binomial error distribution. Statistical analyses were performed with *R* statistical software v2.10.1.

3 Results

*Sitobion avenae* spent significantly more time in the area containing 3-hexenyl acetate at the three tested amounts (1, 10 and 100 µg) whereas they only responded positively to the amounts of 10 and 100 µg of 1-hexanol and 1 and 10 µg of hexenol. The presence of a bias in the bioassay testing 100 µg of hexenol on *S. avenae* did not allow to assess correctly the effect of this dose on the studied pest (Table 1, Fig. 1). On the other hand, methyl-salycilate showed neither attractive nor repellent effect toward this aphid species.

![Fig. 1](image)

*Fig. 1* Mean relative stay durations (in %) of insects - *Sitobion avenae* aphid and both sexes of *Harmonia axyridis* predator - in the area offering the corresponding odour source in the 4 ways olfactometer

A: Methyl-salycilate; B: Cis-3-hexenyl acetate; C: Hexenol; D: 1-hexanol.

Horizontal dash lines in the graphs represent the theoretical mean relative duration for each of the olfactometer area.
Harmonia axyridis females spent significantly more time in the area presenting methyl-salicylate at any of the three tested doses. Concerning the other compounds, such a response was only observed for 100 µg of 3-hexenyl acetate and 10 µg and 100 µg of hexenol. No positive or negative response was observed with 1-hexanol.

The responses of H. axyridis males were different: they spent more time in the area containing 100 µg of chemical, for all the semiochemicals tested. A positive response was also obtained with 10 µg of 3-hexenyl acetate (Table 1). All the other doses of any tested chemicals did not induce a response.

Concerning E. balteatus, significantly higher numbers of insects were attracted by positive control \( (F=4.516, P<0.001; \text{Fig. 2}) \). And they were also attracted by cis-3-hexenyl acetate \( (F=4.513, P<0.001; \text{Fig. 2}) \). There was no significant difference between clear plant and the other odour sources.

4 Discussion

This work highlight semiochemicals originated from wheat, which could be used in push-pull strategies aiming to control aphids. The bioassays performed on aphids indicated that cis-3-hexenyl acetate, hexenol and 1-hexanol induced a positive behavioural response in S. avenae, but as the doses of cis-3-hexenyl acetate and hexenol increased, the duration of stay for aphids became shorter. On the other hand, the opposite behaviour was observed for 1-hexanol as the highest positive response of S. avenae toward this semiochemical was observed for the highest doses. The positive responses of S. avenae toward cis-3-hexenyl acetate are in accordance with the work of Guo and Liu (2005) which showed that a foraging response was induced by this chemical compound in Macrosiphum avenae and Rhopalosiphum padi. Quiroz and Niemeyer (1998) also showed that cis-3-hexenyl acetate is a wheat and oat volatile compound that elicits attraction of apterae aphids. Moreover, 1-hexanol, cis-3-hexenyl acetate and methyl- salicylate elicited statistically significant electrophysiological responses when tested at the dose of 10 µg (Webster et al., 2008).

Research conducted in field showed that methyl-salicylate repels S. avenae (Petterson et al., 1994). Although no significant repellent effect of methyl-salicylate was observed in our experiments, aphids spent shorter time in the area containing this
semiochemical. Other aphid species seem also to be repelled by this compound. Indeed, Hardie et al. (1994) indicated that methyl-salicylate has previously been shown to cause black bean aphids to move away from its source in a linear-track olfactometer.

Males and females of *H. axyridis* presented different olfactory response to low doses of the four tested volatiles whereas at the dose of 100 µg, they generally showed a strong positive response. This fact indicates that the dose is highly important in biological control of pest. Similar results were obtained by testing the odours emitted by various densities of tea aphids on seven-spot ladybirds. Natural enemies perception rely on semiochemical cues only above a threshold, defined by the amount but also by time and space. When the dose reached a certain level, natural enemies (*Chrysopa sinica, Aphidius* sp., *Coccinella septempunctata*) began to respond. The mechanism by which insects receive semiochemicals may be responsible for this fact (Guerrieri et al., 1999; Han and Chen, 2002). Volatile compounds at extremely high concentrations may stimulate olfactory receptor neurons that are not normally specific to those volatiles (Mustaparta, 2002). Therefore, suitable concentrations to apply in field experiments have to be well identified.

As doses of cis-3-hexenyl acetate increase, aphids spent less time in the area offering the odour in the olfactometer set-up. However, ladybirds and hoverflies were strongly attracted by the highest amount of this compound. The use of this chemical cue could then be efficient in push-pull strategy. It could be interesting to study the effect of higher doses of 3-hexenyl acetate on *S. avenae* and its related natural enemies to maybe obtain a more pronounced attractive effect of aphidophagous predators while having no more effect on aphids.

Ladybirds have also been observed to be significantly attracted by odour source of

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**Fig. 2** Mean individuals percentage (in %) of *Episyrphus balteatus* in response to different molecules released in wind-tunnel experiments

*** indicates extremely significant difference (*P* < 0.001).

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methyl-salicylate, on the contrary, aphids had no response. James (2005) have also shown that the ladybird Stethorus punctum picipes was attracted to sticky traps baited with methyl-salicylate and with hexenol. Methyl-salicylate induced the searching behaviour of ladybirds and potentially could be used in infochemical releasers in field for attracting predators such as ladybirds and hoverflies without attracting aphids. In our experiments, hexenol showed an increasing attractive potential toward ladybird and a decreasing one on aphids as the molecule amount became higher. It can then also present good potentials for biological control. Finally, 1-hexanol, which is a common GLV in many plants, was more attractive to aphids than to ladybirds. This molecule is then not suitable for push-pull application in field.

In conclusion, having a good knowledge of the behavioural activity of aphids and their natural enemies towards volatiles is essential to identify the more suitable semiochemicals which could be used in biological strategies, such as push and pull ones, aiming to control aphids in crop fields.

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References


小麦挥发物对蚜虫及其天敌的行为影响
——田间有效挥发性信息化合物的筛选*

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Haubruge Eric2 孙京瑞1  Francis Frédéric2** 陈巨莲1**

【目的】植物挥发性信息化合物能够影响植食性昆虫及其他天敌行为，因此，在植物-植食性昆虫-天敌互作系统中具有重要地位。目前，小麦[Triticum aestivum L. (Gramineae)]中多种挥发物已被鉴定，为明确其对蚜虫及其天敌的行为影响。

【方法】本试验利用四臂嗅觉仪和风洞测定4种不同浓度的小麦挥发物(水杨酸甲酯、3-己酰醋酸酯、己烯醇和1-己醇)对麦长管蚜[Sitobion avenae (Fabricus)]及其两种重要天敌，异色瓢虫[Harmonia axyridis (Pallas) (Coleoptera: Coccinellidae)]和黑带食蚜蝇[Episyrphus balteatus DeGeer] (Diptera: Syrphidae)]的行为反应。

【结果】结果显示，水杨酸甲酯仅吸引异色瓢虫。3-己酰醋酸酯和己烯醇吸引麦长管蚜及异色瓢虫，但随其浓度增加对蚜虫的吸引作用降低，对异色瓢虫吸引作用增强。3-己酰醋酸酯对黑带食蚜蝇也具有吸引作用。另外，与天敌相比，1-己醇对麦长管蚜吸引作用更强。

【结论】总之，本试验明确除1-己醇外，水杨酸甲酯、3-己酰醋酸酯、己烯醇可以作为吸引天敌，控制蚜虫的潜在用于推-拉策略的挥发性化学信息物。

关键词 小麦,挥发物,蚜虫,天敌,行为