RESEARCH ARTICLE

Can the pheromones of predators modulate responses to herbivore-induced plant volatiles?

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Abstract

Biological control of greenhouse pests has been successfully developed and applied. In greenhouse crops, several entomophagous species (predators and parasitoids) are used simultaneously in the crop cycle. One important aspect of these crops, which represent modified ecosystems, is the interactions among complexes of species, including plants, phytophagous insects, and predators. The chemical relationships (semiochemicals: pheromones and kairomones) among these species likely play an important role in greenhouse crops; however, few studies have focused on these relationships. The aim of this study was to analyse the importance of semiochemicals. Three groups of laboratory trials were conducted with two predatory species: *Nabis pseudoferus* and *Nesidiocoris tenuis* (Hemiptera: Nabidae and Miridae, respectively). The results of the first group of trials indicated that the adult females of both species were more attracted to herbivore-induced plant volatiles (HIPVs) than they were to the control plants or plants with artificial damage. Based on the second group of trials, pheromones triggered an attraction in adult females of both species for conspecifics. Finally, based on the interactions of the adult females of the same species, pheromones changed or modulated the predatory responses to HIPVs. The implications of these results for the biological control of pest species in greenhouses are further discussed.

Introduction

Chemical information on the biology of plants and animals, including intraspecific chemical communication and its exploitation by heterospecifics, is important. Semiochemicals are the chemical signals involved in this interaction (Wyatt, 2011, 2014), and they are classified as pheromones when they mediate interactions between organisms of the same species and as allelochemicals when they mediate interactions between organisms of different species (Dicke & Sabelis, 1988). Allelochemicals are subdivided depending on whether the interaction is adaptively advantageous for the receiver (kairomones), transmitter (allomones), or both (synomones) (Dicke & Sabelis, 1988; Vet & Dicke, 1992; Mori, 2010).

The importance of prey or host kairomones in the interactions among phytophagous-entomophagous (predator and parasitoid) insects has been stressed in several studies (e.g. Hagen *et al.*, 1976; Dicke *et al.*, 1990; Vet & Dicke,

1992). Moreover, plants can respond to feeding or egg deposition by phytophagous arthropods by changing the blend of volatiles emitted. These herbivore-induced plant volatiles (HIPVs) can attract entomophagous natural enemies (predator and parasitoid) of herbivores, which is referred to as 'indirect plant defence' (Greenstone & Dickens, 2005; Mumm & Dicke, 2010; James *et al.*, 2012; Rani, 2014). In a predator–prey interaction, because the transmitter is the plant, HIPVs are synomones because they are beneficial to both plants and predatory species (Greenstone & Dickens, 2005). In contrast, prey kairomones only benefit predatory species (Rani, 2014).

Recent studies reviewed the role of allelochemicals in the plant–phytophagous–entomophagous complex, including prey or host kairomones and HIPVs (Honda *et al.*, 2010; Kaplan, 2012a) and the role of HIPVs in pest control (Thacker & Train, 2010) and in biological control (Kaplan, 2012b). Moreover, the roles of HIPVs for both

parasitoid (e.g. Cabello & Vargas, 1985; Colazza & Wajnberg, 2013) and predatory species (e.g. Erbilgin & Raffa, 2001; Poelman & Dicke, 2014) have been studied extensively.

Modulations caused by semiochemicals in relation to phytophagous insects, such as interactions between pheromones and plant volatiles (non-HIPVs), have been reported. More specifically, Reddy & Guerrero (2004) reviewed the modulations by semiochemicals in relation to sex and aggregation pheromones, and Deisig *et al.* (2014) reviewed these chemicals in relation to non-lepidopteran insects. These studies indicate that plant volatiles have inhibitory or repellent effects that interrupt insect responses to pheromones. In contrast, plant volatiles can also synergistically enhance the response to sex pheromones (Reddy & Guerrero, 2004). Furthermore, interactions between HIPVs and pheromones in relation to phytophagous species have also been reported, such as for the two-spotted spider mite *Tetranychus urticae* Koch on lima bean leaves (Dicke, 1986) and the palm weevil *Rhynchophorus* spp. (Deisig *et al.*, 2014).

The modulating effects of semiochemicals are more difficult to establish for entomophagous species. The chemical relationships for entomophagous species are apparently not based on a single semiochemical but include responses to a complex mixture of chemical cues (i.e. kairomones, allomones, and synomones), which act either jointly or sequentially in predator (e.g. Dicke *et al.*, 1990) and parasitoid species (e.g. Vinson, 1976, 1984) and have either a short or a long detection distance (Vet & Dicke, 1992).

In greenhouse biological control programmes, such as those conducted in Europe, several species of natural enemies (predators and parasitoids but predominantly predators) have been used simultaneously to control pest species (van Lenteren & Woets, 1987; van Lenteren, 2012; Vila & Cabello, 2014). Furthermore, for the control of greenhouse aphid pests, studies have implemented a system of 'banker plants', in which cereal plants (barley or wheat) are infested with specific gramineous aphids (e.g. the bird cherry–oat aphid *Rhopalosiphum padi* (L.)) that are the alternative hosts of parasitoids, usually *Aphidius* spp. or *Lysiphlebus testaceipes* (Cresson), until the first infestations with the aphid pest species (the cotton aphid *Aphis gossypii* Glover or the green peach aphid *Myzus persicae* Sulzer) develop in the greenhouse crop (Frank, 2010; Huang *et al.*, 2011). The findings imply that a greater number of volatiles in the greenhouse may interfere with the populations of pest species (Tan & Liu, 2014, 2014) or their natural enemies (Li *et al.*, 2015) and increase the complexity of chemical relationships within the greenhouse, potentially altering the effectiveness of biological control programmes.

Nesidiocoris tenuis (Insecta: Hemiptera: Miridae) is a true omnivore (Sanchez 2008) and represents a case of trophic omnivory in which the consumer feeds on both plants and prey according to the terminology of Coll & Guershon (2002). The distribution of the species was originally paleotropical, and the mirid has been introduced in Europe (Wheeler & Henry, 1992; Rabitsch, 2008). This omnivore is a reputed predator of small arthropod pests (i.e. aphids, whiteflies, and eggs of Lepidoptera) (Valderrama *et al.*, 2007; Perez-Hedo & Urbaneja, 2015) but also causes injuries to vegetative and reproductive plant parts (Sanchez, 2009).

Nabis pseudoferus (Insecta: Hemiptera: Nabidae) is a non-omnivorous zoophagous insect (*sensu stricto*). This species is primarily an aphid predator but also feeds on Lepidoptera and other groups of insects (Puchkov, 1980; Cabello, 2009; Cabello *et al.*, 2009).

Based on the above discussion, the aims of this study were to determine the responses to different chemical cues: Prey odour (kairomones), HIPVs (synomones), and pheromones in two species of heteropteran predators with different diets (zoophagy versus zoophytophagy) and explore the possible implications of their use as control agents for pest species in protected crops.

Materials and methods

Biological material

The *N. pseudoferus* and *N. tenuis* specimens used in the trials were collected from populations in Granada and Almeria (Spain), respectively, and they were reared under laboratory conditions for two generations before their use in laboratory trials. Plastic containers (20 L) were used for rearing with material for dispersion, and the alternative host, the Mediterranean flour moth *Ephestia kuehniella* Keller (Lep.: Pyralidae), was used as prey following the methodology described by Vila & Cabello (2014).

Rhopalosiphum padi (Hem.: Aphididae) was also used as prey, and these insects were purchased (Plantacontrol®; Agrobio S. L., La Mojonera, Almeria, Spain) and raised in cages equipped with mesh covers. Wheat plants, *Triticum aestivum* (Garcia®, Agrusa S.L., Mollerussa, Lleida), were also used following the methodology described by Vila & Cabello (2014). In all trials with plants, this variety of wheat was used. The plants were grown and the insects were reared in the following environmental conditions: $25 \pm 1°C$, 60–80% RH, and 16:8 h of light : dark.

Equipment

For the still-air bioassays, 2- and 4-choice olfactometers were used. Moving-air bioassays were previously conducted with Y-tube (2-choice) and four-arm

olfactometers; however, the results indicated that still-air bioassays were better able to assess the behaviour of both *N. pseudoferus* and *N. tenuis* adults (unpublished data).

The 2-choice still-air olfactometer was the same as that used by Prokopy *et al.* (1995) and Van Tol *et al.* (2002, 2004) for adult curculionids but with different dimensions. The olfactometer consists of a plastic Petri dish (100 mm diameter × 42 mm height) with two holes in the Petri dish lid at 10 mm in diameter and 65 mm apart. Two small plastic tubes (length: 30 mm; diameter: 10 mm) were positioned on top of the holes in the lid. Two plastic cylinders (length: 73 mm; diameter: 45 mm) were placed on the lid such that one of the small plastic tubes was in the centre of each cylinder. A plastic cup (length: 73 mm; diameter: 45 mm) with a rim (length: 30 mm; diameter: 62 mm) was placed on top of each plastic cylinder to interlock the cylinder and cup, with rigid metallic gauze (mesh: 2 μm) clamped between the cylinder and cup (Fig. 1A).

The 4-choice still-air olfactometer was similar to that of the 2-choice except that a plastic container with a rectangular plastic frame (length: 130 mm; width: 130 mm; height: 30 mm) that was closed below and above with two plastic plates replaced the Petri dish. The top plate had four holes at 10 mm in diameter and 65 mm apart. Over each hole, a plastic cylinder and a cup were placed as indicated above for the 2-choice olfactometer (Fig. 1B).

Experimental design and procedure

The experimental design in all trials was completely randomised with only a 'treatment' (chemical cues) factor, with 35 replications performed per trial. All trials were conducted for 24 h in climatic cabinets (Memmert®, Memmert GmbH + Co. KG, Schwabach, Germany, ICP 600) (25°C ± 1, RH: 75–85%, photoperiod: 16:8 h light : dark). In each trial, to prevent the visual influence of the different elements tested in the olfactometers (i.e. plants, aphids, or conspecifics), a plastic black film was placed over the lids of the lower containers (i.e. decision chambers).

The females captured in the trapping device of the olfactometer could not reverse direction during the experiment. The entry hole to this part of the olfactometer was designed to prevent the escape of captured adult females back to the decision chamber as shown in Fig. 1A and Fig. 1B. The possible escape of adult females (from the trapping device) was practically zero because each olfactometer was inspected every 2 h; when an adult female made her choice (she was in the trapping device), the replicate and treatment was concluded, and when a decision was not made, the experiment continued until the next inspection (2 h) up to a limit of 24 h.

Trials 1 and 2: Behavioural responses to plants, prey, and plant damage (prey kairomones and synomones)

A single mated *N. pseudoferus* adult female was used (Trial 1) to evaluate the predator behaviour in response to plants, prey, and damaged plants. The females were obtained from the populations maintained in the laboratory, and they were collected less than 1 week after the last nymphal ecdysis, in the ovipositional period. The females were isolated and kept without food for 24 h before the trial. Only a sponge (1 cm × 1 cm) moistened with distilled water was provided. Subsequently, one mated female was placed in the lower container of each olfactometer in the conditions described above for 24 h. Six 4-choice olfactometers were used in the trial.

The treatments (chemical cues) were as follows: (a) plant, plant + prey, prey, and plant with artificial damage. The plants included a variety of wheat (aforementioned; height = 6–8 cm) sown in containers (Ø 2.8 cm; height 1.5 cm; 7 plants per container). (b) The prey included 10 adult *R. padi* from the populations maintained in the laboratory, with the plant–prey treatments composed of young wheat plants infected with *R. padi* (10 specimens per plant). (c) The artificial damage was performed with 10 needle punctures in the aforementioned number of plants.

In the trial with the other species *N. tenuis* (Trial 2), we also used one mated female. These females were less than one week since the last nymphal ecdysis and were handled in the same manner as the females in the previous trial with the same treatments (Trial 1).

Analyses of the results only included the adult females that made a choice (No. = 32 and 33 adult females, average time = 6.15 ± 1.12 and 7.04 ± 1.60 h and maximum time = 19.83 and 21.50 h for Trials 1 and 2, respectively).

Trials 3 and 4: Effect of the presence of a conspecific (pheromones)

In the trials examining the effects of a conspecific, a single mated female of *N. pseudoferus* (Trial 3) or *N. tenuis* (Trial 4) was used and handled as in previous trials (Trials 1 and 2). One adult female was released in the bottom container (Petri dish) of each olfactometer, remaining at the same temperature and lighting conditions as above for 24 h. The trials were performed with 2-choice still-air olfactometers. Treatments (chemical cues) were a conspecific female (of identical physiological characteristics) and no chemical cue (control). Each trial and treatment had 35 replications. As in previous trials, analyses of results only included adult females that made a choice (No. = 31 and 33 adult females, average time = 3.23 ± 0.89 and 5.06 ± 1.24 h, and maximum time = 21:00 h and 22:00 h for Trials 3 and 4, respectively).
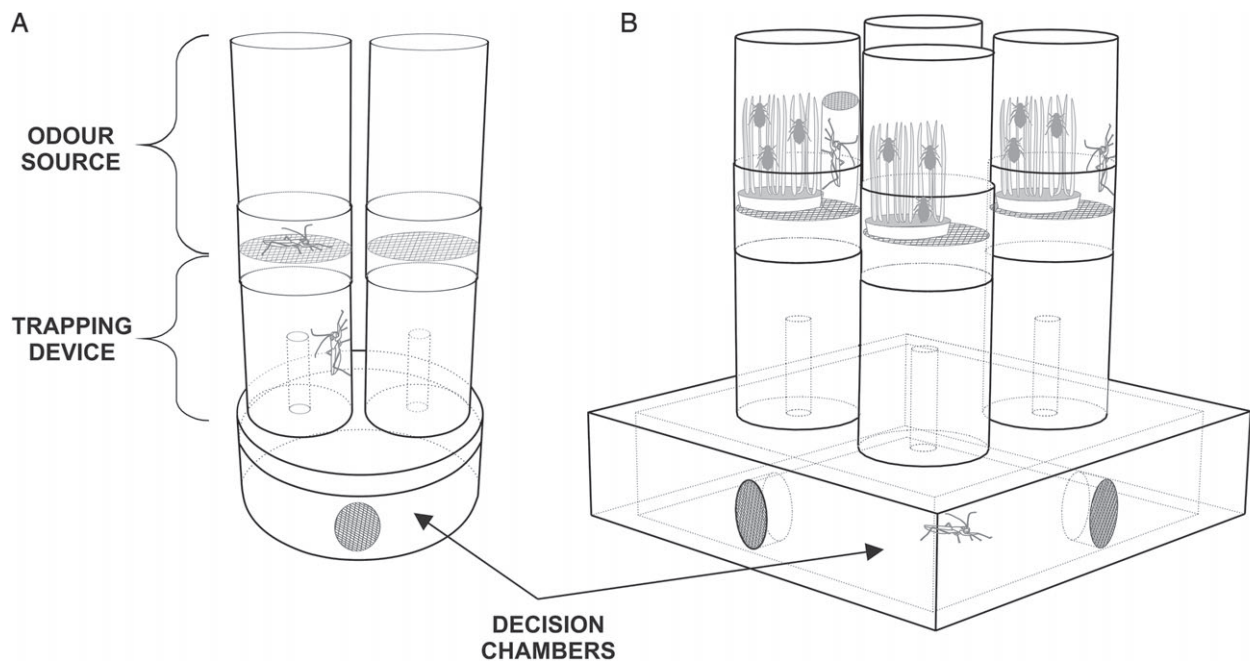
Figure 1 (A) 2-choice still-air olfactometer and (B) 4-choice still-air olfactometer, with the different components identified. Both were used in the trials.

Trials 5 and 6: Effect of a conspecific on plant–prey–predator interaction (pheromones verus synomones)

In the last group of trials, the effect of a conspecific was assessed in the plant–prey–predator interaction (pheromones versus prey kairomones and HIPVs). One adult female of *N. pseudoferus* (Trial 5) and one adult female of *N. tenuis* (Trial 6), with the aforementioned features, were used, and as in Trials 1 and 2, 4-choice olfactometers were used. The treatments in both trials were plant, plant + prey, plant + prey + conspecific, and plant + prey + separated conspecific. Plant and prey treatments were identical to those in the first set of trials (Trials 1 and 2). Furthermore, and depending on the treatment, a conspecific female (of identical physiological characteristics) was either placed with plant and prey (and therefore had the option to feed on prey) or separated from the plant and prey by a mesh (and therefore could not feed on prey) (Fig. 1B).

The analyses of the results only included adult females that made a choice (No. = 32 and 31 adult females, average time = 6.04 ± 1.14 and 6.39 ± 1.15 hours and maximum time = 18.75 and 18.75 hours for Trials 5 and 6, respectively).

Data analyses

The study of arthropod behaviour using olfactometers requires that statistical analyses recognise two primary problems: (a) pseudoreplication, because the replications were not true replications in a completely randomised design because identical equipment was used repeatedly throughout the trial (only one or a few olfactometers were available) (e.g. Ramirez *et al.*, 2000; Wajnberg & Haccou, 2008); and (b) overdispersion (see Dean, 1998), because it is characteristic of data obtained with olfactometers (e.g. Ricard & Davison, 2007; Davison & Ricard, 2011).

Therefore, generalized linear models (GZLMs) were used to examine the functional relationships between the behavioural responses of female predators (response variable) and the different odour sources (explanatory variables) in the two- or four-arm olfactometer trials. Because the data did not conform to the simple variance assumptions implied in using the binomial distribution, we used quasi-likelihood functions to compensate for the overdispersion of female predators within the olfactometers (Turlings *et al.*, 2004). The models were fitted by maximum quasi-likelihood estimation (IBM, 2015) with the GenLin procedure with binomial errors and the logit link function using the IBM SPSS version 22.0 statistical software package. In each trial, the significance of the model was assessed by an Omnibus test (to test whether the explained variance in a data set is significantly greater than the unexplained variance, overall). For each regression effect specified in the model, a Wald statistic was conducted, which is a test based on the linearly independent pairwise comparisons among the estimated marginal
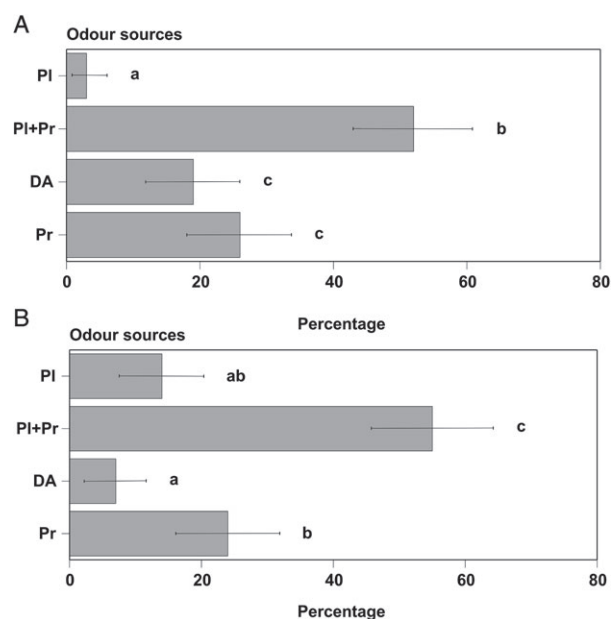
Figure 2 Preference of female adults of *Nabis pseudoferus* (A) or *Nesidiocoris tenuis* (B) in a 4-choice still-air olfactometer for odours of plant (Pl), prey (Pr), plant + prey (Pl + Pr), and artificially damaged plant (AD). Means (±SE) with the same letter are not significantly different from one another at $P = 0.05$.
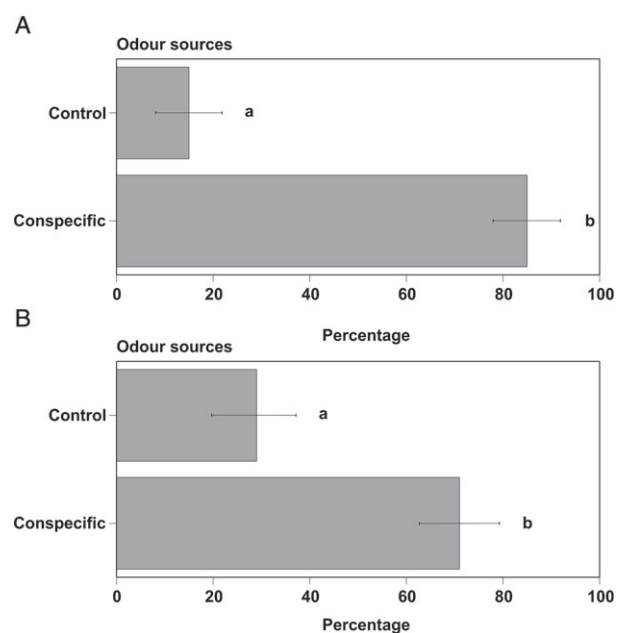

Figure 3 Preference of female adults of *Nabis pseudoferus* (A) or *Nesidiocoris tenuis* (B) in a 2-choice still-air olfactometer for conspecific odour. Means (±SE) with the same letter are not significantly different from one another at $P = 0.05$.

means. Then, the mean values were compared pairwise, with significance indicated at $P = 0.05$ (IBM 2013).

Results

Behavioural responses to plants, prey, and plant damage (prey kairomones and synomones)

Nabis pseudoferus *(Trial 1)*

The percentage response of *N. pseudoferus* females in Trial 1 to the different chemical cues (plant, prey, combination of the two, and artificial damage) is shown in Fig. 2A. In the GZLM analysis, the fitted model was statistically significant (Omnibus test, likelihood ratio $\chi^2 = 19.160$, d.f. = 3; $P < 0.001$). The factor (type of chemical cue) of the model had a significant effect on the selection pattern of the predatory females (χ^2 of Wald = 29.151, d.f. = 3; $P < 0.001$). Adult females were significantly more attracted to the combination of plant and prey (aphids) than to the single prey or the plant with artificial damage. By comparison, the plants were the least attractive to the adult females.

Nesidiocoris tenuis *(Trial 2)*

For *N. tenuis*, the statistical analysis indicated that the model was significant in explaining the variance (Omnibus test, likelihood ratio $\chi^2 = 17.022$, d.f. = 3;

$P < 0.001$). The factor 'type of chemical cue' had a significant effect on the female *N. tenuis* selection pattern (χ^2 of Wald = 19.377, d.f. = 3; $P < 0.001$). As with *N. pseudoferus*, the percentage of females attracted to volatiles emitted by the plant and prey treatments was significantly higher than the percentage of females attracted to the other treatments (Fig. 2B).

Effect of the presence of a conspecific (pheromones)

Nabis pseudoferus *(Trial 3)*

The effects of a conspecific on the behaviour of *N. pseudoferus* females are shown in Fig. 3A. Female predators were significantly ($85.0 \pm 7.1\%$) more attracted to a conspecific than to the control (no presence). In the GZLM analysis, the Omnibus test showed the model was significant in explaining the variance (likelihood ratio $\chi^2 = 27.438$, d.f. = 1; $P < 0.001$). The factor of the analysed model (conspecific) was highly significant in affecting the selection of predatory females (χ^2 of Wald = 47.864, d.f. = 1; $P < 0.001$).

Nesidiocoris tenuis *(Trial 4)*

N. tenuis females (Fig. 3B) were significantly more attracted to a conspecific than to the control (no presence). In the GZLM analysis, the Omnibus test showed

that the model was significant in explaining the variance (likelihood ratio $\chi^2 = 10.626$, d.f. = 1; $P < 0.001$). The factor of the analysed model (conspecific) was highly significant in affecting the selection by female predators (χ^2 of Wald = 12.600, d.f. = 1; $P < 0.001$). However, this attraction among females was less than that shown by *N. pseudoferus*.

Effect of a conspecific on plant–prey–predator interaction (pheromones versus synomones)

Nabis pseudoferus *(Trial 5)*

The percentage of adult female *N. pseudoferus* attracted to the plant–prey factor in the presence of a conspecific is shown in Fig. 4A, together with the values for the plant itself (control, in this trial). The GZLM analysis indicated that the model was highly significant (likelihood ratio $\chi^2 = 21.434$, d.f. = 3; $P < 0.001$). The factor of the analysed model (type of chemical cue) was highly significant in affecting the selection by female predators (χ^2 of Wald = 24.205, d.f. = 3; $P < 0.001$). The percentage of females attracted to the plant–phytophagous insect combination when conspecifics were present but isolated was significantly higher than that for the plant–pest treatment without a conspecific or with the conspecific not isolated. In this trial, a few females were attracted to plant volatiles (as the only chemical cue).

Nesidiocoris tenuis *(Trial 6)*

The percentage of female *N. tenuis* attracted to the plant–prey factor in the presence of a conspecific (either isolated or not) is shown in Fig. 4B. The model was highly significant (likelihood ratio $\chi^2 = 14.820$, d.f. = 3; $P = 0.002$) based on the statistical analysis. The type of chemical cue significantly affected the selection by female predators (χ^2 of Wald = 14.037, d.f. = 3; $P = 0.003$).

Volatiles emitted by the plant in combination with those emitted by the prey attracted significantly more female predators than the plant, plant–pest with non-isolated conspecific and plant–pest with isolated conspecific treatments.

Discussion

The results of Trials 1 and 2 for both predators indicated that adult female *N. pseudoferus* and *N. tenuis* were attracted to the volatiles emitted by plants when prey were feeding (HIPVs) (Fig. 2). Here, they function as synomones, beneficial for both participating parties (plant and predator).

Turlings & Wackers (2004) identified several species of Miridae (no information on Nabidae) that were attracted
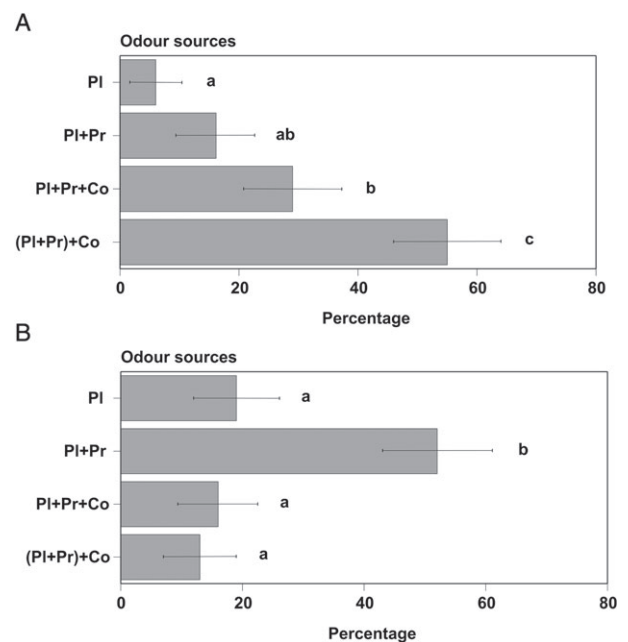

Figure 4 Preference of female adults of *Nabis pseudoferus* (A) or *Nesidiocoris tenuis* (B) in a 4-choice still-air olfactometer for odours of the plants (Pl), plant + prey (Pl + Pr), plant + prey + conspecific with direct contact of conspecific (Pl + Pr + Co), and plant + prey plus conspecific without direct contact ((Pl + Pr) + Co). Means (±SE) with the same letter are not significantly different from one another at $P = 0.05$.

to HIPVs. However, the reviewed literature does not contain examples of Nabidae species attracted to HIPVs. Therefore, this study is the first to report such behaviour in a species of this genus.

However, recent publications indicated that *N. tenuis* and *Macrolophus pygmaeus* (Hem.: Miridae) are attracted to HIPVs induced by the sweet potato whitefly *Bemisia tabaci* (Hem.: Aleyrodidae) and the South American tomato moth *Tuta absoluta* (Lep.: Gelechiidae) (Lins *et al.*, 2014). Similarly, Perez-Hedo & Urbaneja (2015) found that females of *N. tenuis* are apparently more attracted to pepper plants infected by the aphid *Myzus persicae* (Hem.: Aphididae) than to uninfected plants. Therefore, the results in this study for *N. tenuis* are consistent with these examples.

One notable result of this study was that the females of the two species exhibited similar responses to HIPVs (Trials 1 and 2, Fig. 2A and Fig. 2B), although the feeding strategies of the two species were markedly different. The adult females of *N. pseudoferus* feed as strict carnivores; therefore, the attraction to HIPVs was logical and consistent with literature reports for carnivorous species (e.g. Dicke, 1999). However, the adult females of *N. tenuis* have a phytophagous-zoophagous diet (omnivorous); therefore, the results were not as logical. The phytophagous

behaviour of females can be explained by the increased sensitivity of the receptor organs of phytophagous insects to plant volatiles (non-HIPVs), whereas predatory insects are more sensitive to HIPVs (Dickens, 1999). Moreover, certain omnivorous heteropteran predatory species are equally attracted to both non-HIPVs (plant volatiles) and HIPVs (Aldrich, 1998; Lehrman *et al.*, 2013).

Another notable result was the direct effect of a conspecific on both species. For the strict predator *N. pseudoferus*, the conspecific was attractive (Fig. 3A). These results are inconsistent with those reported by Roth *et al.* (2008), who found that females of the same species did not show a significant attraction to conspecifics. Several reasons might explain the differences between the two studies. First, the above authors used females collected directly from the field, which increased the difficulty of determining their age. Second, their trials did not include a previous fasting period, which may have prevented a struggle for territory and reduced the attraction to a conspecific.

The attraction observed among female *N. pseudoferus* might have been caused by non-chemical communication, such as acoustic or vibrational communication. However, vibratory communication in the family Nabidae has not been reported (Gogala, 2006). Stridulation by a species of Nabidae has been frequently mentioned in the literature; however, this report is based on the misidentification of animals and their ascribed behaviours (Wessel *et al.*, 2014). Therefore, in this study, we believe that the response of the females of this species was only caused by chemical cues.

Females of *N. tenuis* were also attracted to one another (Fig. 3B). For this species, although a stridulatory device has been described in several tribes and subfamilies of Miridae (Wessel *et al.*, 2014), stridulation has not been reported for the Dicyphini tribe, to which *N. tenuis* belongs. Furthermore, the functions of acoustical communication in Miridae remain unknown (Gogala, 1984; Goula, 2008).

In both species, the observed behaviours might be related to repugnatorial glands. The primary role of these glands in species of Heteroptera is to produce, store, and subsequently release volatiles. These volatiles are usually odorous (i.e. repugnatorial substances) because of their primary defensive role against predators; however, other roles are also cited, including their roles in sexual or aggregation behaviours (Staddon, 1979; Aldrich, 1988). For species of Miridae, the metathoracic repugnatorial glands in females produce and emit attractive pheromones for males (Aldrich, 1988; Millar, 2005), whereas evidence supporting a similar role for these substance in Nabidae has not been observed.

Finally, the third group of trials was conducted to assess the effect of a conspecific on predator attraction to plant + phytophagous insects, and although both species were greatly influenced by a conspecific, opposite effects were observed for each species. For *N. pseudoferus* (Fig. 4A), when a conspecific was isolated from the plant + prey factor, an attractive response was triggered; however, without this isolation, the response was significantly lower. When the conspecifics were isolated, the volatiles perceived by females likely included two types of allelochemicals – those emitted by the damaged plant (synomones) and those emitted by the prey (kairomone) – as well as potential pheromones emitted by conspecifics. For *N. tenuis*, an apparent repulsive effect was observed (Fig. 4B).

A comparison of these results (Fig 4A) with those obtained in previous trials (Fig 2A) indicated that two questions must be addressed: (a) Were the differences between the treatments with and without the isolation of *N. pseudoferus* conspecifics caused by the emission of different quantities and/or composition of pheromones? (b) Why was the response of *N. pseudoferus* females different from that of *N. tenuis* females?

For the first question, the conspecifics in these trials conducted different activities (depredation or not), which could lead to differences in the quantity or composition of the pheromones emitted. Thus, Wertheim *et al.* (2005) suggest that the response of individual insects is often largely affected by their physiological state. For example, Staples *et al.* (2002) found that calm adults of the mirid *Lopidea robiniae* do not produce detectable volatiles; whereas disturbed adults emit a six-component blend. Similarly, Wardle *et al.* (2003) found that the composition of volatile compounds released by disturbed and calm female *Lygus lineolaris* (Hem.: Miridae) was different. Therefore, our results could be explained by the pheromones emitted by conspecifics inhibiting the responses to HIPVs. As indicated in the Introduction, many examples of pheromone-plant volatile (non-HIPVs) interactions in phytophagous insect species have been reported (Reddy and Guerrero, 2004; Deisig *et al.*, 2014). Our results for the females of *N. pseudoferus* extend these effects to predatory insects and HIPVs.

In relation to the second question, the pheromones in heteropteran species have different functions that include defence, cannibalism inhibition, resource competition inhibition, and alarm or aggregation signalling (Curtis & McCoy, 1964; Aldrich, 1988; Aldrich *et al.*, 1995; Krall *et al.* 1999; Wardle *et al.*, 2003). In this study, the function might be related to cannibalism in *N. pseudoferus* females because cannibalistic behaviour has been previously reported in this species (Fernandez-Maldonado, 2016) and other species of the genus (Perkins & Watson 1972; Puchkov, 1980). Our results and those of the cited studies are consistent with those of Hurd (2008), who

reported that most non-omnivorous predators are cannibalistic. However, the cannibalism rate for the omnivorous species *N. tenuis* is substantially lower (T. Cabello, unpublished data). According to Leon-Beck & Coll (2007) and Laycock *et al.* (2006), omnivorous predators can be sustained by plant food sources in the absence of prey, which prevents cannibalism.

Based on the results of this study, HIPVs were important in the selection of prey by hemipteran-heteropteran predators; however, the response of females varied significantly depending on the presence of other volatiles (pheromones). These results could have consequences for the effectiveness of these predators in biological pest control, which is particularly important because the use of this group of predators has increased in recent years in greenhouse crops (Vila & Cabello, 2014). For example, the use of synthetic pheromones could be useful in the dispersion from points of release in the absence of prey for *N. tenuis*, which has been observed for other heteropteran species (e.g. Sant'Ana *et al.*, 1997).

The use of synthetic HIPVs or products that induce the production of such products in plants could also be used in biological pest control (conservation of the natural enemies) (Kaplan, 2012b). However, based on our study, the use of said products for certain biological control agents, such as *N. pseudoferus*, may not provide adequate control because encounters between conspecifics may increase and subsequently lead to an increase in cannibalism, even when prey are present. This could involve a reduction in their effectiveness.

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