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SHORT COMMUNICATION

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Attraction of two lacewing species to volatiles produced by host plants and aphid prey

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Abstract It is well documented that host-related odors enable many species of parasitoids and predatory insects to locate their prey and prey habitats. This study reports the first characterization of prey and prey host odor reception in two species of lacewings, *Chrysoperla carnea* (Say) and *Chrysopa oculata* L. 2-Phenylethanol, one of the volatiles emitted from their prey's host plants (alfalfa and corn) evoked a significant EAG response from antennae of *C. carnea*. Traps baited with this compound attracted high numbers of adult *C. carnea,* which were predominantly females. One of the sex pheromone components (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol of an aphid species, *Acyrthosiphon pisum* (Harris) attracted only *C. oculata* adults. Single sensillum recordings showed that the olfactory neurons of *C. carnea* responded to both 2-phenylethanol and aphid sex pheromone components, but those of *C. oculata* only responded to the latter.

Introduction

Chrysoperla carnea is the most common lacewing species in the Midwestern USA (Tauber et al. 2000). Adults are glyciphagous and pollinivorous feeding mainly on honeydew from homopteran species and floral nectar (Canard and Principi 1984). Previous studies showed that adults are attractive to host plant odors and tryptophan, a breakdown product of aphid honeydew (Flint et al. 1979; Hagen et al. 1976; Van Emden and Hagen 1976). In alfalfa fields, we observed a high abundance of *C. carnea* adults in early summer, while heavy infestations of pea aphids (*Acyrthosiphon pisum*) occurred (Zhu, unpublished data). This observation indicates that either alfalfa volatiles or certain volatiles induced by aphid infestations may be involved in attraction of them. Both larval and adult stages of *Chrysopa oculata* are predatory, adults are active throughout the summer and early autumn (Jubb and Mastellar 1977; Propp et al. 1969). We observed that in late summer and autumn, *C. oculata* adults attack soybean aphids (*Aphis glycines*) in the field and lay eggs on leaves of the soybean aphid overwintering host plant (*Rhamnus cathartica*), where pheromone-emitting aphids are located (Zhu, unpublished data). This observation suggests that aphid pheromones are involved in prey location by adult *C. oculata.*

Understanding the relationship between structure and function of the olfactory system in predatory insects provides the basis for new semiochemical tools to enhance the efficacy of biological control in sustainable agriculture, and provides new insights into olfaction in predatory insects. Therefore, we examined the response of these two lacewing species to semiochemicals associated with their aphid prey and aphid host plants. Our goals were (1) to identify chemical structures of volatiles associated with alfalfa (*Medicago sativa*, L), a host plant for the pea aphid; (2) to determine if host plant volatiles and aphid pheromones influence host searching behavior; and (3) to characterize the olfactory receptor neurons that respond to volatiles produced by aphid prey and their host plants.

Materials and methods

Insect sources

A laboratory colony of *C. carnea* was established from larvae purchased from Rincon-Vitova Insectary, Ventura, California. Larvae were reared on pea aphids and frozen *Ephestia kuehniella* eggs at 20°C, 16:8, light–dark period until eclosion. Adults were sexed and kept in separate cages until used. *C. oculata* adults were collected from the fields in central Iowa (using a sweep net), and reared in

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the laboratory under the same conditions as described for *C. carnea*.

Collection of host plant volatiles

Volatiles released from alfalfa, *Medicago sativa* L., were collected from non-infested alfalfa plants (1–2-week old) from the field (n=5) using solid-phase microextraction (SPME) with a polymer fiber coated with 100 µm of polydimethylsiloxane (Supelco, Bellefonte, PA). The SPME fiber was pre-conditioned for 2 h at 250°C. The fiber was placed over plants with roots held in a water-filled test tube fixed at the bottom of a ventilated glass container (1 l) supplied with charcoal-filtered air at a flow rate of 50 ml/min. After 6 h, the SPME fiber was immediately analyzed in a gas chromatography-mass spectrometry (GC-MS) system (Hewlett Packard 5972) for chemical structure determination. The GC-MS system was composed of a HP-5890 Series II GC equipped with either a DB-5 or a DB-wax column (30 m × 0.25 mm i.d., J & W Scientific, Folsom, CA), and a HP-5972 MS detector. The injector temperature was 200°C and the split valve was opened 1 min after injection. The column temperature started at 40°C for 1 min following the injection and then increased to 250°C at a rate of 15°C/min. Mass spectra were recorded from 30 to 550 a.m.u. after electronic impact ionization at 70 eV. The chemical identification of the volatile compounds was determined by comparing the retention indices and mass spectra with those of synthetic standards.

Electroantennogram (EAG) tests and single sensillum recordings

For EAG responses, we performed recordings by connecting an electrogel-filled (Spectro 360, Parker Laboratory, New Jersey) glass electrode to the excised head of a *C. carnea* adult. A recording electrode filled with the same gel was connected with the cut tip of the antenna. Solutions of the test compounds (100 µg/10 µl) made in HPLC-grade methylene chloride were applied to filter-paper strips (0.5 cm × 2.5 cm, Whatman No. 1). The filter-paper strips were dried and inserted into Pasteur pipettes (15 cm long). The sequence of the test stimuli puffed over each antenna was randomly determined. A control puff of solvent was applied after each puff of a test stimulus. The amplitudes of EAG responses to each tested compound were measured from antennae of six adults, using an EAG program developed by Syntech (The Netherlands).

The penetration technique (Hubel 1957) with tungsten electrodes (<0.3 µm) was used for single sensillum recordings. An adult lacewing of each species was restrained with metal wire loops on a dental wax stub; antennae were fixed in a desired position with several thin copper wires. The tip of the recording electrode was inserted at the base of a single sensillum trichodeaum. The indifferent electrode was inserted into the abdomen. The recording electrode was connected to a high impedance amplifier (Syntech). The signal was recorded on an FM recorder (Vetter model 420F); the recorded responses were analyzed by an autospike program (Syntech, The Netherlands). Neuron activity was recorded from 25 males and 20 females of the two lacewing species, with successful responses from 12 *C. carnea* adults (5 males and 7 females) and 16 *C. oculata* adults (12 males and 4 females).

Field trapping test

In 1999, field tests were conducted to demonstrate the behavioral activities of candidate attractants associated with host plants and aphid prey in an alfalfa field (12,000 m^2) in Iowa. The first set of experiments (five replicates) was conducted in May, to test the attractiveness of EAG-active corn volatiles to *C. carnea*. The second field experiment from mid June to July (five replicates) examined the response of *C. carnea* adults to the alfalfa produced volatiles. The third test (six replicates) was conducted in autumn to determine the response of adult lacewings to aphid sex pheromones and to previously reported attractants (indole and β-caryophyllene). Traps and dispensers used were the same as described in Zhu et al. (1999). The concentration of plant volatiles used was 100 mg. In the pheromone dispenser, 10 mg of each compound was housed in a borosilicate vial (Chromacol, Connecticut, USA) from which pheromones volatilized through a 1-mm hole was drilled in the plastic cap of the vial. The control trap contained the solvent (methylene chloride)-impregnated dispenser only. Within each replicate, traps were hung from metal fence posts at 1 m height and 10 m apart distance. The traps were checked daily. Dispensers were replaced every other day, except aphid pheromone lures, which were used throughout the experiment (21 days). The trap position within each replicates was randomized to minimize the effects of habitat heterogeneity.

Host plant associated volatiles used in field tests were purchased from Bedoukian Research Inc., Sigma and Aldrich (USA) with purity levels ranging from 95 to 99% (Zhu et al. 1999). The eight volatile compounds tested (Fig. 1a) are released from corn leaves (Buttery and Ling 1984). Nonanal, 2-phenylethanol, (*Z*)-3-hexenyl acetate, methyl salicylate, and 2-phenylethyl acetate were identified as major volatiles released from alfalfa. The blend of volatiles tested contained the above five compounds in their naturally released ratio of 2.1:1.5:7.7:0.9:3.5. The two aphid sex pheromone components tested, (4a*S*,7*S*,7a*R*)-nepetalactone and (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol, were synthesized at Rothamsted Research Laboratory, UK. The purity of these two compounds was approx. 97 and 93%, as analyzed by GC-MS, respectively. Indole (99.9%) was purchased from Supelco.

Results

GC-MS analyses of SPME collections of alfalfa detected nine major volatile compounds (Table 1). Among them,

Table 1 Volatiles identified from alfalfa, *Medicago sativa*, and their EAG activities from adult *Chrysoperla carnea*

Volatiles	EAG activity
Aliphatic compounds	
(*Z*)-3-Hexenol	–
(*Z*)-3-Hexenyl acetate	–
(*E*)-2-Hexenal	–
Nonanal	+
Aromatic compounds	
2-Phenylethanol	+++
Methyl salicylate	–
2-Phenylethyl acetate	–
Terpenes and Terpenoids	
(*E*)-β-Farnesene	++
Caryophyllene	–

"+++" indicates the highest EAG response elicited from antennae of *Chrysoperla carnea* (based on amplitudes of EAG responses, see details in the result)

2-phenylethanol and β-farnesene elicited significantly higher EAG responses from antennae of *C. carnea* (7.3±0.62 and 5±0.34 mV), compared to 0.2±0.03 mV from the control (n=6, t=2.57, P<0.0001, Student's t test). A moderate, but significant EAG response by *C. carnea* was also observed for a third compound, nonanal (2.6±0.03 mV; n=6, t=2.01, P<0.001, Student's t test).

Field assays showed that traps baited with 2-phenylethanol caught significantly higher numbers of *C. carnea,* compared to traps with the other tested compounds (Fig. 1a, n=5, for females, F=13.01, P<0.0001, for males, F=3.029, P<0.018, ANOVA followed by Fisher's PLSD test). Compared to the number of male *C. carnea* caught, twice as many female *C. carnea* were caught by 2-phenylethanol (Fig. 1a, n=5, P<0.01, Student's t test). Traps baited with a blend of alfalfa volatiles and 2-phenylethanol were also highly attractive to *C. carnea* adults (Fig. 1b, n=5, F=3.581, P<0.001, ANOVA followed by Fisher's PLSD test), compared to the remaining compounds tested. Only three adult *C. oculata* were caught in the above field tests. In autumn, significantly higher numbers of *C. oculata* adults were caught in traps baited with (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol (Fig. 1c, n=6, for, F=19.649, P<0.0001, ANOVA followed by Fisher's PLSD test), compared to control traps. Adult *C. carnea* were only attractive to traps baited with 2-phenylethanol (Fig. 1c, n=6, F=3.803, P<0.01, ANOVA followed by Fisher's PLSD test).

Among single sensillum recordings, about 60% of recordings showed clear responses to the tested compounds. Receptor neurons of both sexes of *C. carnea* only responded to the two aphid pheromone components, 2-phenylethanol and the alfalfa blend of volatiles, but not to the control (Fig. 2). No differences in spike amplitudes were observed in neurons responding to plant volatile and aphid pheromone compounds. In contrast, in *C. oculata*, we only found receptor neurons tuned to the two aphid sex pheromone components (Fig. 2).

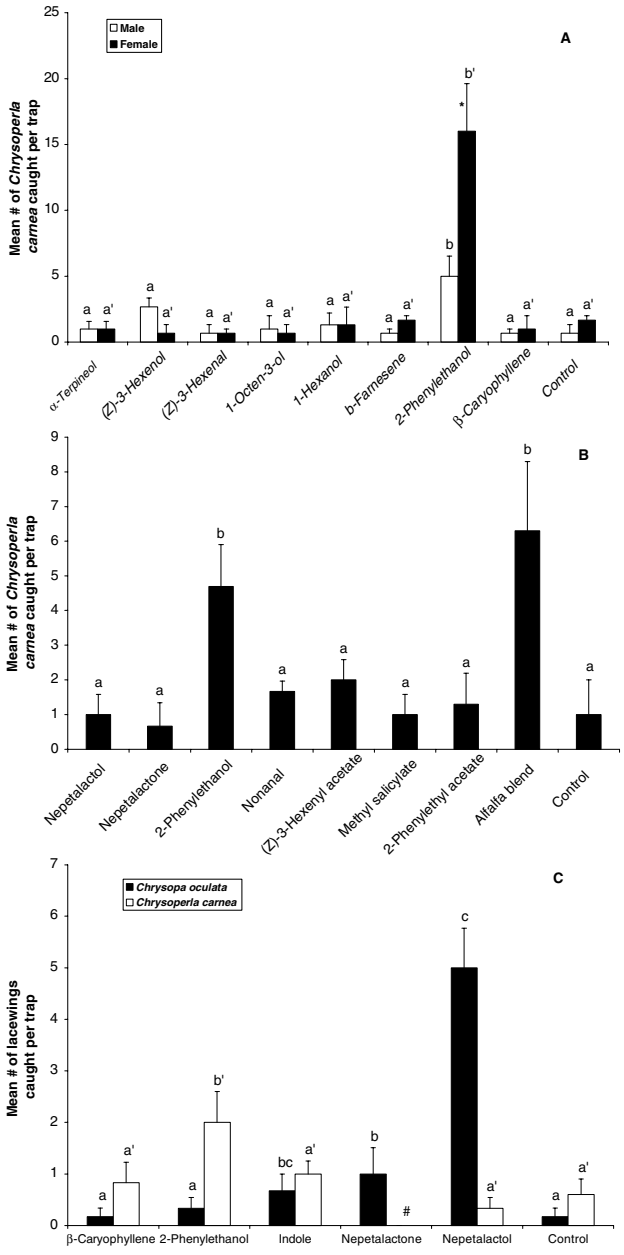

Fig. 1 Mean number (± standard error) of male and female *Chrysoperla carnea* caught in traps baited with selected EAG active corn volatiles (**a**); mean number of lacewings caught in traps baited with alfalfa volatiles and aphid sex pheromones (**b**); mean numbers of the two lacewing species caught in traps baited with aphid sex pheromones and some previously reported lacewing attractants (**c**). Columns with different *letters* indicate significant differences (ANOVA followed by Fisher's PLSD test). Asterisks on the column of 2-phenylethanol treatment in (**a**) indicate significant differences (Student's t test). # was not included in the ANOVA test as no lacewings were caught in traps baited with this compound

Discussion

Among the green leaf and floral volatile compounds released from alfalfa, 2-phenyethanol, previously detected from alfalfa pods, leaves, and flowers (Srinivas 1988; Wong and Teng 1995), was highly attractive to *C. carnea* adults.

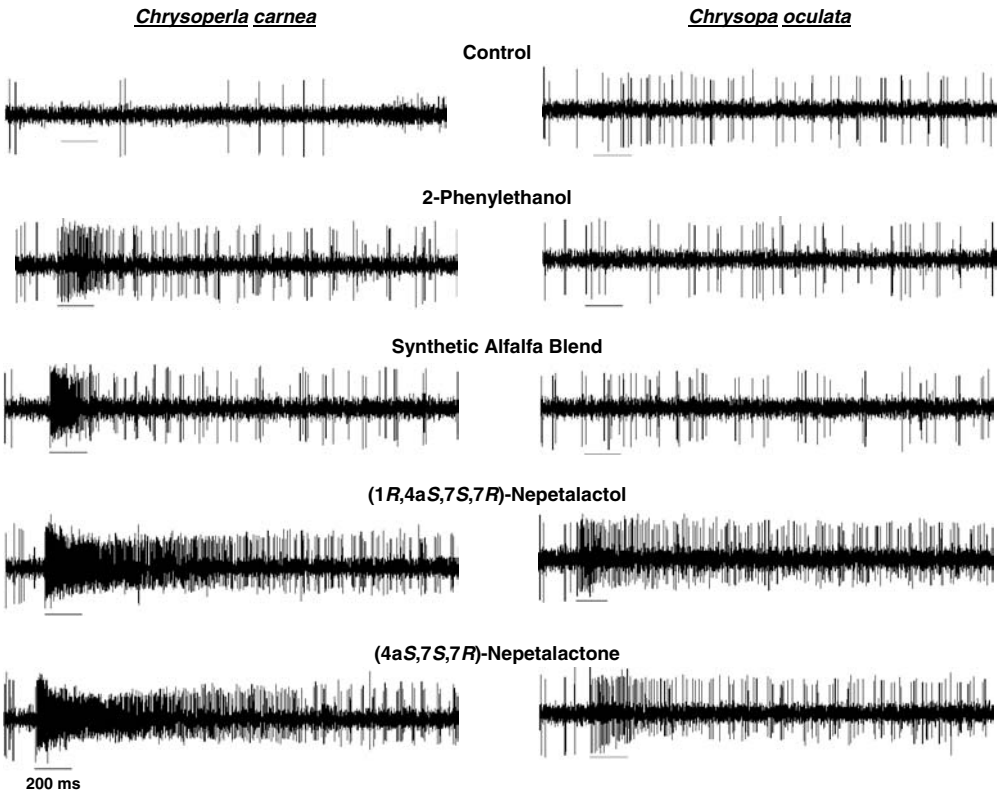

Fig. 2 Typical single sensillum responses, as recorded from female trichodea sensilla of *Chrysoperla carnea* and *Chrysopa oculata* with stimulation of 100 µg of selected alfalfa volatiles and prey aphid pheromones. Horizontal bars represent 200 ms of stimulation

(*Z*)-3-Hexenyl acetate, the most common volatile produced by many forage legumes, was also detected, but it was not attractive to *C. carnea*. This is the first report of nonanal and 2-phenylethyl acetate being released from alfalfa. One unexpected finding in this study was that β-caryophyllene, a previously reported volatile attractive to *C. carnea* adults (Flint et al. 1979), showed no attraction to *C. carnea* adults in this study. This discrepancy might be due to geographic variation in *C. carnea* responses, the different dosage used (2 g vs. 100 mg in this study), or to the different crop habitat (cotton vs. alfalfa).

Adult *C. carnea* typically feed on nectar and pollen from flowers. Gravid females of *C. carnea* fly towards and lay eggs on vegetation of preferred plant species (Duelli 1980; New 1984). Results from our trapping tests in the alfalfa field demonstrated that significant numbers of female *C. carnea* were caught in traps baited with 2-phenylethanol. This indicates that 2-phenylethanol may be used by *C. carnea* as a signal for locating food or oviposition sites. A study of orientation and oviposition behavior of several lacewing species has documented that female *C. carnea* laid significantly higher numbers of eggs on sunflower, compared to cotton or pigeonpea (Ballal and Singh 1999). SPME analyses of volatiles from the sunflower revealed that 2-phenylethanol was also released from this species (Zhu, unpublished data).

Chrysopa oculata is an aphid-feeding lacewing that is typically abundant during late summer and early autumn in Midwest of USA (Tauber and Tauber 1973; Jubb and Masteller 1977). During this period oviparous females of many aphid species produce sex pheromones to attract conspecific males (Dawson et al. 1990). Our results demonstrated that adult *C. oculata* are attracted to the aphid sex pheromone component, (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol. The same compound has been identified as a sex pheromone component in several aphid species and has recently been shown to have a similar function in soybean aphids (Picket et al. 1992; Zhu et al., unpublished data). Several other predatory lacewing species are attracted to this aphid pheromone component (Boo et al. 1998; Hooper et al. 2002). Relatively higher numbers of gravid female *C. oculata*, compared to males, caught in traps with this compound (Zhu et al., unpublished data) support the hypothesis that *C. oculata* uses the aphid pheromone as a kairomone to locate prey and oviposition sites. Female *C. oculata* achieve higher level of fecundity after consuming aphids (Burke and Martin 1956). Although significantly lower numbers of *C. carnea* were caught in traps with 2-phenylethanol in autumn, compared to numbers during the summer, 2-phenylethanol was the most attractive compound tested. The lower number of *C. carnea* captured in autumn may be related to seasonal patters of abundance, or to a portion of the population entering reproductive diapause.

Antennae of *C. carnea* possess long trichoid sensilla (Zhu et al. 2000), which are usually involved in pheromone- and host-volatile reception in arthropods (Keil and Steinbrecht 1984). Responses of *C. carnea* receptor neurons to 2-phenylethanol could be explained by the glyciphagous and pollinivorous nature of adults. The existence of receptor neurons in *C. oculata* that only respond to aphid sex pheromone components is most likely related to the predatory behavior of adults. Furthermore, females of *C. oculata*

need certain proteins for egg development that can only be acquired from feeding on aphids (Tauber and Tauber 1973). Our results provide the basis for a better understanding of the chemical ecology and sensory physiology of these two predacious lacewing species for enhanced biological control.

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