Conspecific and Heterogeneric Lacewings Respond to (Z)-4-Tridecene Identified from Chrysopa formosa (Neuroptera: Chrysopidae)

Sándor Koczor[1] & Ferenc Szentkirályi[1] & József Vuts[2] & John C. Caulfield[2] & David M. Withall[2] & John A. Pickett[2] & Michael A. Birkett[2] & Miklós Tóth[1]

Abstract

Green lacewings (Chrysopidae) are predators of soft-bodied pest insects and are among the most important biological control agents in crop protection. Chrysopa spp. are of special importance since, unlike most green lacewing species, adults are also predatory. The current study was undertaken in search of Chrysopa formosa compounds with semiochemical activity. Using coupled gas chromatography-electroantennography (GC-EAG), head and thorax extracts of C. formosa elicited EAG responses to a compound subsequently identified by coupled GC/mass spectrometry, microchemistry, chemical synthesis and GC peak enhancement as (Z)-4-tridecene. In field experiments, this compound decreased attraction of adult C. formosa to (1R,4aS,7S,7aR)-nepetalactol and that of Chrysoperla carnea species-complex to a ternary floral lure, with the inhibitory effect found to be dose-dependent. Our results suggest that (Z)-4-tridecene may serve as a general warning signal among multiple green lacewing species. Perspectives for potential practical applications are discussed.

Keywords Chrysopa formosa · Chrysoperla carnea species-complex · (Z)-4-tridecene · (1R,4aS,7S,7aR)-nepetalactol · Synthetic ternary floral bait · Repellency · Biological control · Predator

Introduction

Green lacewings (Chrysopidae) comprise a species-rich family with more than 1200 taxa described worldwide (Brooks and Barnard 1990). Their larvae are predatory and are of interest for biological control of agricultural pests (Pappas et al. 2011). Chrysoperla spp. for instance, are considered important agents of biological control (Pappas et al. 2011), and are available commercially. Nevertheless, it is important to note that the taxon previously referred to as 'Chrysoperla carnea' is composed of several species (Henry et al. 2001); hence, the name C. carnea species-complex or C. carnea s. lat. Besides Chrysoperla spp., Chrysopa spp. are also of importance for biological control since, unlike most green lacewings, adults are also predatory (Bozsik 1992; Canard 2001).

To maximize the control impact of green lacewings, de-tailed knowledge of their chemical ecology is of key importance (Aldrich and Zhang 2016). To date, studies have mainly reported on behavioral responses of lacewings to plant or aphid semiochemicals (e.g., Flint et al. 1979; Hooper et al. 2002; Tóth et al. 2006). Despite the identification of several compounds from different green lacewing species (e.g., Aldrich et al. 2009), reports on the behavioral responses of green lacewings to these semiochemicals are scarce. (1R,2S,5R,8R)-Iridodial from extracts of male Nearctic lace-wings Chrysopa oculata Say, 1839 (Zhang et al. 2004) and C. nigricornis Burmeister, 1839, was reported as a male attrac-tant (Zhang et al. 2006), and it has also been found to be attractive to other green lacewing species (Aldrich and Zhang 2016). Interestingly, Aldrich et al. (2016) found that this com-pound was not produced by laboratory-reared C. oculata, but only by field-collected individuals. Zhu et al. (2000) identified (Z)-4-tridecene (TRIDEC) from a Nearctic Chrysoperla sp. and reported that it elicited arrestment in a Y-tube olfactometer, and decreased attraction to 2-phenylethanol, a floral volatile, in a preliminary field experiment. Reduced oviposition of

* Sándor Koczor
koczor.sandor@agrar.mta.hu

1 Plant Protection Institute, Centre for Agricultural Research, HAS, H-1022 Herman Ottó u. 15, Budapest, Hungary

2 Department of Biointeractions and Crop Protection, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK

Chrysopa commata Kis & Újhelyi, 1965, C. oculata, C. perla (Linnaeus, 1758) and 'C. carnea (Stephens)' on surfaces where hatched first-instar larvae had been present has been reported (Ruzicka 1994, 1996, 1998, 2010). However, poten-tial semiochemicals have not been identified. Interestingly de-spite the reports on the effect of freshly hatched larvae on oviposition, later studies conducted with Chrysoperla species did not verify the effect of previously laid eggs on oviposition by females (Fréchette et al. 2006; Koczor et al. 2017).

Previous work has shown that Chrysoperla spp. green lace-wings respond to plant volatiles (e.g., Flint et al. 1979; Tóth et al. 2006 and references therein). For instance, a ternary plant volatile blend was more attractive to males and females of European populations of the C. carnea species-complex (i.e., C. carnea complex) than previously published attractants (Tóth et al. 2009). The ternary blend, also, was found to increase oviposition in the vicinity of baits (Jaastad et al. 2010; Koczor et al. 2015a), and adults preferred overwintering shelters baited with the blend (Koczor et al. 2015a).

The current study focused on C. formosa Brauer 1850, one of the most common Chrysopa species in Hungary, which is also of significance in agroecosystems of arable crops and orchards throughout Europe and Asia (Duelli 2001; Szentkirályi 2001). Given the important ecological role that C. formosa has, as a predator of soft-bodied insects, our aim was to investigate semio-chemicals from C. formosa with a view to using the identified compounds as potential tools in ecological pest management.

Methods and Materials

Preparation of Lacewing Extracts

Male C. formosa were collected in the field at Halásztelek (Hungary) with CSALOMON® VARL+ funnel traps (Plant Protection Institute, CAR, HAS, Budapest, Hungary, http://www.csalomontraps.com) baited with (1R,4aS,7S,7aR)-nepetalactol (NEPOH; Botanix Ltd., Paddock Wood, Kent, UK) in PVC rope formulation (5% loading w:w; Agrisense-BCS Ltd., Pontypridd, Wales, UK). This trap design and for-mulation is effective at catching C. formosa males (Koczor et al. 2010, 2015b). For the preparation of extracts, 25 live males were frozen at −20 °C, dissected into head, thorax and abdomen, which were then immersed in hexane at room tem-perature. The supernatant was placed in glass microcapillaries, which were closed by heat sealing, and stored at -20 °C until required for electrophysiological and chemical studies.

Electrophysiology (EAG)

Extracts were tested for EAG activity with antennae from male C. formosa in a preliminary screening. For presenting the stim-uli to the antenna, a stainless steel tube (teflon coated inside) was used with a constant humidified airflow of ca. 0.7 l.min^{-1}. An antenna was freshly amputated at the base from a live lace-wing and mounted between two glass capillary electrodes con-taining Ringer solution. The antennal preparation was placed ca. 3 mm from the effluent airflow. One of the electrodes was grounded, while the other was connected to a high-impedance DC amplifier (IDAC-232, Syntech, Kirchzarten, Germany). Aliquots (10 µl) of head, thoracic or abdominal extracts were administered onto a 10 mm diam. Rotilabo filter disc (RKTech Kft., Budapest, Hungary) inside a Pasteur pipette. Methyl sa-licylate (10 µl of 1 µg.µl^{-1} hexane solution) was used as a standard, and was tested before and after the other stimuli, for normalizing response amplitudes. Solvent (hexane) and air were the controls. For a stimulus, 1 ml of air was pushed through the Pasteur pipette into the airstream flowing toward the antenna. Stimuli were administered at ca. 20–30 s intervals.

Coupled Gas Chromatography-Electroantennography (GC-EAG)

A thoracic extract was purified on silica gel (hexane) to remove contamination from high molecular weight contaminants, and was subjected to coupled GC-EAG using an Agilent 6890 N gas chromatograph equipped with a DB-WAX column (J&W Scientific, Folsom, CA, USA, 30 m × 0.32 mm i.d.). Helium was the carrier gas and injection was performed in the splitless mode. The temperature program started at 60 °C and increased to 220 °C by 10 °C.min^{-1}. The column effluent was split be-tween the flame ionization detector (FID) and a heated transfer line to the EAG apparatus. For each test, 1 µl aliquots of an extract and 10 ng tetradecyl acetate in 1 µl hexane as internal standard, were co-injected. A compound was defined as EAG-active if it evoked an antennal response, distinguishable from background noise, in at least three replicates.

Coupled GC/Mass Spectrometry (GC/MS) Analysis

Thoracic extracts were analyzed on an HP 6890 GC, equipped with a cool-on-column injector and FID, and fitted with a 30 m × 0.32 mm inner dia. × 0.5 µm film thickness polar DB-WAX column, or a 50 m × 0.32 mm inner dia. × 0.52 µm film thickness non-polar HP-1 column (J & W Scientific, Folsom, CA, USA). The oven temperature was maintained at 30 °C for 2 min and then programmed at 10 °C.min^{-1} to 250 °C. The carrier gas was hydrogen. For tentative identification of EAG-active peaks from thoracic ex-tracts, GC/MS analysis was performed on a Micromass Autospec Ultima magnetic sector mass spectrometer (Waters, Milford, MA, USA), attached to an Agilent 6890 N GC (fitted with a 30 m × 0.32 mm inner dia. × 0.5 µm film thickness polar DB-WAX column, J & W Scientific, Folsom, CA, USA) equipped with a cool-on-column injector. Ionization was by electron impact (70 eV, 220 °C). The GC oven temperature

was maintained at 30 °C for 5 min and then programmed at 5 °C.min^{-1} to 250 °C. Tentative identifications were obtained by comparison of mass spectra with the NIST mass spectral database (2011), and confirmed by comparison of KI values and GC peak enhancement with an authentic sample, obtained by chemical synthesis of TRIDEC (see below). Quantification of compounds was achieved using the single-point external standard method with a series of C7-C22 alkanes.

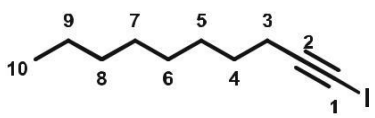
Dimethyl Disulphide (DMDS) Derivatization

Prior to alkylthiolation to determine double bond position, a thoracic extract was purified on silica gel (hexane) to remove high molecular weight contaminants. The hexane fraction was then concentrated to 100 µl and stored at -20 °C until analysis. DMDS derivatization of the extract was conducted as described by Attygalle (1998). First, 50 µl of thoracic extract was placed carefully in a 1.1 ml pointed vial (Kinesis Ltd., St. Neots, Cambridgeshire, UK), to which 1 µl DMDS (Sigma-Aldrich, Gillingham, Dorset, UK) and 50 µl 5% iodine solution (in diethyl ether) was added. The mixture was left at ca. 20 °C for 24 h, then 50 µl saturated $Na_2S_2O_3$ solution added. A four µl aliquot from the upper organic layer was analyzed by GC/MS.

Chemical Synthesis

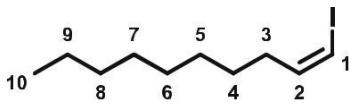
For the synthesis of TRIDEC, all chemicals were purchased from Sigma-Aldrich (Gillingham, Dorset, UK), Alfa Aesar (Heysham, Lancashire, UK) or TCI (Oxford, Oxfordshire, UK) and used without further purification unless otherwise stated. All solvents were purchased from Sigma-Aldrich or Fisher Scientific (Loughborough, Leicestershire, UK) and used without further purification unless otherwise stated. Dry tetrahydrofuran (THF) and diethyl ether were prepared by treatment with pre-activated 4 Å molecular sieves and allowed to stand overnight. All ^{1}H- and ^{13}C–NMR spectra were obtained on a Bruker DRX500 fitted with a 5 mm BBO BB-1H probe. Deuterated solvents were purchased from Goss Scientific (Crewe, Cheshire, UK) and used without fur-ther purification. Residual solvent peaks were used as an in-ternal calibration standard for NMR spectra.

The synthetic route to TRIDEC is shown in Scheme 1.



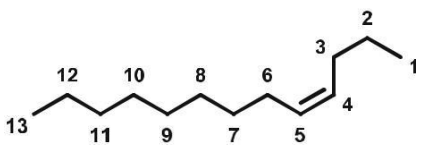
*1-Iododec-1-yne **2**.*

To a solution of 1-decyne 1 (5.00 g, 36.17 mmol) in hexane (200 ml), cooled to -78 °C under nitrogen, was added 1.6 M n-BuLi solution (32.2 ml, 43.40 mmol) and the mixture stirred for 60 min. Iodine (11.00 g, 43.40 mmol) in Et_2O (100 ml) was added slowly to the mixture before being allowed to warm to room temperature over 3 h. The reaction was quenched by addition of water and the organic layer separated. The organic layer was washed with water, saturated sodium thiosulphate, dried ($MgSO_4$) and concentrated under vacuum. The crude product was purified on silica gel (100% petroleum ether) to give 1-iodo-dec-1-yne 2 (9.18 g, 96% yield) as a colorless oil. ^{1}H–NMR ($CDCl_3$, 500 MHz): 2.38 (t, 2H, J = 7.1 Hz, H-8), 1.53 (m, 2H, H-7), 1.39–1.30 (m, 10H, H-2 to 6), 0.91 (t, 3H, J = 6.8 Hz, H-1); ^{13}C–NMR ($CDCl_3$, 125 MHz): 94.90 (C-9), 31.84, 29.17, 29.06, 28.81, 28.50 (C-7), 22.68, 20.84 (C-8), 14.14 (C-1), 7.67 (C-10).

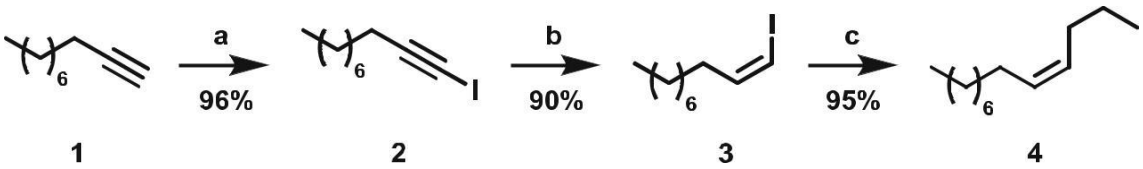


*(Z)-1-Iododec-1-ene **3**.*

To a solution of borane-dimethyl sulphide complex (1.62 g, 21.32 mmol) in Et_2O (60 ml), cooled to 0 °C under nitrogen, was added cyclohexene (3.50 g, 42.64 mmol) and the solution stirred for 15 min before being warmed to room temperature for a further 60 min. The reaction was cooled back to 0 °C before 1-iododec-1-yne 2 (5.36 g, 20.30 mmol) in Et_2O (30 ml) was added. After stirring for 30 min, the cooling bath was removed and the reaction allowed to warm to room temperature for 90 min. The reaction mixture was again cooled to 0 °C and acetic acid (15 ml) added and stirred for 2 h. Water was added to the reaction mixture and extracted with petroleum ether. The combined organics were washed with saturated $NaHCO_3$, dried ($MgSO_4$) and concentrated under vacuum. The crude product was purified on silica gel (100% petroleum ether) to give (Z)-1-iododec-1-ene 3 (4.82 g, 90% yield) as a colorless oil. ^{1}H–NMR ($CDCl_3$, 500 MHz): 6.19 (m, 2H, H-9 and 10), 2.15 (m, 2H, H-8), 1.45 (m, 2H, H-7), 1.36–1.27 (m, 10H, H-2 to 6), 0.91 (t, 3H, J = 7.1 Hz, H-1); ^{13}C–NMR ($CDCl_3$, 125 MHz): 141.54 (C-9), 82.14 (C-10), 34.73 (C-8), 31.89, 29.44, 29.26, 29.15, 27.98, 22.70 (C-7), 14.15 (C-1)



*(Z)-4-Tridecene **4**.*



Scheme 1 The synthetic route to (Z)-4-tridecene 4. a n-BuLi, I_2, Et_2O, −78 °C up to RT; b i. $BH_3.SMe_2$, Cyclohexene, Et_2O, 0 °C ii. 2, Et_2O, 0 °C iii. AcOH, 0 °C up to RT; c PrMgBr, TMEDA, $Fe(acac)_3$, THF, −78 °C

To a suspension of magnesium turnings (2.19 g, 59.86 mmol) and a single iodine crystal in THF (100 ml), under nitrogen, was added bromopropane (6.69 g, 54.42 mmol) and the mixture heated to 70 °C for 60 min. To a solution of (Z)-1-iododec-1-ene 3 (4.82 g, 18.14 mmol) and N, N, N′, N′-tetramethylethylenediamine (2.11 g, 18.14 mmol) in THF (50 ml), cooled to -78 °C under nitrogen, was added tris(acetylacetonato)iron(III) (0.64 g, 1.81 mmol) followed by the previously prepared Grignard solution. The solution was stirred for 30 min before the reaction was quenched by addition of saturated ammonium chloride. The reaction mixture was poured into water, extracted with petroleum ether and the combined organics dried ($MgSO_4$) and concentrated under vacuum. The crude product was purified on silica gel (100% petroleum ether) to give TRIDEC 4 (5.67 g, 95% yield) as a colorless oil. ^{1}H–NMR ($CDCl_3$, 500 MHz): 5.39 (m, 2H, H-9 and 10), 2.05 (m, 4H, H-8 and 11), 1.44–1.31 (m, 14H, H 2 to 7 and 12), 0.95–0.90 (m, 6H, H-1 and 13). ^{13}C–NMR ($CDCl_3$, 125 MHz): 130.14, 129.64, 31.93, 29.80, 29.54, 29.34, 29.31, 27.24, 22.92, 22.71, 22.65, 14.15, 13.84.

Preparation of Baits

For the preparation of ternary floral baits, synthetic compounds were obtained from Sigma-Aldrich Kft (Budapest, Hungary). Polyethylene (PE) bag dispensers (CSALOMON®, Plant Protection Institute, CAR, HAS, Budapest Hungary) were prepared as follows: compounds were loaded onto a 1 cm piece of dental roll (Celluron®, Paul Hartmann AG, Heidenheim, Germany) and put into a PE bag (ca 1.0 × 1.5 cm) made of 0.02 mm linear polyethylene foil (FS471– 072, Phoenixplast BT, Pécs, Hungary). The ternary floral blend was composed of 100 mg each of phenylacetaldehyde (≥90% chemical purity as per the manufacturer), methyl salic-ylate (≥99%) and acetic acid (≥99%), in a 1:1:1 ratio (Tóth et al. 2009). The dispensers were heat-sealed. For Experiment 3., 100 mg phenylacetaldehyde alone was formulated into PEbag dispensers, as described above. For preparation of NEPOH baits, NEPOH was obtained from Botanix Ltd. (Paddock Wood, Kent, UK) and formulated into polyethylene vial dispensers as follows: 100 mg of compound was loaded onto a 1 cm piece of dental roll (Celluron®, Paul Hartmann AG, Heidenheim, Germany) and put into a 0.7 ml PE vial with lid (No. 730, Kartell Co., Noviglio, Italy). The lids of the dispensers were closed. Samples of NEPOH used to prepare baits were checked for purity by GC/MS, using a capillary GC column (50 m × 0.32 mm i.d. × 0.32 μm film thickness, J & W Scientific, Folsom, CA, USA) di-rectly coupled to a magnetic sector mass spectrometer (Micromass Autospec Ultima). Ionization was by electron impact (70 eV, 250 °C).

For Experiment 1, 100 mg of TRIDEC was formulated into PEbag dispensers as described above. For the dose experiment (Experiment 2), 1, 10 or 100 mg of TRIDEC was formulated into PE vial dispensers similarly to NEPOH baits as described above. For Experiment 3, 50 mg of TRIDEC was formulated into PE vial dispensers as described above.

For each formulation type (PE bag, PE vial, PVC rope), dispensers were attached to 8 × 1 cm plastic strips for easy handling when assembling the traps. Baits were then wrapped singly in pieces of aluminum foil and stored at −18 °C until used. PE bag baits were changed at 2–3 week intervals, and PE vial dispensers were replaced at 4-week intervals, as pre-vious experience showed that similar dispensers did not lose their attractiveness during this period (e.g., Tóth et al. 2009; Koczor et al. 2015b).

Field Tests

Field experiments were conducted in 2015 and 2016 in a sour cherry orchard at Halásztelek (Hungary). For the experiments, CSALOMON® VARL+ funnel traps were used (produced by Plant Protection Institute, CAR, HAS, Budapest, Hungary), which are suitable for catching green lacewings (Koczor et al. 2015a, 2015b; Tóth et al. 2006, 2009). In both experi-ments, one replicate of each treatment was incorporated into a block, so that individual treatments were 5–8 m apart. Within each block, the arrangement of treatments was randomized. Distance between blocks was 15–20 m. Traps were suspended in the lower part of the canopy at a height of ca 1.5–1.8 m. As a rule, traps were checked twice weekly, and rotated every second week.

To prevent caught insects from escaping, a small piece (1 × 1 cm) of household anti-moth strip (Chemotox®, Sara Lee; Temana Intl. Ltd., Slough, UK; active ingredient 15% dichlor-vos) was placed in the container. Captured green lacewings were brought to the laboratory and determined to species, according to Aspöck et al. (1980). Lacewings of the

C. carnea complex have been treated as one taxonomic unit in the data analysis.

Details of Experiments

Experiment 1: The aim was to test the effect of TRIDEC on the attraction of green lacewings to the ternary floral bait and to NEPOH. Treatments included were: ternary floral bait alone, NEPOH bait alone, TRIDEC bait alone, ternary floral bait + TRIDEC, NEPOH + TRIDEC, and unbaited traps. The experiment was run from 16 July–31 August 2015, with 5 blocks.

Experiment 2: This experiment tested the effect of addition of different doses of TRIDEC on the attraction of green lacewings to NEPOH or to the ternary floral bait. Treatments included ternary floral bait alone, NEPOH bait alone, ternary floral bait with either 1, 10 or 100 mg dose of TRIDEC, NEPOH bait with 1, 10 or 100 mg of TRIDEC, and unbaited traps. The experiment was run from 5 July–6 September 2016, with 4 blocks. Comparison of treatments was restricted to those that were meaningful for the respective species; that is, at-tractive stimulus alone, attractive stimulus in combina-tion with different doses of TRIDEC and unbaited traps as control.

Experiment 3: This experiment tested the effect of addition of TRIDEC to phenylacetaldehyde on captures of green lacewings and noctuid moths. Noctuid moths were chosen because of previous reports on attraction of several species to phenylacetaldehyde (Landolt et al. 2013). In the current experiment, noctuid moths were treated as a taxonomic unit of phytophagous insects, and not determined to species level. Treatments included phenylacetaldehyde alone, phenylacetaldehyde and TRIDEC, and unbaited traps. The experiment was run from 8 August–3 October 2016, with 5 blocks.

Statistical Analysis

To exclude positional effects, catches were summed for trap rotation periods; i.e., for periods in which traps were at the same position. Data of experiments were tested for normality by Shapiro-Wilk test and for homogeneity of variances by Bartlett test. Since none of the experimental data met the criteria of parametric tests, nonparametric tests were used. Data were analyzed by Kruskal-Wallis test, and differences between treatments evaluated by pairwise Wilcoxon test with Bonferroni correction (Sprent and Smeeton 2007). For dose-response studies, data of the respective treatments containing 1, 10 or 100 mg of TRIDEC were also tested by Spearman's rank correlation (Hollander et al. 2014). Statistical procedures were conducted using the software R (R Core Team 2016).

Results

Analyses of Extracts and Identification of EAG-Active Constituent

Hexane extracts from head and thorax of male C. formosa elicited higher EAG responses from conspecifics than abdominal extracts (Fig. 1a). Head and thorax extracts also elicited EAG responses from C. carnea complex lacewings (Online Resource Fig. 1). Thoracic extracts were chosen for GC-EAG analysis. Only one compound, at Kovats index 1344, elicited consistent responses from the antennae of C. formosa (Fig. 1b). GC/MS analysis of the thoracic extract, and comparison of the MS data with the NIST library, suggested that the EAG-active compound was a monounsaturated C_{13} alkene (tridecene) with a molecular ion (M^+) at m/z 182 (Fig. 2a). DMDS derivatization of a purified extract containing the EAG-active peak generated a new compound with a M^+ at m/z 276, and major fragment ions at m/z 103 and 173, indicating the formation of a C_{13}-DMDS adduct thiomethylated at the C_4 and C_5 positions (Fig. 2b). Confirmation of the identity of the EAG-active peak as a 4-tridecene isomer, specifically with (Z) stereochemistry, was confirmed by GC peak enhance-ment with an authentic sample of TRIDEC obtained by chem-ical synthesis. Quantitative analyses calculated the approxi-mate amount of TRIDEC to be 21 ng from the thorax of a single male. TRIDEC was also found in head extracts (ca. 50 ng/male), but was missing from abdominal extracts. Skatole was also present in both the head and thoracic extracts (Online Resource Fig. 2).

Field Tests

In the experiment testing addition of TRIDEC to attractants (Experiment 1), only treatments containing NEPOH attracted more C. formosa males than unbaited traps (Table 1); no fe-males were caught in the test. Addition of TRIDEC to NEPOH resulted in decreased catches, and no catches were observed in traps baited with TRIDEC only (Table 1). On the other hand, only treatments containing the ternary floral bait attracted more C. carnea complex males and females than did unbaited traps. Addition of TRIDEC to the ternary floral bait decreased catches of both sexes, and no lacewings were caught in traps baited with only TRIDEC (Table 1).

In the dose-response experiment (Experiment 2), relatively low catches of C. formosa males were recorded, and again no females were caught. Only traps baited with NEPOH alone caught more C. formosa than unbaited traps (Fig. 3a). Catches of traps containing NEPOH +1 mg TRIDEC did not differ from those containing NEPOH alone (Fig. 3a). The correla-tion of catches with increasing doses of TRIDEC was negative and marginally insignificant at p=0.05 level (Spearman's

rho = −0.248, p = 0.056). As for the C. carnea complex, both males and females were caught. Traps containing the ternary floral bait alone caught the most lacewings, and all treatments containing TRIDEC attracted fewer lacewings (Fig. 3b). Catches in traps baited with the ternary floral bait +100 mg TRIDEC were not different from those of unbaited traps (Fig. 3b). Catches were negatively correlated with increasing dose of TRIDEC for both males (Spearman's rho = −0.459, p < 0.001) and females (Spearman's rho = −0.506, p < 0.001).

In the experiment testing the effect of addition of TRIDEC to phenylacetaldehyde (Experiment 3), all lace-wings caught belonged to the C. carnea complex and were only found in traps baited with phenylacetaldehyde alone; catches of this treatment were higher than for those of other treatments (Table 2). In terms of non-target noctuid moths, both treatments containing phenylacetaldehyde caught more than did unbaited traps (Table 2). However, traps baited with phenylacetaldehyde alone caught more moths than those baited with phenylacetaldehyde + TRIDEC.

Discussion

Among green lacewings, Chrysopa spp. are well known for the strong odor they emit when disturbed. This odor is due to ska-tole emitted from the prothoracic glands (Aldrich et al. 2009). The odorous secretion of the Nearctic C. oculata is believed to have a defensive function against potential vertebrate and in-vertebrate predators, and skatole and 1-tridecene have been reported from prothoracic gland extracts (Blum et al. 1973). Aldrich et al. (2009) confirmed the presence of skatole in pro-thoracic gland extracts of C. oculata, but reported 1-tridecene to be erroneous and identified the compound as TRIDEC. Blum et al. (1973) suggested skatole, the strong-smelling component of the secretion, was responsible for the defensive function.

From an ecological point of view, emission of a compound released upon attack may convey important information to conspecifics on the presence of a potential threat, for instance, a predator. Detection of such a warning signal could then result in dispersion and decreased predation, as is the case of

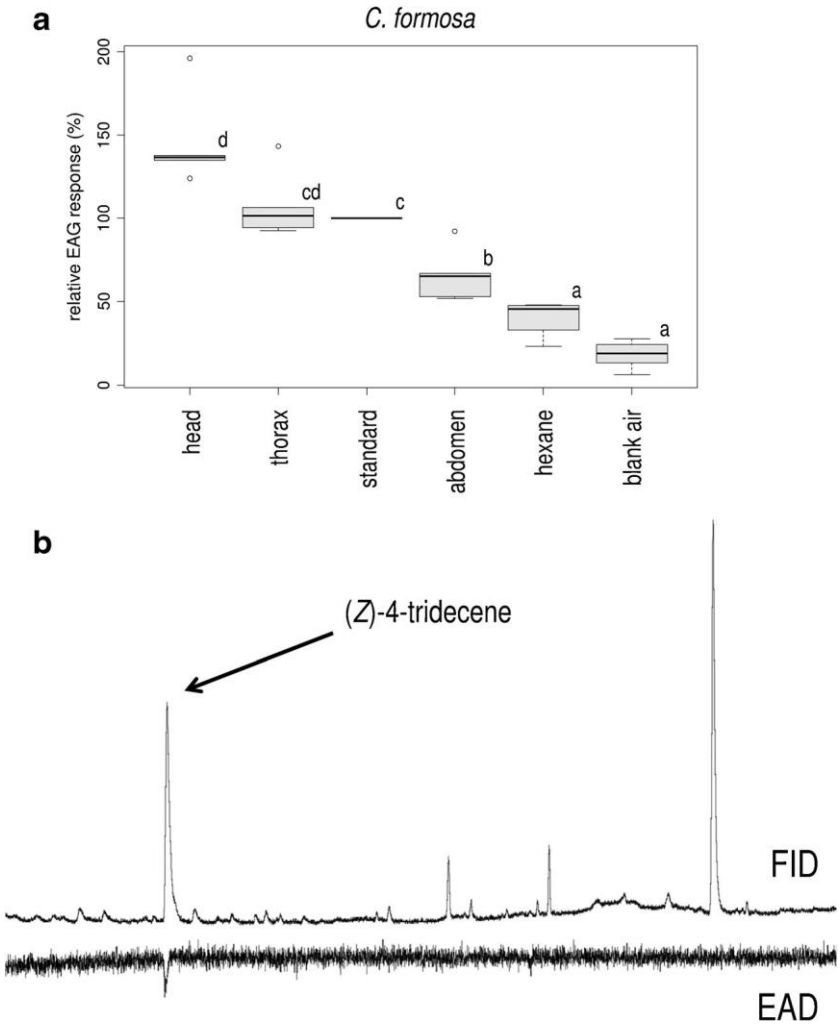


Fig. 1 Electroantennographic (EAG) activity of Chrysopa formosa extracts. a EAG screening of head, thoracic and abdominal extracts against a standard compound (methyl salicylate), solvent control (hexane) or blank air, using antennae of male C. formosa (N = 6). Treatments marked with the same letter are not different (Kruskal-Wallis test, pairwise comparisons by Wilcoxon test with Bonferroni correction at p = 0.05). b gas chromatographic-EAG analysis of C. formosa thorax extract tested on a C. formosa male antenna. Upper trace, flame ionization detector (FID); lower trace, electroantennographic detector (EAD) response

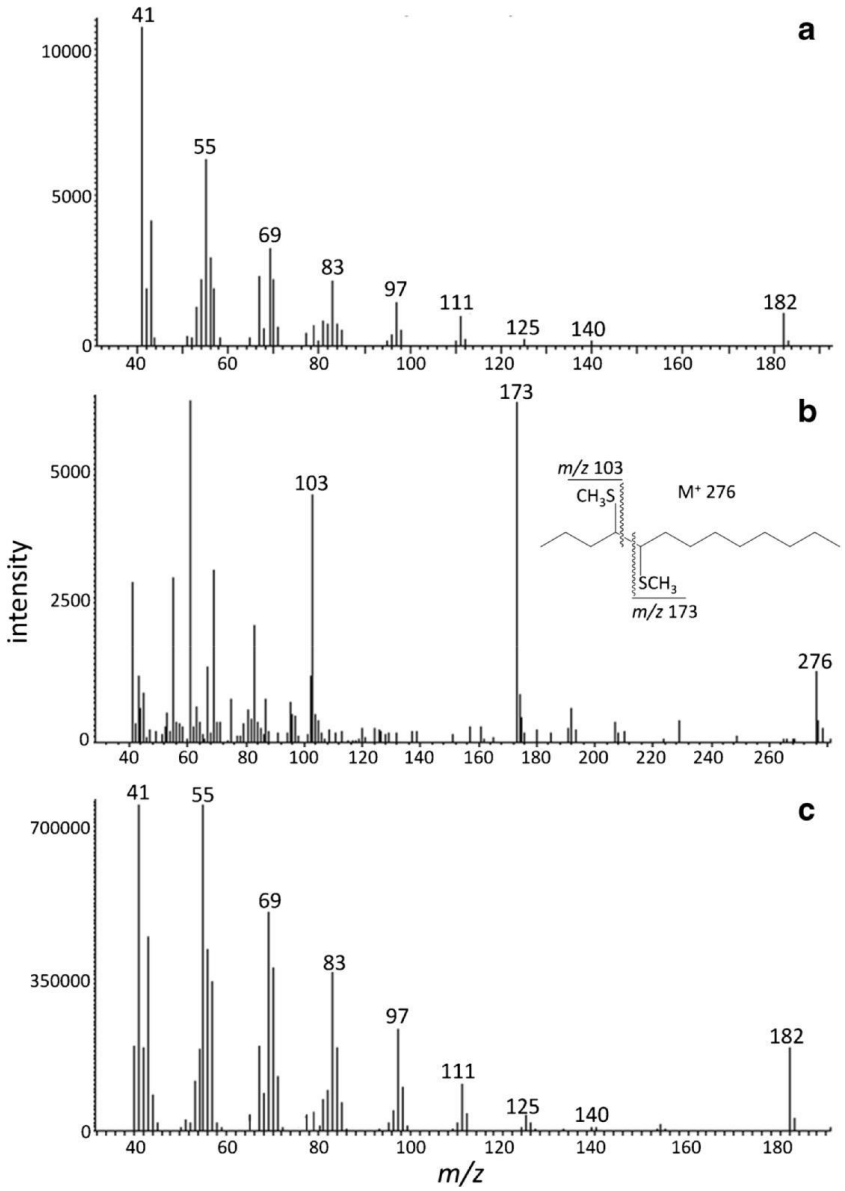


Fig. 2 Mass spectra of electroantennogram-active compound in Chrysopa formosa thorax extract (a), that of its dimethyl disulphide adduct (b), and synthetic (Z)-4-tridecene (c)

Table 1 Catches of Chrysopa formosa and Chrysoperla carnea complex lacewings in traps baited with (1R,4aS,7S,7aR)-nepetalactol (NEPOH) or a ternary floral bait, with (Z)-4-tridecene (TRIDEC). Catches compared by Kruskal-Wallis test, with pairwise comparisons by Wilcoxon test with Bonferroni correction at p = 0.05. To exclude positional effects, catches were summed for trap rotation periods; i.e., for periods in which traps were at the same position. As a rule, traps were rotated every second week

	catch / trap / trap rotation period (mean ± SE)		
	C. formosa *	C. carnea complex	
treatment	males	males	females
NEPOH bait	11.07 ± 1.39c	0 ± 0a	0 ± 0a
NEPOH bait + TRIDEC bait	2.6 ± 0.5b	0 ± 0a	0 ± 0a
ternary floral bait	0 ± 0a	5.87 ± 0.96c	25 ± 2.51c
ternary floral bait + TRIDEC bait	0 ± 0a	0.67 ± 0.23b	4.67 ± 0.82b
TRIDEC bait	0 ± 0a	0 ± 0a	0 ± 0a
no bait	0 ± 0a	0 ± 0a	0.2 ± 0.11a

*Only male C. formosa were caught

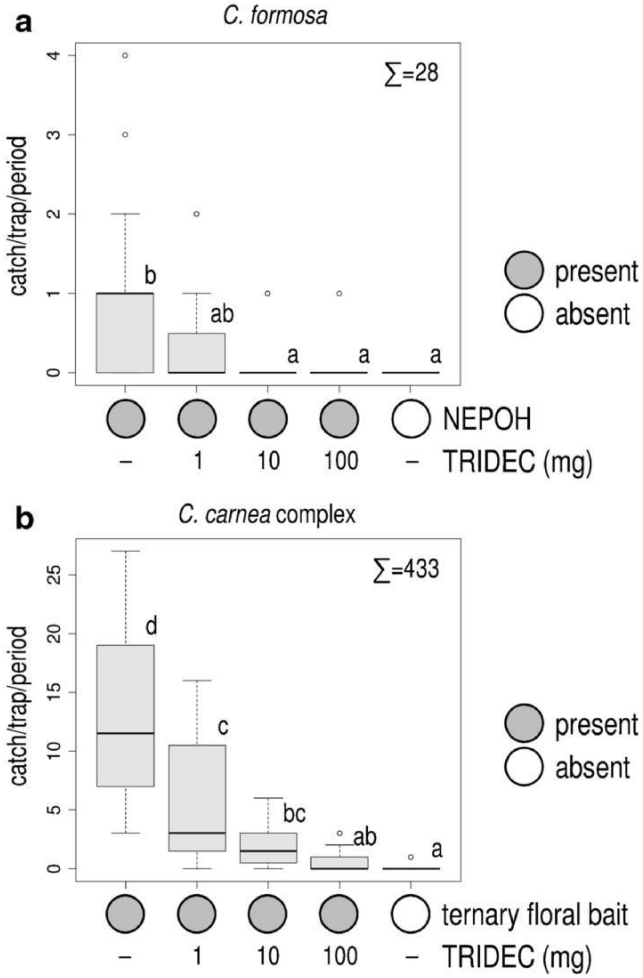


Fig. 3 Trap catches of Chrysopa formosa and Chrysoperla carnea complex lacewings in dose-response trials with (Z)-4-tridecene (TRIDEC) and (1R,4aS,7S,7aR)-nepetalactol (NEPOH). Differences in catch were tested by Kruskal-Wallis test, with pairwise comparisons by Wilcoxon test with Bonferroni correction at p = 0.05.'∑' indicates the total number of individuals caught of the respective species in the experiment

several aphid species that use (E)-β-farnesene as an alarm pheromone (Pickett et al. 2013). In the current study, head and thoracic extracts of C. formosa showed high EAG activ-ity, which may be attributed to the only EAG-active constituent, TRIDEC. In field experiments, addition of this compound caused decreased catches of C. formosa in traps baited with NEPOH, an aphid sex pheromone component highly attractive for the species (Koczor et al. 2010, 2015b). The inhibitory effect of TRIDEC was more pronounced with increasing dose. To our knowledge, this is the first report of the behavioral responses of a Chrysopa species to TRIDEC.

TRIDEC, together with skatole, has been reported from prothoracic extracts of other Chrysopa spp. such as C. incompleta Banks, 1911, C. nigricornis, C. oculata and C. quadripunctata Burmeister, 1839, from North America, and C. septempunctata from Eastern-Asia (Aldrich et al. 2009). According to Aspöck et al. (2001), C. septempunctata is a synonym of C. pallens Rambur, 1838. Although skatole was identified from C. formosa in our study (data not shown), it did not show any effect on attraction of C. formosa to NEPOH in field tests (Koczor et al. 2015b). The presence of TRIDEC in head extracts of C. formosa may be explained by the close proximity of the prothoracic gland openings to the head. Furthermore, Blum et al. (1973) observed that the head and anterior part of lacewings can become covered with the secretion. In the current experiments, TRIDEC also showed a strong negative dose-dependent effect on the attraction of C. carnea complex lacewings to a ternary blend of floral compounds, a strong attractant for both sexes (Tóth et al. 2009). Our results are consistent with those of Zhu et al. (2000), who identified TRIDEC from a Nearctic Chrysoperla species and reported that the compound decreased attraction to the floral attractant 2-phenylethanol in a field experiment (Zhu et al. 2000). Nevertheless, it is important to note that according to the current understanding of the taxonomy of Chrysoperla spp., the taxon previously referred to as 'Chrysoperla carnea' includes a multitude of species in different geographic regions and, thus, the taxon mentioned in North American studies as 'C. carnea' probably refers to the Nearctic C. plorabunda (Fitch, 1855) (Duelli 2001; Henry et al. 2001). Furthermore, TRIDEC has also been identified from prothoracic gland extracts of lacewings in other genera, namely Ceraeochrysa cubana Hagen, 1861, from South America, and Plesiochrysa ramburi Schneider, 1851, and a Mallada sp. from Australia

Table 2 Catches of Chrysoperla carnea complex lacewings and noctuid moths in traps baited with phenylacetaldehyde alone or in combination with (Z)-4-tridecene (TRIDEC). Catches compared by Kruskal-Wallis test, with pairwise comparisons by Wilcoxon test with Bonferroni correction at p = 0.05. To exclude positional effects, catches were summed for trap rotation periods, that is for periods in which traps were at the same position. As a rule, traps were rotated every second week

treatment	catch / trap / trap rotation period (mean ± SE)	
	C. carnea complex	Noctuidae
phenylacetaldehyde	4.45 ± 0.84b	3.75 ± 0.45c
phenylacetaldehyde + TRIDEC	0 ± 0a	1.3 ± 0.29b
no bait	0 ± 0a	0 ± 0a

(Aldrich et al. 2009). Thus, it is possible that this compound has a more widespread alarm function across lacewing taxa.

In detection and monitoring of pests, plant volatiles are becoming increasingly important (e.g., Hári et al. 2011; Pickett and Khan 2016; Vuts et al. 2014). For example, in crops in which key pests are managed by mating disruption, pheromone traps may not detect or monitor the respective pest species, whereas powerful blends of plant volatiles could (e.g., Hári et al. 2011). A considerable advantage of these baits is that, unlike most pheromone baits, they provide valuable means for monitoring females, leading to more precise prediction of population dynamics (e.g., Hári et al. 2011; Pickett and Khan 2016). However, floral baits may also attract a multitude of other insects; thus, evaluation of catches may require more taxonomic knowledge than in the case of pheromone traps (e.g., Jósvai et al. 2016). Beside phytophagous insects, beneficial organisms, in-cluding some green lacewings, are attracted to plant vol-atiles (e.g., Flint et al. 1979; Tóth et al. 2006; Zhu et al. 1999). Thus, monitoring of pests with these volatiles may also result in increased mortality of beneficial insects.

Common green lacewings (C. carnea complex) are of special interest as biological control agents due to their abundance in agroecosystems (e.g., Duelli 2001). Their larvae are effec-tive predators of several pests, especially aphids (Canard 2001; Pappas et al. 2011). Since a female can lay several hundred eggs and one single individual may devour hundreds of aphids during its development (Atlihan et al. 2004), the killing of these predators should be avoided. For this reason, a decrease in catch of such beneficial insects in traps used for pest monitoring is desirable.

Phenylacetaldehyde attracts several noctuid moths (e.g., Landolt et al. 2013), and it is also attractive to males and females of C. carnea complex lacewings (Tóth et al. 2006). In the current study, addition of TRIDEC to phenylacetaldehyde decreased lacewing catches to zero while, at the same time, the combination still attracted considerable numbers of noctuid moths. It is important to note that the combination attracted fewer moths than phenylacetaldehyde alone; however, since TRIDEC was tested in a dose (50 mg) comparable to that of phenylacetaldehyde (100 mg), it might have masked the attractiveness of phenylacetaldehyde to some extent, thereby resulting in decreased moth catches. Since TRIDEC was found to elicit strong avoidance by green lacewings, even at lower doses (Experiment 2), this effect could be further optimized by finding a dosage that decreases lacewing catches without considerably impacting target moth catches.

In conclusion, our results suggest that TRIDEC might serve as a common alarm signal among multiple green lacewing species. The presence of the compound has been reported from several genera from different geographic regions; thus, future studies should test the behavioral response to the compound for other species. For practical application, the compound may be used for decreasing the mortality of green lacewings (e.g., Chrysoperla spp.) in plant volatile-baited traps. Nevertheless, to achieve optimal efficacy, the response of the respective pest species to TRIDEC also needs to be examined.

Acknowledgements The current research was partially supported by the National Research Development and Innovation Office (NKFIH) grant PD115938, the SMARTCROP project of the Research Council of Norway and the János Bolyai Research Scholarship of the Hungarian Academy of Sciences. Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council (BBSRC) of the United Kingdom.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest

References

Aldrich J, Zhang QH (2016) Chemical ecology of Neuroptera. Annu Rev Entomol 61:197–218

Aldrich JR, Le TC, Zhang QH, Torres J, Winterton SL, Han B, Miller GL, Chauhan KR (2009) Prothoracic gland semiochemicals of green lacewings. J Chem Ecol 35:1181–1187

Aldrich JR, Chauhan K, Zhang QH (2016) Pharmacophagy in green lacewings (Neuroptera: Chrysopidae: Chrysopa spp.)? PeerJ 4: e1564

Aspöck H, Aspöck U, Hölzel H (1980) Die Neuropteren Europas. Eine zusammenfassende darstellung der systematik, ökologie und chorologie der Neuropteroidea (Megaloptera, Raphidioptera, Planipennia) Europas. 2 volumes. Goecke & Evers, Krefeld

Aspöck H, Hölzel H, Aspöck U (2001) Familie Chrysopidae. In: Aspöck H, Hölzel H and Aspöck U (eds) Kommentierter Katalog der Neuropterida (Insecta: Raphidioptera, Megaloptera, Neuroptera) de r West pa läa rkt is . Denis ia 02, Biologiece nt rum des Oberösterreichischen Landesmuseums, Linz, Austria, pp 69-124

Atlihan R, Kaydan B, Özgökçe MS (2004) Feeding activity and life history characteristics of the generalist predator, Chrysoperla carnea (Neuroptera: Chrysopidae) at different prey densities. J Pest Sci 77: 17–21

Attygalle AB (1998) Microchemical techniques, In: Millar JG and Haynes KF (eds) Methods in Chemical Ecology, Volume 1, Chemical Methods. Kluwer, Boston, pp 207–294

Blum MS, Wallace JB, Fales HM (1973) Skatole and tridecene: identifi-cation and possible role in a chrysopid secretion. Insect Biochem 3: 353–357

Bozsik A (1992) Natural adult food of some important Chrysopa species (Planipennia: Chrysopidae). Acta Phytopathol Entomol Hung 27: 141–146

Brooks SJ, Barnard PC (1990) The green lacewings of the world: a generic review (Neuroptera: Chrysopidae). Bull Br Mus nat Hist Entomol 59:117–286

Canard M (2001) Natural food and feeding habits of lacewings. In: McEwen PK, New TR, Whittington A (eds) Lacewings in the crop environment. Cambridge University Press, Cambridge, pp 116–128

Duelli P (2001) Lacewings in field crops. In: McEwen PK, New TR, Whittington A (eds) Lacewings in the crop environment. Cambridge University Press, Cambridge, pp 158–171

Flint HM, Salter SS, Walters S (1979) Caryophyllene: an attractant for the green lacewing. Environ Entomol 8:1123–1125

Fréchette B, Coderre D, Lucas É (2006) Chrysoperla rufilabris (Neuroptera: Chrysopidae) females do not avoid ovipositing in the presence of conspecific eggs. Biol Control 37:354–358

Hári K, Pénzes B, Jósvai J, Holb I, Szarukán I, Szólláth I, Vitányi I, Koczor S, Ladányi M, Tóth M (2011) Performance of traps baited with pear ester-based lures vs. pheromone baited ones for monitor-ing codling moth Cydia pomonella L. in Hungary. Acta Phytopathol Entomol Hung 46:225–234

Henry CS, Brooks SJ, Thierry D, Duelli P, Johnson JB (2001) The com-mon green lacewing (Chrysoperla carnea s. lat.) and the sibling species problem. In: McEwen PK, New TR, Whittington A (eds) Lacewings in the crop environment. Cambridge University Press, Cambridge, pp 29–42

Hollander M, Wolfe DA, Chicken E (2014) Nonparametric statistical methods. Wiley, Hoboken

Hooper AM, Donato B, Woodcock CM, Park JH, Paul RL, Boo KS, Hardie J, Pickett JA (2002) Characterization of (1R,4S,4aR,7S, 7aR)-dihydronepetalactol as a semiochemical for lacewings, includ-ing Chrysopa spp. and Peyerimhoffina gracilis. J Chem Ecol 28: 849–864

Jaastad G, Hatleli L, Knudsen GK, Tóth M (2010) Volatiles initiate egg laying in common green lacewings. IOBC/wprs Bull 54:77–82

Jósvai JK, Koczor S, Szabóky C, Ladányi M, Tóth M (2016) Microlepidoptera caught in traps baited with lures containing pear ester and acetic acid in Hungary. Acta Phytopathol Entomol Hung 51:255–266

Koczor S, Szentkirályi F, Birkett MA, Pickett JA, Voigt E, Tóth M (2010) Attraction of Chrysoperla carnea Complex and Chrysopa spp. lace-wings (Neuroptera: Chrysopidae) to aphid sex pheromone compo-nents and a synthetic blend of floral compounds in Hungary. Pest Manag Sci 66:1374–1379

Koczor S, Knudsen GK, Hatleli L, Szentkirályi F, Tóth M (2015a) Manipulation of oviposition and overwintering site choice of com-mon green lacewings with synthetic lure (Neuroptera: Chrysopidae). J Appl Ent 139:201–206

Koczor S, Szentkirályi F, Pickett JA, Birkett MA, Tóth M (2015b) Aphid sex pheromone compounds interfere with attraction of common green lacewings to floral bait. J Chem Ecol 41:550–556

Koczor S, Szentkirályi F, Fekete Z, Tóth M (2017) Smells good, feels good: oviposition of Chrysoperla carnea-Complex lacewings can be concentrated locally in the field with a combination of appropri-ate olfactory and tactile stimuli. J Pest Sci 90:311–317

Landolt PJ, Tóth M, Meagher RL, Szarukán I (2013) Interaction of acetic acid and phenylacetaldehyde as attractants for trapping pest species of moths (Lepidoptera: Noctuidae). Pest Manag Sci 69:245–249

Pappas ML, Broufas GD, Koveos DS (2011) Chrysopid predators and their role in biological control. J Entomol 8:301–326

Pickett JA, Khan ZR (2016) Plant volatile-mediated signalling and its application in agriculture: successes and challenges. New Phytol 212:856–870

Pickett JA, Allemann RK, Birkett MA (2013) The semiochemistry of aphids. Nat Prod Rep 30:1277–1283

R Core Team (2016) R: a language and environment for statistical com-puting. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Ruzicka Z (1994) Oviposition-deterring pheromone in Chrysopa oculata (Neuroptera: Chrysopidae). Eur J Entomol 91:361–370

Ruzicka Z (1996) Oviposition-deterring pheromone in Chrysopidae (Neuroptera) intra- and interspecific effects. Eur J Entomol 93: 161–166

Ruzicka Z (1998) Further evidence of oviposition-deterring allomone in chrysopids (Neuroptera: Chrysopidae). Eur J Entomol 95:35–39

Ruzicka Z (2010) Detection of oviposition-deterring larval tracks in Chrysopa oculata and Chrysopa perla (Neuroptera: Chrysopidae). Eur J Entomol 107:65–72

Sprent P, Smeeton NC (2007) Applied nonparametric statistical methods. Chapman & Hall/CRC, Boca Raton

Szentkirályi F (2001) Lacewings in fruit and nut crops. In: McEwen PK, New TR, Whittington A (eds) Lacewings in the crop environment. Cambridge University Press, Cambridge, pp 172–238

Tóth M, Bozsik A, Szentkirályi F, Letardi A, Tabilio MR, Verdinelli M, Zandigiacomo P, Jekisa J, Szarukán I (2006) Phenylacetaldehyde: a chemical attractant for common green lacewings (Chrysoperla carnea s.l., Neuroptera: Chrysopidae). Eur J Entomol 103:267–271

Tóth M, Szentkirályi F, Vuts J, Letardi A, Tabilio MR, Jaastad G, Knudsen GK (2009) Optimization of a phenylacetaldehyde-based attractant for common green lacewings (Chrysoperla carnea s.l., Neuroptera: Chrysopidae). J Chem Ecol 35:449–458

Vuts J, Furlan L, Bálintné Csonka É, Woodcock CM, Caulfield JC, Mayon P, Pickett JA, Birkett MA, Tóth M (2014) Development of a female attractant for the click beetle pest Agriotes brevis. Pest Manag Sci 70:610–614

Zhang QH, Chauhan KR, Erbe EF, Vellore AR, Aldrich JR (2004) Semiochemistry of the goldeneyed lacewing Chrysopa oculata: at-traction of males to a male-produced pheromone. J Chem Ecol 30: 1849–1870

Zhang QH, Schneidmiller RG, Hoover DR, Young K, Welshons DO, Margaryan A, Aldrich JR, Chauhan KR (2006) Male-produced pheromone of the green lacewing, Chrysopa nigricornis. J Chem Ecol 32:2163–2176

Zhu J, Cossé AA, Obrycki JJ, Boo KS, Baker TC (1999) Olfactory reac-tions 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 behavioral responses. J Chem Ecol 25:1163–1177

Zhu JW, Unelius RC, Park KC, Ochieng SA, Obrycki JJ, Baker TC (2000) Identification of (Z)-4-tridecene from defensive secretion of green lacewing, Chrysoperla carnea. J Chem Ecol 26:2421–2434