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ORIGINAL PAPER

Smells good, feels good: oviposition of *Chrysoperla carnea*-complex lacewings can be concentrated locally in the field with a combination of appropriate olfactory and tactile stimuli

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Abstract Larvae of common green lacewings (*Chrysoperla carnea* species-complex) are important generalist predators, and can serve as valuable biological control agents. Since only larvae are predatory, oviposition is of crucial importance. Recently, a ternary floral bait was described, attractive to females that would then lay their eggs in the vicinity of the baits. Earlier studies have shown that females prefer to lay their eggs on spines and hairs of plants. In the present study, we tested whether the number of eggs laid could be increased by combining chemical, visual and tactile stimuli. Common green lacewings were caught equally well in baited funnel traps covered with white, yellow, fluorescent yellow, light blue or black sheets, showing no preference to any of the colours. In further tests the field effect of the ternary floral bait on the oviposition site choice of common green lacewings was confirmed, as in the absence of the bait only negligible numbers of eggs were recorded on egg-laying plates. In the presence of the ternary floral bait, lacewings laid more eggs on spiny than on smooth surfaces. Removal of previously laid eggs did not affect the number of eggs laid afterwards. Our studies demonstrated that by combining the ternary floral bait and an appropriate spiny surface, oviposition of common green lacewings can be increased and concentrated to a relatively small area. Possible practical applications are discussed.

Keywords Common green lacewings · Ternary floral bait · Oviposition · Spiny surface · Colour preference · Chrysopidae

Key message

- Common green lacewings are among the most important predatory species in biological pest management, since their larvae are effective, generalist predators, thus from the applied point of view, site of oviposition is of crucial importance.
- Based on our findings, with combination of appropriate olfactory and surface stimuli, oviposition can be concentrated to a chosen site, where after hatching larvae will prey in the vicinity.

Introduction

Common green lacewings (*Chrysoperla carnea* species-complex, i.e. *C. carnea*-complex) form a taxon of predatory insects that contains important biological control agents (Hagley 1989; McEwen et al. 1998; Maisonneuve and Marrec 1999; Zaki et al. 1999; Canard 2001; Pappas et al. 2011). Larvae of these lacewings are generalist predators, feeding on aphids and other soft-bodied insects, such as lepidopteran eggs and larvae (Obrycki et al. 1989; Canard 2001; Atlihan et al. 2004). However, the adults are not predatory and feed on pollen, nectar and honeydew (Bozsik 1992; Canard 2001). In accordance with the

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feeding habits of adults, plant-derived compounds have been tested as attractants (Flint et al. 1979; Zhu et al. 1999; Tóth et al. 2006). A ternary bait comprising synthetic floral compounds was also developed, which showed higher attractiveness to *C. carnea*-complex adults than previously published attractants (Tóth et al. 2009). Unlike several other green lacewing attractants, the ternary floral bait attracted females as well (Tóth et al. 2009; Koczor et al. 2010). Furthermore, it was found that females laid their eggs on baited traps (Tóth et al. 2009; Jaastad et al. 2010) and in the vicinity of the baits (Koczor et al. 2015).

The present study was undertaken with the objective of increasing the number of eggs laid by lacewings attracted to the ternary bait. We set out to investigate the possible effect of visual and tactile stimuli which could be used in combination with the chemical stimulus of the baits, on one hand to attract more female lacewings to the baits, and on the other, to increase egg-laying intensity in the vicinity of the baits by attracted females.

Many insects show visual preference and are attracted to different colours (e.g. Prokopy and Owens 1983; Schmera et al. 2004; Jenser et al. 2010), so a part of our experiments was designed to investigate colour preference of green lacewings. As for tactile stimuli, green lacewings are often observed to lay their eggs on protruding parts of plants, including the top of spines and hairs (Szentkirályi 2001a). In the present study, we aimed to test different artificial surfaces to see whether the effect of the ternary floral bait (Tóth et al. 2009) on oviposition can further be increased by providing appropriate surface characteristics.

Materials and methods

Ternary floral bait

All synthetic compounds (>90 % chemical purity as per the manufacturer) were obtained from Sigma–Aldrich Kft (Budapest, Hungary). For preparing baits, compounds were loaded onto a 1 cm piece of dental roll (Celluron®, Paul Hartmann AG, Heidenheim, Germany), which was put into a polyethylene bag (ca 1.0 × 1.5 cm) made of 0.02-mm linear polyethylene foil (FS471-072, Phoenixplast BT, Pécs, Hungary). The bait consisted of phenylacetaldehyde, acetic acid and methyl salicylate, in a 1:1:1 ratio. The loading of each compound was kept at 100 μl. The dispensers were heat sealed and attached to 8 × 1 cm plastic handles for easy handling when assembling the traps. For storage, dispensers were wrapped singly in pieces of aluminium foil and kept at −18 °C until used. For application, baits were removed from the aluminium foil and fixed to the traps or to the plates. Baits were fixed to the traps through the middle hole of the roof with a rubber stopper. To the plates baits were fixed with a peg; for this purpose, small holes were made on both the plates and the bait handle. In the field, baits were changed at 2–3 week intervals, as previous experience showed that they do not lose their attractiveness during this period (Tóth et al. 2009).

Experimental design

Field experiments were performed between 2007 and 2012 in Hungary at Halásztelek (47°21′11″N, 19°0′20″E) in a sour cherry orchard and at Újfehértó (47°49′21″N, 21°39′57″E) in an apple orchard.

For the experiment on colour preference of adults CSALOMON® VARL + funnel traps were used (produced by Plant Protection Institute, CAR, HAS, Budapest, Hungary), which proved to be suitable for catching green lacewings (Tóth et al. 2006, 2009; Koczor et al. 2010).

For studying the effect of tactile stimuli, different surfaces including plastic plants (TetraPlantastics®, Melle, Germany) and velcro (Orsó Textil Kft. Péteri, Hungary) were applied to smooth plastic plates with wires or a clincher, respectively. On the top of each plate, three holes were drilled and a wire was put through the external ones so that the plate could be tied to a branch. Plates were positioned vertically. The ternary bait was fixed to the plate through the middle hole with a peg.

In all field experiments 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 randomised. Distance between the blocks was 20–30 meters. Traps or egg-laying plates were suspended in the lower part of the canopy of trees at a height of ca 1.5–1.7 m. As a rule, traps or plates were checked twice weekly.

Colour preference

Experiment 1 For testing colour preference of adult common green lacewings, CSALOMON® VARL + funnel traps were set up with ternary floral bait and the outside of the traps was covered by sheets of five different colours, each colour representing a different treatment. Colours included blue, white, yellow, fluorescent yellow and black. Yellow, fluorescent yellow, blue and white traps are commercially available for monitoring of different insects species (see for instance www.csalomontraps.com). Black colour was included as a potentially neutral stimulus. For reflectance spectra of colours used (excluding black) refer to Schmera et al. (2004) and Jenser et al. (2010). The experiment was run from June 14 to September 6, 2007 at Újfehértó with 4 blocks. A small piece (1 × 1 cm) of household anti-moth strip (Chemotox®, Sara Lee; Temana

Intl. Ltd, Slough, UK; active ingredient 15 % dichlorvos) was placed in the container to kill the captured insects.

Effect of surface characteristics (tactile stimuli)

Experiment 2 The aim of this experiment was to test the effect of different surface characteristics on oviposition site choice of common green lacewings. Three different surface characteristics were applied to 20 × 20 cm plastic plates, including: spiny plastic plants, a simple spiny surface (velcro, both loop and hook sides) and control (plastic plates without any additional surface structure). Plates with different surface characteristics were set up both with and without the ternary floral bait, hence resulting in six treatments in total. Plastic plants were chosen instead of dry natural plants in order to avoid possible confounding effects of the odour of natural plants. Since to our knowledge common green lacewings lay their eggs on many different plant species, showing no preference for any particular species (Szentkirályi 2001a), we chose the plastic plants for the tests by their structure, not by resemblance to any given plant species. Plastic plants were green and to provide higher structural diversity plastic plants of 3 different forms were fixed at each plate of the respective treatments (Fig. 1).

Spiny surface and plastic plants covered a similar surface of the plates. At each inspection numbers of eggs were counted and afterwards the eggs were removed. Beside total number of eggs, numbers on the plates themselves and on the additional surface elements (velcro, plastic plants) were also compared statistically. The experiment was run from July 23 to August 6, 2009 at Halásztelek, with five blocks.

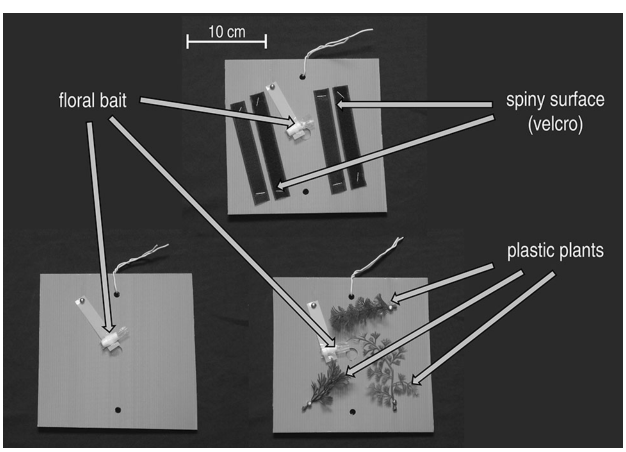

Fig. 1 Plastic plates (20 × 20 cm) with different surface characteristics (Experiment 2). Treatments included three different surfaces: plastic plants, spiny surface (velcro) and control (plastic plates without additional surface structure). Different surface characteristics were tested with and without the ternary floral bait (here only the baited treatments are shown)

Experiment 3 The aim of this experiment was to test whether surfaces covered densely (velcro, both loop and hook sides, 64/cm^2) with smaller (1 mm) spines or surfaces covered sparsely (14/cm^2) with larger (3 mm) spines were preferred for oviposition. Different surfaces (similar coverage) were applied to 7 × 12 cm smooth plastic plates. Plates with different surface characteristics, including densely covered spiny surface, sparsely covered spiny surface and smooth control were set up both with and without the ternary floral bait, hence resulting in six treatments in total. At each inspection total numbers of eggs per plate were counted and the eggs were removed. The experiment was run from August 17 to September 3, 2009 at Halásztelek, with five blocks.

Experiment 4 The aim of this experiment was to test the effect of different coverage with spiny surfaces and also to compare the loop and hook side of the velcro. Treatments were applied to 21 × 10 cm smooth plastic plates. Plates were equipped with the ternary floral bait and either with large (20 × 10 cm) or small (5 × 5 cm) coverage of either loop or hook side of velcro, hence resulting in four treatments in total. At each inspection total numbers of eggs per plate were counted and eggs were removed. The experiment was run from June 11 to 20, 2011 at Halásztelek, with ten blocks.

Experiment 5 The aim of this experiment was to test whether previously laid eggs affected further oviposition of common green lacewings. Treatments were applied to 20 × 10 cm sheets of velcro (hook side) equipped with the ternary floral bait. The experiment was run in three consecutive periods. At the beginning of each period empty sheets were set up with baits and lacewings laid their eggs on them. After the first 3 days, in one treatment previously laid eggs were removed gently with a forceps. This procedure was repeated at each inspection (3–4 days), and in the other treatment eggs were only removed at the end of a 2-week period. Partition of the total period of the experiment at 2-week intervals was necessary because hatched eggs were lost after some time making it difficult to count the total number of eggs in the treatment where previously laid eggs had not been removed. The test was run from June 29 to August 27, 2012 at Halásztelek, with five blocks.

Statistics

Data of all experiments were tested for normality by Shapiro–Wilk tests. Since none of the experimental data were found to be normally distributed, nonparametric tests were used. Catches in Exp 1 and number of eggs in Exp 2, 3, 4 were analysed by Kruskal–Wallis tests. Level of significance was $p = 0.05$. If significant differences were found, differences between treatments were evaluated by pairwise Wilcoxon rank sum tests with Bonferroni correction (Sprent and Smeeton 2007). In Exp 5, treatments were compared with Wilcoxon rank sum test. All statistical procedures were conducted using the software R version 2.15.2, basic package (R Core Team 2012).

Results

Colour preference

In this experiment despite the large number of lacewings captured (in total 2241 lacewings were caught in the test), no significant difference was found in catches between the colours tested (Kruskal–Wallis Chi-square $d = 5.9294$, $p = 0.204$).

Effect of surface characteristics

In Exp 2, significantly more eggs were found in treatments with the ternary floral bait than in the parallel unbaited ones (Fig. 2). There was no significant difference between unbaited treatments. Among baited treatments lacewings laid significantly more eggs on plates with spiny surface than on those with smooth surface, and the number of eggs laid on plates with plastic plants did not differ from either (Fig. 2). If only the number of eggs laid on the plates themselves was considered, this did not differ among baited treatments (Kruskal–Wallis Chi-square $d = 0.0809$, $p = 0.96$). Numbers of eggs laid on the loop and hook side of velcro were also similar (Wilcoxon rank sum test, $W = 10.5$, $p = 0.753$) and differences between plastic plants were marginally significant (Kruskal–Wallis Chi-square $d = 6.832$, $p = 0.033$).

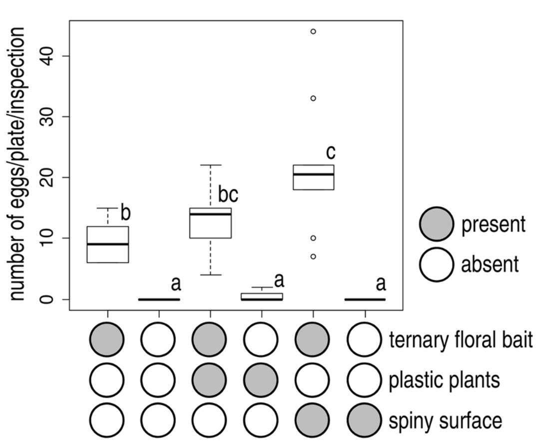

Fig. 2 Numbers of lacewing eggs laid on baited (ternary floral bait) and unbaited plastic plates (20 × 20 cm) with different surface characteristics (plates with smooth surface, with plastic plants or with spiny surface) (Experiment 2). Plastic plants used in the test are shown in Fig. 1. Treatments marked with the same letter are not significantly different (Kruskal–Wallis test, followed by pairwise comparisons by Wilcoxon rank sum test with Bonferroni correction at $p = 0.05$). A total of 433 lacewing eggs were recorded in the test

In Exp 3, there was no significant difference among unbaited treatments (Table 1). Among baited treatments significantly more eggs were found on the surface densely covered with small spines than on the smooth surface, and the latter did not differ from the number of eggs laid on unbaited plates. Surfaces covered sparsely with larger spines did not differ from plates densely covered with small spines, nor from smooth surfaces either (Table 1).

In Exp 4, lacewings laid more eggs on plates with 20 × 10 cm area of spiny surface than on those with 5 × 5 cm spiny surface area (Fig. 3). There was no difference between hook or loop side velcros at either coverage (Fig. 3).

In Exp 5, no difference was found among treatments irrespective of whether previously laid eggs were left there or removed (Wilcoxon rank sum test, $W = 352$, $p = 0.439$).

Discussion

Common green lacewings are important agents of biological control, these species are present in a wide variety of habitats, including agroecosystems (e.g. Duelli 2001; Szentkirályi 2001a, b, c). For recruitment of common green lacewings the use of semiochemicals (e.g. Tóth et al. 2009; Koczor et al. 2015) could offer a promising perspective. Nevertheless, besides semiochemicals, visual stimuli might also have importance as it has been found in many different insects (e.g. Prokopy and Owens 1983).

Previous physiological studies found differences in sensitivity of common green lacewings to different colours, with the highest sensitivity to bright green (Kral and Stelzl 1998). On the other hand, a laboratory study found that *Chrysoperla carnea* females laid higher proportion of eggs on black rather than coloured cloths as compared to glass walls (Sattar and Abro 2011). Despite this and previous reports of colour preference by North American species of the *C. carnea*-group (Maredia et al. 1992; Blackmer et al. 2008), in the present study no preference for either tested colour was found, that is colours did not affect attraction of these lacewings to the ternary floral bait. Consequently the colours tested cannot be exploited as visual stimuli for increasing female lacewing numbers attracted by the

Table 1 Numbers of lacewing eggs laid on baited (ternary floral bait) and unbaited plastic plates (7 × 12 cm) with smooth surface, covered densely with smaller spines or covered sparsely with larger spines (Experiment 3). Treatments marked with the same letter are not significantly different (Kruskal–Wallis test, followed by pairwise comparisons by Wilcoxon rank sum test with Bonferroni correction at $p = 0.05$)

Surface characteristics	Ternary floral bait	Mean number of lacewing eggs/plate/inspection ± SE
Smooth	Present	0.3 ± 0.21a
Dense spines	Present	4.1 ± 0.86b
Sparse spines	Present	1.9 ± 0.81ab
Smooth	Not present	0.0 ± 0.0a
Dense spines	Not present	0.2 ± 0.13a
Sparse spines	Not present	0.0 ± 0.0a

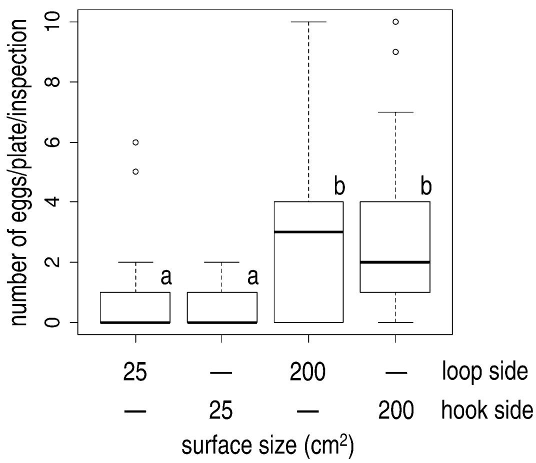

Fig. 3 Numbers of lacewing eggs laid on plastic plates (21 × 10 cm) baited with the ternary floral bait, with 20 × 10 cm and 5 × 5 cm areas covered by spiny surface (velcro loop or hook side) (Experiment 4). Treatments marked with the same letter are not significantly different (Kruskal–Wallis test, followed by pairwise comparisons by Wilcoxon rank sum test with Bonferroni correction at $p = 0.05$). A total of 206 lacewing eggs were recorded in the test

chemical stimulus of the bait. Our findings are consistent with the results of several North American sticky trap experiments, in which no semiochemicals were applied (Capinera and Walmsley 1978; Capinera 1980; Udayagiri et al. 1997). However, it is important to note that the species mentioned in North American studies as *C. carnea* most probably refers to the Nearctic *Chrysoperla plorabunda* (Fitch, 1855) (Duelli 2001; Henry et al. 2001).

The present experiments confirmed the effect of the ternary floral bait (Tóth et al. 2009) on the oviposition site choice of common green lacewings. In our experiments in treatments without baits only a negligible number of eggs were recorded, irrespective of surface structure. On the other hand if the ternary floral bait was in combination with a spiny surface, the number of eggs increased. The present results are in accordance with and can be explained by previous field observations (Szentkirályi 2001a), reporting that lacewings prefer to lay their eggs on protruding parts of plants, including spines and hairs. Furthermore if a larger area was covered with spiny surface, more eggs were laid on plates of the same size (Experiment 4). This suggests that surfaces with higher coverage of spines could be the preferred oviposition sites for common green lacewings if other attractive (e.g. chemical) stimuli are also provided. Although females laid similar numbers of eggs on both loop and hook sides of velcro, when considering movement of hatched larvae, considerable differences might exist. Movement of larvae seemed laborious on the loop side; however no such difficulties were observed in their movement on the hook side (personal observation, data not shown).

In our tests spiny plastic plants did not perform better than an artificial spiny surface not resembling plants. Furthermore, although not statistically different, numerically more eggs were found on the latter. This suggests that surface structure is an important cue for oviposition site choice; however, the shape of the object on which the eggs are laid might have lesser significance.

Previous studies reported intra- and inter-specific effects of semiochemicals produced by hatching larvae of some green lacewing species, which deterred other females from laying their eggs at the specific site (e.g. Ruzicka 1996). Nevertheless, this was not supported by later studies performed with a Nearctic *Chrysoperla* species (Fréchette et al. 2006). In the present study we did not find any effect of the presence of previously laid eggs on later oviposition of *C. carnea*-complex females, despite the fact that during the experiment most of the eggs not removed did hatch.

In summary, our experiments have shown that chemical stimuli from the ternary floral bait and tactile stimuli provided by different surface characteristics can affect oviposition site choice of common green lacewings. This, besides its potential scientific significance can make it possible that with the combination of these artificial stimuli, reproducible egg-laying plates could be produced. Such an egg-laying device may also be applied in everyday agriculture by concentrating eggs, hence concentrating hatching larvae of these important predatory insects to the place where they are most needed.

Conclusions

Our study provides an example of the effect of simplified artificial stimuli on a predatory insect. We found that ternary floral baits and tactile stimuli provided by spiny surfaces can improve oviposition of *C. carnea*-complex in a site of your choice. Furthermore, by providing movable egg-laying plates, the eggs can be moved to greenhouses for biological control purposes. In addition, monitoring of common green lacewings by the number of eggs can be performed in an environment-friendly way, providing better estimation for activity of predatory larvae, compared to other methods such as suction traps (e.g. Vas et al. 1999), from which only data on adult numbers can be gathered.

Author contribution statement

SK, FSz and MT conceived and designed the experimental setup. SK and ZF conducted the experiments. SK analysed data and wrote the manuscript. All authors read, contributed to and approved the manuscript.

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Compliance with ethical standards

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

Research involving human and animals All applicable international, national guidelines for the care and use of animals were followed.

Informed consent Informed consent does not apply to these studies.

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