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ORIGINAL CONTRIBUTION

Manipulation of oviposition and overwintering site choice of common green lacewings with synthetic lure (Neuroptera: Chrysopidae)

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Abstract

Common green lacewings (*Chrysoperla carnea* species complex) are important predators of several insect pests, particularly aphids. Due to their potential in biological control, several efforts have been made to find means to retain common green lacewing adults or even to increase their numbers at a given site. In the course of field experiments conducted in Hungary and Norway, we tested the effect of a known ternary bait (phenylacetaldehyde, methyl salicylate, acetic acid) on oviposition and overwintering site choice of common green lacewings. In an orchard in Hungary, overwintering chambers were set up with and without baits. For the experiment on oviposition in Norway, a number of lacewing eggs were compared on trees with and without baits in selected sectors of the canopy. With the ternary bait, more common green lacewings could be attracted to overwintering chambers, and also significantly more eggs were found in the vicinity of baits than in other selected parts of the canopy, suggesting that this bait has the potential to manipulate both overwintering site choice and oviposition site choice of these important beneficial insects.

Introduction

Green lacewings are important predators of several insect pests, particularly aphids. The common green lacewing (*Chrysoperla carnea* species complex, i.e. *carnea* complex) is a widely spread taxon also present in agro-ecosystems, which comprises several species (Henry et al. 2001). Due to their potential in biological control (Hagley 1989; Maisonneuve and Marrec 1999; Atlihan et al. 2004; Pappas et al. 2011), several efforts have been made to find applicable methods to preserve common green lacewing adults or even to increase their numbers at a given site and thus enhance the population density of their predatory larvae by the higher rate of egg-laying (McEwen et al. 1998).

The species of the *carnea* complex overwinter as hibernated adults. To increase their survival in the hibernation period, the use of overwintering chambers was suggested to provide shelter from the adverse winter conditions and to increase survival (Sengonca and Frings 1989; Sengonca and Henze 1992). Overwintering chambers also provide the possibility to move overwintering lacewings, with the chamber to the desired site with the aim of establishing a population from the beginning of next spring (Sengonca and Frings 1989). Although the increase of survival up to 100% reported by Sengonca and Frings (1989) was not supported by later studies (Sengonca and Henze 1992; McEwen et al. 1999; Weihrauch 2008), overwintering chambers may give a possibility to increase abundance of common green lacewings locally.

Another major scientific goal was to develop an effective bait attracting *carnea* complex adults. Several compounds were suggested as attractants for these lacewings (e.g. Flint et al. 1979; Zhu et al. 1999; Tóth et al. 2006). Some compounds (e.g. caryophyllene, camphor) were also tested whether they affect overwintering site choice of common green lacewings; however, no such effect was found (McEwen et al. 1999).

As common green lacewings are predatory in the larval stage (Pappas et al. 2011), oviposition is of particular importance for practical aspects of biological control. Several studies were performed on oviposition site choice of green lacewings including *Chrysoperla* species (Ruzicka 1996; Petersen and Hunter 2002; Fréchette et al. 2006; Kunkel and Cottrell 2007). Recently, sprays of food sources and synthetic attractants (caryophyllene, tryptophan) were tested for the Nearctic *Chrysoperla rufilabris*; nevertheless, none of the treatments showed consistent effect on oviposition of females compared to the control (Kunkel and Cottrell 2007).

Recently, a three-component bait consisting of phenylacetaldehyde, methyl salicylate and acetic acid was developed, which attracted common green lacewing adults more efficiently in field conditions than any previously published attractant (Tóth et al. 2009). Furthermore, female lacewings laid eggs in traps baited with the ternary blend (Tóth et al. 2009; Jaastad et al. 2010), suggesting that this combination of chemical stimuli may have a strong effect on behaviour of common green lacewing adults.

In the present study, we aimed to test whether the ternary bait affects the behaviour of common green lacewings during oviposition and overwintering site choice. According to our predictions, if the ovipositing females and individuals taking shelter for overwintering are responsive to these stimuli, then females should lay more eggs on baited trees and baited overwintering chambers should hold higher number of individuals, which could provide new perspectives for biological control.

Material and Methods

Preparation of baits

All synthetic compounds (≥95% 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 comprised of three components, that is, phenylacetaldehyde, acetic acid and methyl salicylate, in a 1 : 1 : 1 ratio. The loading of each compound was kept at 100 mg. The dispensers were heat-sealed and attached to 8 × 1 cm plastic handles for easy handling when assembling the traps. Dispensers were wrapped singly in pieces of aluminium foil and stored at −18°C until used.

Description of field trials

Field experiments with overwintering chambers

The objective of these tests was to determine whether the ternary bait has the potential to increase the number of common green lacewing individuals taking shelter in artificial overwintering chambers. Experiment 1 was performed in an approximately 3 ha sour cherry orchard in Halásztelek (N 47°21′, E 19°00′; Pest County, Hungary). The orchard is part of a complex of orchards including apple and mixed orchards and is surrounded by urban and agricultural landscape. Forty-eight overwintering chambers were set out on 2nd October 2008 and collected on 2nd March, 2009. Twenty-four chambers were operated with two bait dispensers attached to the outside of the box (after 3 weeks, baits were replaced by new ones), while another 24 boxes served as controls without baits. Overwintering chambers were 21 ×21 × 16 cm size, made up of plastic cardboard with six horizontal entrance holes on the front and the back side, each of 60 × 15 mm size. Chambers were filled with corrugated cardboard sheets to provide shelter for overwintering lacewings. Corrugated cardboard was used to provide similar circumstances in all chambers. Chambers were hung on trees at a height of 1.8–2 m, as lacewings were observed to fly frequently at this height in the orchard. Baited and unbaited chambers were placed alternately at least 8–10 metres from each other. At the end of the trial, overwintering chambers were collected and thoroughly searched for green lacewings at a cool place (below 5°C). All collected individuals were preserved in alcohol and identified to species level after the works of Henry et al. (1996, 2001, 2002) and Thierry et al. (1992, 1998). Within the *carnea* complex, there are two nomenclatural approaches (Henry et al. 2001; Canard and Thierry 2005). In our work, we adopted the nomenclature of Henry et al. (1996, 2001, 2002).

Field trials on oviposition site preference of C. carnea complex to lures

Experiment 2 was conducted to study the number and distribution of eggs laid in the tree canopy with and without ternary lures. This test was conducted in a small (approximately 0.25 ha) mixed orchard with pears, apples and cherries in Ås, Eastern Norway (N 59°40′, E 10°46′). The orchard is surrounded by mixed deciduous and conifer forest on two sides. In the experiment seven trees were equipped with ternary baits and six trees were only equipped with control markers. The experiment was set up in three rows of cherries at the orchard. The tree height was approximately 3 m with a canopy of ca. 1.8–2.3 m diameter. Distance between trees selected for the study was approximately 8 m. The registration census of eggs was performed in seven sectors of the canopy. Sectors for census were dispenser or marker point in the centre (C) and all others in 30 cm distance in the directions north (N), south (S), west (W), east (E), over (O) and under (U) the dispenser or marker. Dispensers with treatment and control markers were all placed in the centre of the tree canopy. Eggs were counted in the area of an imaginary sphere, 10 cm diameter, around the seven respective check points. Sectors were checked for *C. carnea* eggs 16 times from the 13th of May until the 1st of July 2008. During the experiment, baits were changed every 3 weeks as previous experience had shown that they do not lose their attractiveness during this period (Tóth et al. 2006, 2009).

Statistical analyses

Data sets from both experiments were fitted into a generalized linear model (GLM), for Poisson distributed data (Crawley 2002). In experiment 2, mean separation of the seven sectors in the tree canopy was performed using a post hoc test, Tukey's honestly significant difference (HSD) test (Zar 1984). Statistical procedures were conducted using the software R Version 1.4-2 (R Development Core Team 2008).

Results

In experiment 1, more lacewings were found in baited (mean ± SE 5.61 ± 1.12) than in unbaited (mean ±SE 2.38 ± 0.76) overwintering chambers; baits showed a significant effect on the number of lacewings (GLM, z = 5.403, df = 45, P < 0.001). All lacewings collected belonged to the *Chrysoperla carnea* complex with *C. pallida* and *C. carnea* s. str. being more numerous and *C. lucasina* present with fewer individuals (Table 1). Both males and females of all species were found in both baited and unbaited chambers (Table 1).

In experiment 2, the mean value ±SE in tree canopies holding dispensers loaded with the ternary blend was 4.86 ± 0.86 eggs per lure per inspection. This is a significantly higher number of eggs than in the control treatment (Tukey's HSD test; P < 0.001). For trees with lures, significantly higher numbers of eggs were

Table 1 Total number of individuals of each species of the *Chrysoperla carnea* species complex recorded in overwintering chambers with (n = 24) or without (n = 24) the synthetic ternary bait (Exp. 1.).

	Chambers with bait		Chambers without bait	
Species	Total number	Female %	Total number	Female %
Chrysoperla carnea (Stephens, 1836)	45	68.9	19	73.7
C. lucasina (Lacroix, 1912)	16	37.5	3	66.7
C. pallida Henry et al. 2002	68	51.5	35	51.4
All three species	129	55.8	57	59.6

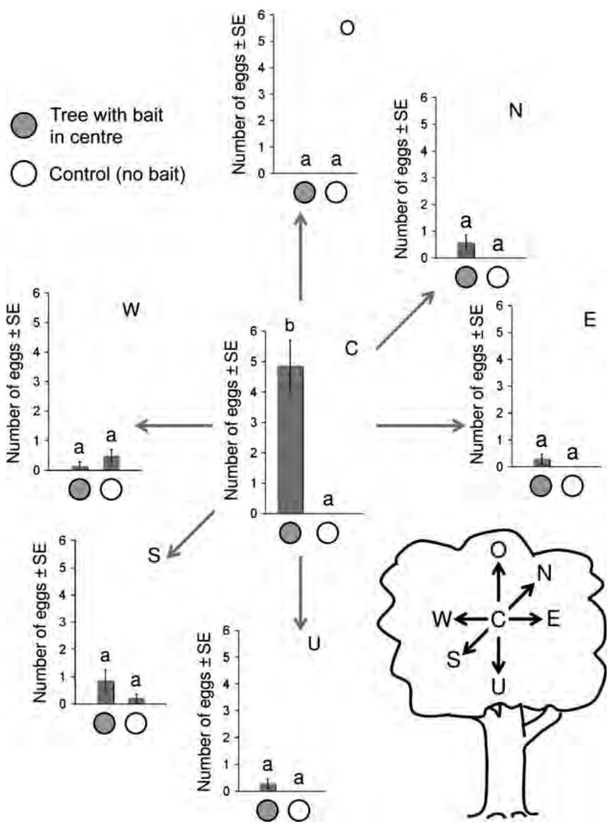

Fig. 1 Number of lacewing eggs in the sectors of the canopy in baited (N = 7) and unbaited (N = 6) trees. Letter codes of sectors are as follows: centre (C) and north (N), south (S), west (W), east (E), over (O) and under (U) the dispenser or marker. Means were calculated per bait/marker per inspection. Values marked with the same letter are not statistically different (ANOVA, Tukey's HSD, P = 0.05)

recorded in the centre (C) of the canopy as compared to the other sectors (fig. 1, Tukey's HSD test; $P < 0.001$). Eggs were found laid in close proximity around the lures centrally placed in the tree canopy, and none of the differences in means between north (N), south (S), east (E), west (W), over (O) and under (U) were significant (fig. 1. Tukey's HSD test; $P = 0.726$).

Discussion

Our results confirm the strong behavioural effect of the synthetic ternary bait on common green lacewings, which proved to be highly attractive in previous studies (Tóth et al. 2009).

Contrary to previous reports of attractants known from previous literature (McEwen et al. 1999), the synthetic ternary lure affected overwintering site choice of common green lacewings in the present study. This opens up practical perspectives to collect higher numbers of adult common green lacewings in overwintering chambers and preserve them to the next spring at the specific site.

Although in our experiments, the number of lacewings in overwintering chambers was relatively low, the difference between baited and unbaited chambers was shown clearly. Several factors may influence the number of lacewings in overwintering chambers, including local population density, habitat conditions and the designs of the chambers. During our tests, we used a simplified overwintering chamber design that in our opinion allowed a clear and standardized comparison of chambers with or without the synthetic bait. We decided to omit some features (e.g. straw as fill and chambers made of painted plywood) suggested by previous authors (Sengonca and Frings 1989; McEwen and Sengonca 2001; Thierry et al. 2002), as these design characters in our view could be less easy to standardize, and as additional odour sources possibly could have had a confounding effect as well. Nevertheless, we are aware that these features may also be important and efficiency of overwintering chambers could be further increased by applying all favourable stimuli (chemical from our synthetic bait and physical from design and fill material, etc.) together in a highly effective chamber design.

Our results have also confirmed that the ternary bait affects oviposition site choice of common green lacewings. Trees equipped with baits holded a higher number of lacewing eggs than trees without baits, which suggests that with the ternary lure, common green lacewings can be attracted to lay their eggs on a selected tree. Furthermore, the distribution of the eggs within the tree canopy showed that eggs were laid in close proximity of the baits, indicating that oviposition activity of common green lacewings can be concentrated using the ternary lure.

Previous reports are available on the effect of food sprays on common green lacewings (e.g. Hagen et al. 1976; Tassan et al. 1979); however, the effect of this methods seemed to decrease in a few days (Hagen et al. 1976); furthermore, later studies did not confirm conclusive effect on oviposition (Kunkel and Cottrell 2007).

Nevertheless, attracting predators and parasitoids of pests is a very promising research area. Several attempts are made to develop methods for using compounds which attract natural enemies of pests (Pickett et al. 2012). According to these studies, plant volatiles seem to have special importance by attracting a variety of predators and parasitoids (e.g. Han and Chen 2002; James and Price 2004; Hare 2011).

However, to our knowledge, despite the reports on attractive effect of several volatiles, results on longer term effect of attractants in local maintenance of natural enemies (e.g. by oviposition and overwintering) are scarce. Thus, we believe our results may contribute to this research area, and this knowledge may be exploited in everyday agricultural practice to increase the impact of biological control.

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