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DYNAMICS AND PREDATION EFFICIENCY OF *CHRYSOPERLA EXTERNA* (NEUROPTERA: CHRYSOPIDAE) ON *ENNEOTHRIPS FLAVENS* (THYSANOPTERA: THRIPIDAE)

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Abstract

The dynamics of predation by the green lacewing *Chrysoperla externa* Hagen (Neuroptera: Chrysopidae) on *Enneothrips flavens* Moulton (Thysanoptera: Thripidae) was investigated by placing embryonated eggs and first-instar larvae of *C. externa* on potted peanut plants (*Arachis hypogaea* L.; Fabales: Fabaceae) in a greenhouse. The plants that received either embryonated eggs or larvae of *C. externa* showed significant reductions in the mean numbers of thrips, about 9 days after the release of the predator on the plants. The potential of *C. externa* as a biological control agent for thrips is discussed.

Key Words: green lacewings, biological control, peanuts, predator performance

Resumo

Este estudo teve como objetivo investigar a dinâmica de predação de *Chrysoperla externa* Hagen (Neuroptera: Chrysopidae) sobre *Enneothrips flavens* Moulton (Thysanoptera: Thripidae), por meio da liberação de ovos embrionados e larvas de primeiro instar de *C. externa* em plantas de amendoim cultivadas em casa de vegetação. As plantas que receberam ovos embrionados e larvas de primeiro instar de *C. externa* apresentaram redução significativa no número médio de tripes nove dias após a liberação do predador. O potencial de *C. externa* como agente controlador foi discutido no contexto de controle biológico de tripes.

Palavras-Chave: crisopídeos, controle biológico, desempenho do predador

Among the pests of peanuts (*Arachis hypogaea* L.; Fabales: Fabaceae), the thrips, *Enneothrips flavens* Moulton (Thysanoptera: Thripidae), has received increasing attention because of the serious economic damage that it causes (Moraes et al. 2005; Dalastra et al. 2011; Michelotto et al. 2013). *Enneothrips flavens* lives in the closed buds or enclosed parts of the plant, and punctures and sucks the cell contents. Consequently, peanut buds are deformed and distorted, exhibiting streaks and discolorations, which result in major crop losses (Gallo et al. 2002). Although chemical control is frequently used, its intense and increasing application contributes to environmental contamination (Bhanti & Taneja 2007), decline of pollinators, and the development of pesticide resistance (Fournier 2005; Henry et al. 2012; Whitehorn et al. 2012). Biological control has been used as an alternative to chemical control, and in some instances it is an efficient tool for pest management (Jonsson et al. 2012).

The green lacewing, *Chrysoperla externa* Hagen (Neuroptera: Chrysopidae), is an important natural enemy of several pest species, because it is tolerant to some pesticides and is a voracious predator (Brettell 1982; Freitas 2001a; Rimoldi et al. 2012; Silva et al. 2012). It has been found in different agroecosystems and has shown significant potential as a biological control agent of phytophagous insects (Carvalho & Souza 2000; Freitas 2002; Bonani et al. 2009).

A growing body of research has shown the importance of releasing green lacewings as control agents for the management of pests, including

thrips (Carvalho & Souza 2000). By releasing second-instar larvae of *Chrysoperla carnea* Stephens, Hassan (1978) demonstrated successful control of *Myzus persicae* Sulzer (Hemiptera: Aphididae) on eggplant (*Solanum melongena* L; Solanales: Solanaceae) grown in greenhouses. The apple aphid, *Aphis pomi* De Geer (Hemiptera: Aphididae), has been controlled by releasing eggs of *C. carnea* on apple cultivars (Hagley 1989). The control of various pests in North America by augmentative release of *C. carnea* has been reported. In cotton, *C. carnea* has suppressed *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) and *Heliothis virescens* (F) (Lepidoptera: Noctuidae) (Ridgway & Jones 1969), and, as also demonstrated with *C. rufilabris,* has significantly suppressed the thrips *Scritothrips citri* Moulton (Thysanoptera: Thripidae) on mango (Khan & Morse 2001).

This study investigated the efficacy of using *C. externa* against *E. flavens,* for reducing the latter's population size in response to the release of *C. externa* eggs and larvae onto peanut plants grown in a greenhouse.

Materials and Methods

Chrysoperla externa Populations

Green lacewing adults were collected by means of an entomological net in a grass field. The field is located near a plantation of *Pinus* sp. in the municipality of Jaboticabal, São Paulo, Brazil. The insects were identified in the taxonomy laboratory at the Universidade Estadual Paulista, Jaboticabal, São Paulo, Brazil. The green lacewings were allowed to mate and males and females were maintained at 25 ± 2 °C, 65 ± 10% RH and 12:12 h L:D. The insects were reared using the methodology developed by Freitas (2001b). The eggs were placed in individual glass bottles (4 × 1 cm) and the newly hatched larvae were used in the experiments.

Growing Peanut Plants

Peanut plants were grown in a greenhouse in 5 L plastic containers containing soil and sand in a 3:1 ratio. Ten seeds of the variety 'Runner IAC 886' were sown per container. Fifteen days after germination, the peanut seedlings were thinned, leaving only 1 plant per container. No pesticide was applied to the plants.

Enneothrips flavens Populations

Twenty-day-old peanut plants were infested with *E. flavens* by placing branches containing thrips on the plants. Taking into account that only 1 infestation might not be enough, a new infestation was performed after 5 days. The thrips used for infestation were obtained from 25 day-old peanut fields at the Universidade Estadual Paulita, Jaboticabal, São Paulo, Brazil. When the experimental plants were 44 days old, the first sample was obtained. The number of thrips on each plant was recorded by observing 6 closed buds on the central branch of the plant. After this initial sample, the predator was released.

Bioassay

The experiments were set up using a fully randomized design with 20 replicates and 3 treatments as follows: Control (no *C. externa* eggs or larvae), T2 (*C. externa* eggs) and T3 (*C. externa* larvae). Each experimental unit was 1 peanut plant, observed after 0, 4, 9 and 15 days, totaling 360 observations. The release of *C. externa* occurred as follows. The control consisted of plants that received no *C. externa* individual. Treatment T2 was composed of plants receiving 4 *C. externa* eggs/plant, and treatment T3 of plants that received 3 newly hatched *C. externa* first-instar larvae/plant. The eggs were placed in a plastic container (height 4 cm × diam 5 cm) with shredded paper to minimize cannibalism. The larvae were released by catching them with a brush and placing them on the plants. All experimental units (containers) were covered with voile bags tied over the plant, to prevent contamination with other plants or insects.

Before the release of the predator, one sampling was done. After the release of the green lacewings, samples were obtained after 4, 9 and 15 days, for a total of 4 samples. The selection of days for sampling was based on the lacewing life cycle: the larva requires 4 days to hatch, and the 1st, 2nd and 3rd stadia each last for 3 days under laboratory conditions.

Statistical Analysis

There was wide variability among the treatments and within each treatment (Fig. 1). Given the dependence of the observations taken in the same experimental unit over time, the nonlinear behavior of the data, as well as the assumption that the mean number of thrips per plant decreases over time, an asymptotic mixed-effects regression model (Pinheiro & Bates 2000) was used. This model can be written as:

$$y_{ijk} = \varphi_{1i} + [(\varphi_{2i} + b_{2ij}) - \varphi_{1i})] \exp[-\exp(\varphi_{3i})t_k] + \varepsilon_{ijk} \quad (M1),$$
$$i = 1, 2, 3, j = 1, \ldots, 20, k = 0, 4, 9, 15,$$

where y_{ijk} is the mean number of thrips (Table 1) for the *i*-th treatment, *j*-th replicate, and *k*-th time period, φ_{1i} is the asymptote for the i-th

TABLE 1. MEAN NUMBER OF *ENNEOTHRIPS FLAVENS* (NYMPHS AND ADULTS) FOUND IN CLOSED *ARACHIS HYPOGAEA* BUDS IN 3 TREATMENTS: CONTROL *A. HYPOGAEA* PLANTS (WITHOUT *CHRYSOPERLA EXTERNA*), ON *A. HYPOGAEA* PLANTS THAT RECEIVED *C. EXTERNA* EGGS, AND ON *A. HYPOGAEA* PLANTS THAT RECEIVED *C. EXTERNA* LARVAE.

	Time (days)	Control plants	Plants with eggs	Plants with 1st instar larvae
Thrips/plant before release (Control)	0	7.75	11.85	13.90
Thrips/plant after release	4	6.70	5.20	6.90
	9	8.20	3.35	3.55
	15	5.95	4.50	3.95

treatment, φ_{2i} is a scaling parameter for the i-th treatment, $b_{2ij} \sim N(0, \sigma^2_{b2})$ is the random effect associated with φ_{2i}, φ_{3i} is logarithm of the rate constant for the i-th treatment, t_k is the time and ε_{ijk} is the error.

To test for treatment differences, two submodels, namely $M2$ and $M3$, were fitted to the data. In $M2$, the $T2$ (*C. externa* eggs) and $T3$ (*C. externa* larvae) treatments were grouped; and in $M3$, the linear predictor is given by

$$y_{ijk} = \varphi_1 + (\varphi_2 + b_{2j}) - \varphi_1) \exp[-\exp(\varphi_3)t_k] + \varepsilon_{ijk} \qquad (M3),$$

that is, no treatment effect was assumed. The models were compared using likelihood-ratio tests (Verbeke & Molenberghs 2000).

Model $M2$ did not differ statistically from model $M1$, and so treatments $T2$ (*C. externa* eggs) and $T3$ (*C. externa* first-instar larvae) did not differ statistically ($p = 0.05$), see Table 2. Also, model $M3$ fit the data poorly compared to model $M2$ (Table 2). Therefore, the control treatment differed from the $T2$ and $T3$ group ($p = 0.05$).

The parameter φ_3 estimate for treatments $T2$ and $T3$ was not significant ($F_{1,171} = 0.10$, $p = 0.75$), so two submodels were fitted to the data: model M 4 with a linear predictor given by

$$y_{ijk} = \varphi_{1i} + (\varphi_{2i} + b_{2ij}) - \varphi_{1i}) \exp[-\exp(\varphi_3)t_k] + \varepsilon_{ijk} \quad (M4),$$

that is, parameter φ_3 is the same for all treatments; and model $M5$, with the linear predictor given by

$$y_{ijk} = \varphi_{1i} + (\varphi_{2i} + b_{2ij}) - \varphi_{1i}) \exp(-t_k) + \varepsilon_{ijk} \qquad (M5),$$

TABLE 2. LIKELIHOOD-RATIO TESTS FOR NESTED MODELS M1, M2 AND M3.

Model	df	2 × logLik	Test	L. Ratio	p-value
M1	16				
M2	10	M2-M1	4.32	4.32	0.63
M3	5	M3-M2	27.99	27.99	< 0.01*

*indicates significant difference ($p < 0.05$).

TABLE 3. LIKELIHOOD-RATIO TESTS FOR NESTED MODELS M2, M4 AND M5.

Model	df	2 × logLik	Test	L. Ratio	p-value
M2	10	474.76			
M4	9	475.56	M4-M2	0.81	0.37
M5	8	480.48	M4-M5	4.93	0.03*

*indicates significant difference ($p < 0.05$).

that is, parameter φ_3 is set to zero. The likelihood-ratio tests (Table 3) showed that the fit from model $M4$ did not differ from that of model $M2$; however, the fit of model $M5$ was significantly different (Table 2). Therefore, model $M4$ fit the data as well as model $M2$ and could be used as a final model.

Table 4 shows the parameter estimates and associated standard errors for model $M4$, which can be written as

$1.072 + 0.208e^{-0.515t}$ if the treatment is the control

$0.659 + 1.506e^{-0.515t}$ if the treatment is $T2$ or $T3$

RESULTS AND DISCUSSION

On day zero, each control plant had a mean of 7.75 thrips, the plants that subsequently received *C. externa* eggs had 11.85 thrips/plant, and the group that subsequently received lacewing larvae

TABLE 4. PARAMETER ESTIMATES (STANDARD ERRORS) FOR MODEL M4 (φ_1 IS THE ASYMPTOTE, φ_2 IS A SCALING PARAMETER, φ_3 IS LOGARITHM OF THE RATE CONSTANT AND σ^2_{b2} IS THE VARIANCE OF THE RANDOM EFFECT ASSOCIATED WITH φ_2)

Treatment	φ_1	φ_2	φ_3	σ^2_{b2}
Control	1.072 (0.071)	1.280 (0.258)	-0.664 (0.159)	1.046
T2 and T3	0.659 (0.089)	2.165 (0.359)	-0.664 (0.159)	1.918

had 13.90 thrips/plant (Table 1). The passage of time did not influence the population of thrips in the control, but there were significant differences in the effects of *C. externa* releases over time on thrips densities at 0 to 4, 9 and 15 days post-release (Figs. 1 and 2).

These results suggest that *C. externa* requires some time after being released in order to show significant impacts on the pest population. On the sampling dates, the mean number of *E. flavens* thrips was significantly reduced on plants that had received *C. externa* when compared with the control plants. These results provided evidence for the potential of *C. externa* as a biological control agent of *E. flavens,* under specific conditions on potted peanuts in a greenhouse. The statistical modeling confirmed that the thrips population decreased in the presence of *C. externa,* as shown in Fig. 1. This result is easily observed by comparing the 2 trends, the constant line describing the *E. flavens* population in the control and the curves describing the *E. flavens* populations under the influence of *C. externa* that had been released either as eggs or larvae (Figs. 1 and 2).

In the absence of predators, it is expected that the mean number of thrips in closed peanut buds will increase, as seen with *M. persicae* aphids on eggplants (Hassan 1977). In the current study, the number of *E. flavens* thrips in the control remained stable, while in the other treatments the number was reduced. On day 15, *C. externa* third-instars started to pupate, and in response the thrips population increased slightly. It is important to use appropriate intervals between predator releases, in order to prevent the pest from persisting when the *C. externa* larvae are in a post-feeding period. The appropriate release intervals for the control of *M. persicae by C. carnea* have been estimated from 2 to 5 weeks (Hassan 1977). However, for *E. flavens* these release intervals would vary from 9 to 15 days, based on the results of the current study.

A few studies involving the genus *Chrysoperla* and thrips have been designed to investigate preferences between different prey. In a recent study, Shrestha & Enkegaard (2013) analyzed the prey choice by 3rd-instar *C. carnea* on the western flower thrips *Frankliniella occidentalis* and the lettuce aphid *Nasonovia ribisnigri* (Mosley) (Aphididae) in the laboratory, by using different prey ratios. The results of the study suggest a slight preference of *C. carnea* for aphids compared to thrips. However, the results were also significantly influenced by the predator-prey ratios; and at some ratios, no preference was observed (Shrestha & Enkegaard 2013). Although this result indicated an apparent weak interaction between *C. carnea* and thrips, survey results have shown that members of the genus *Chrysoperla* are frequently present on plants of different species containing thrips (Bettiol et al. 2004; Mann et al. 2010; Saeidi & Adam 2011), encouraging studies to evaluate the probable interaction dynamics between these species. Unfortunately, the lack of studies investigating possible interactions between populations of *C. externa* and *E. flavens* make any specific comment about the interaction strength between them impossible. To our knowledge, studies examining the biological control of *E. flavens* are not common, and ours is a pioneer study on the use of *C. externa* for this purpose.

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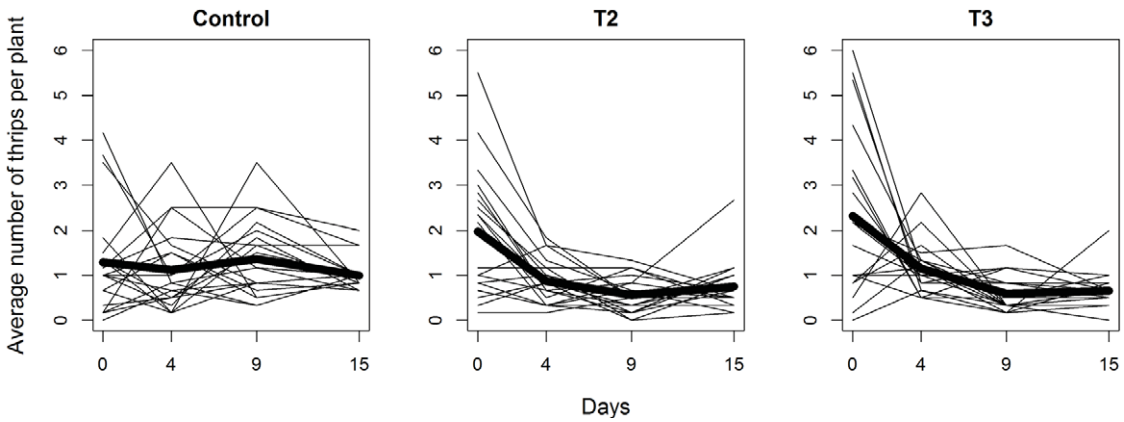

Fig. 1. Biological control of *Enneothrips flavens* with *Chrysoperla externa*, showing daily trends in each of 20 replicates. The control consisted of *Enneothrips flavens* thrips-infested peanut plants that received no *Chrysoperla externa* individuals. In treatment T2 the thrips-infested peanut plants received 4 *C. externa* eggs/plant, and in treatment T3 the thrips-infested peanut plants received 3 newly hatched *C. externa* first-instar larvae/plant.

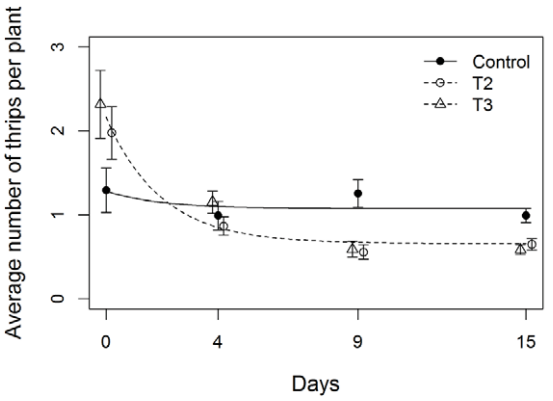

Fig. 2. Reduction of *Enneothrips flavens* on peanut plants by *Chrysoperla externa*. The average (± SE) numbers of thrips per peanut plant were fitted the curve using model M4. The control consisted of *Enneothrips flavens* thrips-infested peanut plants that received no *Chrysoperla externa* individuals. In treatment T2 the thrips-infested peanut plants received 4 *C. externa* eggs/plant, and in treatment T3 the thrips-infested peanut plants received 3 newly hatched *C. externa* first-instar larvae/plant.

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