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Functional response of the green lacewing, *Chrysoperla carnea* larvae on two aphid pests of the citrus: *Aphis spiraecola* and *Aphis gossypii*

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Abstract

The green lacewing, *Chrysoperla carnea*, plays an important role in biological control of various aphid species. In this study, the functional responses of the second and third instar larvae of *C. carnea* were studied on the citrus aphid, *Aphis spiraecola* and melon aphid, *A. gossypii*. The experiments were carried out on orange (*cv*. Thompson Navel) leaves in a growth chamber at 27 ± 2°C, 65 ± 5% RH and a photoperiod of 16 L: 8 D h. Different densities of the preys were offered to the predators, and the number of consumed preys was calculated after 24 h. Based on the logistic regression analysis, both second and third larval instars of the predator exhibited a Type II functional response to both aphid species. With increasing of the larval instar, the attack rate (a) of the predator on *A. spiraecola* was significantly increased and the handling time (T_h) decreased. Attack rate of second larval instar of the predator on *A. gossypii* was significantly higher than that on *A. spiraecola*. Furthermore, the third instar larvae fed on *A. gossypii* showed significantly lower handling time and higher attack rate compared to *A. spiraecola*. The results of this study revealed that the larvae of *C. carnea*, especially the third instar, had a good predation potential in controlling *A. spiraecola* and *A. gossypii*. However, further field-based studies are needed for a comprehensive estimation of biocontrol abilities of *C. carnea* toward these two aphids on the citrus.

Key Words: Biological Control, Citrus Aphid, Functional Response, Green Lacewing, Melon Aphid

واکنش تابعی لاروهای بالتوری سبز، *Chrysoperla carnea* نسبت به دو گونه از شته‌های آفت مرکبات: شته سبز مرکبات *Aphis spiraecola* و شته جالیز *Aphis gossypii*

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چکیده

بالتوری سبز *Chrysoperla carnea* نقش مهمی در کنترل بیولوژیک گونه‌های مختلف شته‌ها دارد. در این تحقیق، واکنش تابعی لاروهای سنین دوم و سوم *C. carnea* نسبت به شته سبز مرکبات، *Aphis spiraecola* و شته جالیز، *A. gossypii* مورد بررسی قرار گرفت. آزمایش‌ها روی برگ‌های پرتقال (رقم تامسون ناول) در اتاقک رشد در دمای ۲ ± ۲۷ درجه سلسیوس، رطوبت نسبی ۵ ± ۶۵ درصد و دوره نوری ۱۶ ساعت روشنایی و هشت ساعت تاریکی انجام شد. تراکم‌های مختلفی از طعمه‌ها در اختیار شکارگرها قرار داده شد. تعداد طعمه‌های خورده شده بعد از ۲۴ ساعت محاسبه و ثبت شد. بر اساس نتایج تجزیه رگرسیون لجستیک، هر دو سن لاروی شکارگر واکنش تابعی نوع دوم را نسبت به هر دو گونه شته نشان دادند. با افزایش سن لاروی شکارگر، نرخ حمله (a) آن نسبت به *A. spiraecola* به طور معنی‌داری افزایش و زمان دستیابی (T_h) آن کاهش یافت. نرخ حمله لارو سن دوم شکارگر روی *A. gossypii* به طور معنی‌داری بیشتر از مقدار متناظر روی *A. spiraecola* بود. همچنین زمان دستیابی لارو سن سوم شکارگر در تغذیه از *A. gossypii* در مقایسه با *A. spiraecola* به طور معنی‌داری کمتر و نرخ حمله آن بیشتر بود. نتایج این تحقیق نشان داد که لاروهای بالتوری سبز به ویژه سن سوم آن، از توانایی خوبی برای کنترل این شته‌ها برخوردار هستند. با این حال، برای برآورد جامعی از توانایی‌های کنترل بیولوژیک *C. carnea* نسبت به این دو گونه شته روی مرکبات، باید آزمایش‌های بیشتری در شرایط صحرایی انجام گیرد.

کلمات کلیدی: بالتوری سبز، شته جالیز، شته سبز مرکبات، کنترل بیولوژیک، واکنش تابعی

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Introduction

Aphids comprise a diverse group of sap-sucking insects, and are normally distributed in temperate regions like North America, Europe, and Asia. Worldwide, there are about 4700 different species of Aphididae, which can cause serious damage on different agricultural crops like citrus (Blackman & Eastop 2007; Vacante & Gerson 2012). In Mediterranean regions, the most common species of aphids on citrus trees are citrus aphid, *Aphis spiraecola* Patch and melon aphid, *A. gossypii* Glover (Tena & Garcia-Mari 2011). *Aphis spiraecola* is one the most important pests of citrus in north of Iran, the large population of which can cause curling, crinkling and distortion of young leaves, especially on young citrus trees. Furthermore, this species is a potential vector of citrus tristeza virus (Soroushmehr 2004). *Aphis gossypii* is a polyphagous species with almost 100 different host plants (Blackman & Eastop 2000), which not only causes direct damage to the host plants but also indirectly transmits more than 76 plant viruses (Garzo *et al.* 2002; Pervez & Omkar 2005). In citrus trees, *A. gossypii* can cause damage by colonizing young shoots and buds (Aghajanzadeh *et al.* 1997).

The lacewings, Chrysopidae, are known as beneficial insects of agricultural ecosystems, which can be effectively used in biological control programs of pests in orchards, fields, and greenhouses. The larvae attack various pests like aphids, coccids, psyllids, thrips, spider mites, as well as eggs and young larvae of Lepidoptera and Coleoptera (Canard *et al.* 1984). This family includes more than 1200 species, the predatory behavior of which makes them promising candidates for biological control programs (Brooks & Barnard 1990). Based on a review of literature, 46 species of Chrysopidae have been reported from Iran (Farahi *et al.* 2009). The green lacewing, *Chrysoperla carnea* Stephens, is one of the most important species of this family that is known as the predator of aphids and many soft-bodied insects (Reddy 2002; Carrilo & Elanov 2004). Tolerance to pesticides, voracious larval feeding capacity as well as wide prey range make this predator more compatible with most IPM systems (Syed *et al.* 2008).

There are various criteria for assessing the efficiency of natural enemies (Waage 1990), including the study of relationship between prey density and predator consumption, which is defined as functional response (Jervis & Kidd 1996). The functional response is a quantitative description of the behavior of a predator when it encounters different prey species, which can be used for better understanding of prey-predator interactions to predict the effectiveness of prospective biological control agents (Houck & Strauss 1985).

Holling (1959) categorized the functional response into Type I, Type II, and Type III. Later, some researchers reported other type of functional response as Type IV (a dome-shapes response) (Luck 1985). Types II and III have received the most attention among these four types of functional response. In Type II model, the predator shows an inverse density dependent response, in which the proportion of prey attacked declines monotonically with prey availability. The Type III model shows a sigmoid relationship where the proportion of consumed prey is positively density-dependent over some regions of prey density (Jervis & Kidd 1996; Pervez & Omkar 2005).

Functional response models are of interest to

IPM practitioners who conventionally have attempted to identify predators showing Type III functional response because the mortality caused by these predators on prey species is thought to stabilize prey populations (Murdoch *et al.* 2003).

To better understand the predatory behavior of the green lacewing on *A. spiraecola* and *A. gossypii*, in this study, the functional response and consumption rate of second and third larval instars of *C. carnea* were assessed to different densities of these aphids.

Materials and methods

Aphids' culture

The initial populations of *A. gossypii* and *A. spiraecola* were collected from unsprayed citrus orchards near Ghaemshahr (36° 27′ 47″ N, 52° 51′ 36″ E), Mazandaran province, Iran. The collected aphids were transferred onto buds of young citrus trees (*Citrus sinensis* L. variety Thompson Navel) and reared for several generations. The aphids' cultures were maintained in a greenhouse at 27 ± 2 °C, 70 ± 10 % RH and 16L: 8D photoperiod.

Predator culture

The colony of *C. carnea* was initiated with larvae of the predator that were collected from aphid-infested leaves in citrus orchards near Ghaemshahr, Mazandaran province, Iran. Adult lacewings were maintained in plastic containers (11 cm diameter; 19 cm height) covered on top by a fine-mesh net for ventilation as well as egg laying substrate. About 10 pairs (male and female) of the predator were released into each container. Adults were fed on an artificial diet consisting of brewer's yeast, honey and distilled water in a 5:7:4 ratios (Vogt et al. 2000) coated as a paste on transparent plastics (8 cm length × 1.5 cm width) and then hung from the top of the containers. Extra water was provided by placing a wet sponge on the top of the container above the net. Eggs laid on the inner side of the container or on the net were transferred by a brush to Petri dishes (9 cm diameter with a fine-net-covered hole in the lid for ventilation). The larvae were separately reared in Petri dishes and fed *ad libitum* with frozen eggs of Mediterranean flour moth, *Ephestia kuehniella* Zeller. The predator was reared in a growth chamber at 27 ± 2°C, 65 ± 5% RH and 16L: 8D photoperiod for more than three generations.

Experimental procedure

To study the functional response of the green lacewing, the plastic Petri dishes (9 cm in diameter) were used. Each Petri dish had a hole (2 cm diameter) in the lid, which was covered with fine-mesh net for ventilation and its bottom lined with a layer of solidified agar solution (2 %) as a substrate to prevent from desiccation of citrus leaves (*C. sinensis cv.* Thompson Navel; with an area of 16.5 cm^2). The experiments were conducted with the second and third instar larvae of *C. carnea* (24 h old) on a mixture of third and fourth instars nymphs of each *A. spiraecola* or *A. gossypii*. Both the second and third instar larvae of the green lacewing were individually provided with *A. spiraecola* in densities of 2, 4, 8, 12, 16, 32, 64, and 96. *Aphis gossypii* nymph densities for the second and third larval instars of *C. carnea* were 2, 4, 8, 12, 16, 32, 64 and 96; and 2, 4, 8, 12, 16, 32, 64, 96 and 128, respectively. Petri dishes were sealed with parafilm to prevent insects from escaping. The Petri dishes were kept in a growth chamber at 27 ± 2°C, 65 ± 5% RH and 16L: 8D. Each prey density was replicated

10 times for each predator larval instars. After 24 h, the predators were removed from each Petri dish and the number of prey consumed (total minus alive) was evaluated.

Statistical analysis

The data were analyzed in two separate steps (Juliano 2001) using SAS program (SAS Institute 2002). First, the type of functional response was determined by a logistic regression of the proportion of prey consumed (N_e) as a function of initial prey density (N_0) as follows:

$$\frac{N_e}{N_0} = \frac{\exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)}{1 + \exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)} \qquad [1]$$

where P_0, P_1, P_2, and P_3 are the intercept, linear, quadratic and cubic coefficients, respectively. These coefficients were estimated using the method of maximum likelihood. The linear coefficient sign (P_1) was used to determine the type of functional response. A negative P_1 indicates Type II functional response, whereas a positive P_1 shows Type III functional response. After determining the type of functional response, the next step is to estimate the handling time (T_h) and attack rate (a) parameters. Since the experiment was carried out without prey replacement during the experiments, random predator equation was used to estimate the parameters (Rogers 1972):

$$N_e = N_0 \{1- \exp [a (T_h N_e - T) \qquad [2]$$

where N_e is the number of prey consumed, N_0 is the initial prey density, a is the attack rate, T_h is the handling time, and T is the total time of exposure.

Pairwise comparisons of functional response parameters for different larval instars or prey types were performed using the indicator variable method (Juliano 2001) as follows:

$$N_e = N_0 \{1- \exp [- (a + D_a (j))(T - (T_h + D_{Th} (j))N_e)]\} \qquad [3]$$

where j is an indicator variable that has a value of 0 for the first data set and 1 for the second data set. The parameters D_a and D_{Th} estimate the differences between data sets being compared for the values of a and T_h parameters, respectively. In other words, the handling time for one data set is T_h, and that for another data set is $T_h + D_{Th}$. Testing for a significant difference in handling times between two data set is accomplished by testing the null hypothesis that D_{Th} includes 0 (Juliano 2001).

Data on the predation rates of *C. carnea* larvae upon encounter with different prey densities were analyzed using one-way ANOVA followed by Tukey's test at $P < 0.05$, and those of both larval instars were subjected to independent-sample *t*-test (SPSS 2007).

Results

The functional responses of the second and third larval instars of *C. carnea* to *A. spiraecola* and *A. gossypii* are illustrated in Figure 1. The results of logistic regression analyses of second and third larval instars of *C. carnea* to varying densities of *A. spiraecola* and *A. gossypii* are presented in Table 1. In all cases, the linear coefficient of equation (1) was negative and significantly different from 0, indicating a Type II functional response.

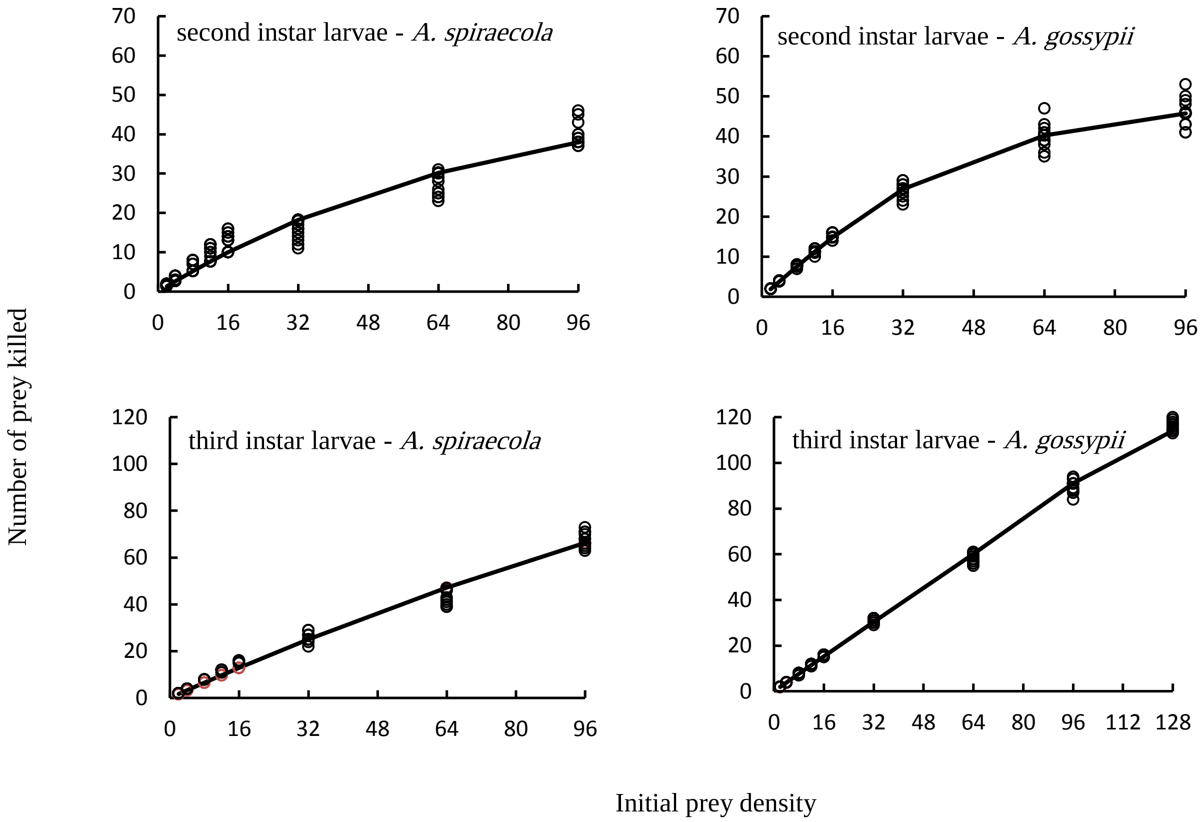

Figure 1. Functional response of second and third larval instars of *Chrysoperla carnea* to different densities of *Aphis spiraecola* and *A. gossypii*. The data points and solid lines represent the number of preys killed and the predictions of the best-fitted type II functional response model, respectively.

The coefficients of attack rate (a) and handling time (T_h) of *C. carnea* larvae were affected by both prey types. The estimated parameters showed that the third instar larvae of *C. carnea* fed on *A. spiraecola* had higher attack rate and shorter handling time compared with the second instar larvae (Table 2). The asymptotic 95% confidence interval for their D_a and D_{Th} did not include 0, which means that there were significant differences between attack rates as well as handling times of second and third larval instars (Table 3). Handling time (T_h) of the third instar larvae of *C. carnea* feeding on *A. gossypii* was shorter than that of the second instar larvae (Table 2). In this case, the asymptotic 95% confidence interval for D_a included 0 but that for D_{Th} did not, which shows that there were significant differences between handling times of second and third larval instars (Table 3). Comparison of functional response parameters of *C. carnea* larvae while feeding on both prey species yielded significant pairwise differences in attack rates of the second instar larvae as well as both handling time and attack rate of the third instar larvae (Table 3). The attack rate of the second instar larvae on *A. gossypii* was significantly higher than that on *A. spiraecola*. Furthermore, the third instar larvae feeding on *A. gossypii* had significantly shorter handling time and higher attack rate than on *A. spiraecola*. The maximum number of aphids that could be consumed by different instars of *C. carnea* (T/T_h) increased with the increase in larval instar (Table 2). This parameter was highest for third instar larvae feeding on *A. gossypii* (Table 2).

The consumption rates of the second and third instar larvae of *C. carnea* were increased by rising densities of *A. spiraecola* ($F_{7,72}$ = 484.24, $P < 0.0001$; $F_{7,72}$ = 1.56, $P < 0.0001$, respectively) and *A. gossypii* ($F_{7,72}$ = 644.99, $P <$ 0.0001; $F_{7,72}$ = 6.95, $P < 0.0001$, respectively), reaching a maximum number at the density of 96 or 128 (Table 4). Furthermore, the consumption rate of the second instar larvae feeding on *A. spiraecola* and *A. gossypii* was not significant at lower densities of the preys, but from density of 16 to 96, the predator consumed larger numbers of *A. gossypii* in comparison with *A. spiraecola* (Table 4). Such trend (from density of 32 to 96) was also found for the third instar larvae (Table 4).

Table 1. Maximum likelihood estimates from logistic regression analyses of the proportion of *Aphis spiraecola* and *A. gossypii* consumed by *Chrysoperla carnea* larvae as a function of initial prey density.

Prey	Larval instar of predator	Parameters	Estimate	SE	χ^2	P-value
A. spiraecola	2nd instar	Constant	5.8377	0.5251	123.60	< 0.0001
		Linear	-0.3297	0.0362	82.83	< 0.0001
		Quadratic	0.00539	0.000703	58.80	< 0.0001
		Cubic	-0.00003	4.004E-6	46.54	< 0.0001
	3rd instar	Constant	7.8159	0.1462	46.50	< 0.0001
		Linear	-0.3352	0.0707	22.45	< 0.0001
		Quadratic	0.00503	0.00128	15.54	< 0.0001
		Cubic	-0.00002	6.936E-6	11.88	0.006
A. gossypii	2nd instar	Constant	5.8695	0.8144	51.95	< 0.0001
		Linear	-0.2029	0.0533	15.40	< 0.0001
		Quadratic	0.00280	0.000999	7.84	0.0051
		Cubic	-0.00001	5.551E-6	5.63	0.0177
	3rd instar	Constant	6.3829	0.8670	54.20	< 0.0001
		Linear	-0.1436	0.0402	12.79	0.0003
		Quadratic	0.00171	0.000561	9.32	0.0023
		Cubic	-6.53E-6	2.36E-6	7.66	0.0057

Table 2. Attack rate (a), handling time (T_h), and maximum predation rate (T/T_h) of *Chrysoperla carnea* larvae fed on *Aphis spiraecola* and *A. gossypii*.

Prey	Larval instar of predator	a		T_h		T/T_h	R^2
		Estimate ± SE	95% CI[*]	Estimate ± SE	95% CI		
A.spiraecola	2nd instar	0.0483 ± 0.00523	0.0379 - 0.0587	0.3575 ± 0.0404	0.2771 - 0.4378	67.13	0.9339
	3rd instar	0.0721 ± 0.00561	0.0609 - 0.0833	0.1167 ± 0.0226	0.0717 - 0.1616	205.65	0.9751
A. gossypii	2nd instar	0.1465 ± 0.0133	0.1201 - 0.1730	0.4282 ± 0.0133	0.4017 - 0.4547	56.05	0.9839
	3rd instar	0.1319 ± 0.00839	0.1152 - 0.1486	0.0455 ± 0.0117	0.0222 - 0.0688	527.47	0.9982

[*] CI: Confidence Interval.

Table 3. The parameters estimated using combined equation for comparison of attack rate and handling time of *Chrysoperla carnea* larvae feeding on *Aphis spiraecola* and *A. gossypii*.

Treatments	Parameter	Estimate	SE	Approximate 95% CI*	
				Lower	Upper
2nd instar-3rd instar (on *A. spiraecola*)	D_a	0.0238	0.00789	0.00821	0.0394
	D_{Th}	-0.2408	0.0446	-0.3289	-0.1526
2nd instar-3rd instar (on *A. gossypii*)	D_a	-0.0147	0.0153	-0.0448	0.0155
	D_{Th}	-0.3827	0.0177	-0.4177	-0.3477
2nd instar (on *A. gossypii*)-2nd instar (on *A. spiraecola*)	D_a	-0.0982	0.0190	-0.1358	-0.0607
	D_{Th}	-0.0708	0.0380	-0.1458	0.00421
3rd instar (on *A. gossypii*)-3rd instar (on *A. spiraecola*)	D_a	-0.0598	0.0122	- 0.0838	-0.0358
	D_{Th}	0.0712	0.0242	0.0234	0.1190

*CI: Confidence Interval.

Table 4. Prey consumption (mean ± SE) by *Chrysoperla carnea* larvae when feeding on various densities of *Aphis spiraecola* and *A. gossypii*.

Prey Density	2nd instar larvae		3rd instar larvae	
	A. spiraecola	*A. gossypii*	*A. spiraecola*	*A. gossypii*
2	2.00 ± 0.00^{Af}	2.00 ± 0.00^{Ag}	2.00 ± 0.00^{Ag}	2.00 ± 0.00^{Ah}
4	3.90 ± 0.10^{Af}	4.00 ± 0.00^{Ag}	4.00 ± 0.00^{Ag}	4.00 ± 0.00^{Ah}
8	7.70 ± 0.15^{Ae}	7.80 ± 0.13^{Af}	8.00 ± 0.00^{Af}	7.80 ± 0.13^{Ag}
12	11.00 ± 0.33^{Ad}	11.70 ± 0.21^{Ae}	11.80 ± 0.13^{Ae}	11.90 ± 0.10^{Af}
16	14.30 ± 0.36^{Bc}	15.50 ± 0.26^{Ad}	15.60 ± 0.16^{Ad}	15.80 ± 0.13^{Ae}
32	15.00 ± 0.77^{Bc}	26.60 ± 0.65^{Ac}	26.00 ± 0.69^{Bc}	31.40 ± 0.34^{Ad}
64	26.50 ± 0.88^{Bb}	39.90 ± 1.11^{Ab}	43.00 ± 1.03^{Bb}	58.50 ± 0.65^{Ac}
96	40.40 ± 1.01^{Ba}	46.00 ± 1.27^{Aa}	67.90 ± 1.05^{Ba}	89.80 ± 0.99^{Ab}
128	-	-	-	117.00 ± 0.81^{a}

Means in the same column followed by different lowercase letters are significantly different ($p < 0.05$, Tukey's test). Means in the same row (for each larval instar) followed by different uppercase letters are significantly different ($p < 0.05$, *t* test).

Discussion

Studying the efficiency of natural enemies is of high importance in biological control programs. The functional response is one of the criteria used for determining the effectiveness of natural enemies. Functional response shows the ability of a predator to alter its feeding behavior in response to changes in prey density and can be used for comparing the potential predatory behavior of a single predator to different prey types or multiple predators to one or more prey species (Moeezipoor *et al.* 2008).

The results of the current study revealed that the functional responses of different larval instars of *C. carnea* to *A. spiraecola* and *A. gossypii* were Type II, suggesting that the larvae showed an inverse density dependent response to both prey types. In small experimental units (such as the present study), the prey density is artificially high and the predator can have easy access to the prey with low search requirements (O'Neil 1997; Montoya-Alvarez *et al.* 2010), and if the prey is a desirable food for the predator, the predator may exhibit Type II functional response. Type II functional response is also reported for different larval instars of the green lacewing preying on *Hyalopterus pruni* Geoffer (Atlihan *et al.* 2004), on *A. gossypii* (Santos *et al.* 2005 & Montoya-Alvarez *et al.* 2010), on *Brevicoryne brassicae* Linnaeus, *Aphis pomi* De Geer, and *Aphis craccivora* Koch (Mushtaq & Khan 2010 a,b), on *Aphis fabae* Scopoli (Hassanpour *et al.* 2015), on *Myzus persicae* Sulzer and *A. craccivora* (Bayoumy & Awadalla 2018), and on *Saissetia oleae* (Olivier) (Mahzoum *et al.* 2020). The second and third instar larvae of *C. carnea* in combination with the third or fourth instar larvae or adult female of *Hippodamia variegata* (Goeze) showed Type II functional response to *A. fabae* nymphs at the second and third days of the experiment (Zarei *et al.* 2019). Typically, predators that exhibit a Type II functional response can be effective at low prey densities (Holling 1965). However, literature on Type II and III functional responses for different larval instars of *C. carnea* are available (Hassanpour *et al.* 2011; Sultan & Khan, 2014; Ail-Catzim *et al.* 2019; Sajjad *et al.* 2021). These results reveal that a predator species may show different functional responses depending on predator and prey characteristics as well as experimental conditions.

Based on these results, increasing the larval instar significantly affected the functional response parameters. The third instar larvae by feeding on *A. gossypii* had shorter handling time (T_h), and by feeding on *A. spiraecola*, they had higher attack rate (a) and shorter handling time (T_h) than the second instar larvae. An increase in attack rate and a decrease in handling time may indicate that the predator is becoming more efficient at capturing the prey (van Alphen & Jervis 1996). The natural enemies with higher attack rate can maintain the prey population below the economic injury level, but those with low attack rate may act vice versa (Hassell 1982). Handling time is a good indicator to estimate the maximum predation rate and effectiveness of a predator (Atlihan & Guldal 2009). It is evident that by decreasing the handling time, the theoretical maximum predation rate (T/T_h) increases and thus the curve of functional response can rapidly reach the asymptote (Nordlund and Morrison 1990). In the present study, the theoretical maximum predation rate (T/T_h) of the third instar larvae was 205.65 and 527.47 for *A. speiraecola* and *A. gossypii*, respectively. This parameter was calculated 67.13 and 56.05 for the second instar. The observed data were lower than the estimated values. According to Curry and Feldman (1979), Roger's model does not correctly forecast the expected number of consumed prey. The handling time that is estimated based on the functional response models differs from the true handling time because this parameter encompasses the time spent not only on actual prey handling but also on the other non-searching activities (Hassell 1978). It seems that the larger size of the third instar larvae and the ability to overcome prey defenses may account for its shorter handling times on both prey types compared to the second instar larvae (Chakraborty & Korat 2010).

Based on the results of pairwise comparisons, the handling time of the predator on *A. gossypii* was shorter than on *A. spiraecola*. In this study, two prey types with different body sizes were offered to the green lacewing larvae. *Aphis spiraecola* is larger than *A. gossypii*; therefore, the shorter handling time on *A. gossypii* may be related to its smaller size. Prey size can influence prey choice by the predators because the encounter rate is a function of prey body size. Normally, preference toward larger preys may be related to the point that the larger prey is a more profitable food for the predator (Charnov 1976), but in some cases, the predation rate on larger preys may be decreased because of the prey's defense or escape capability (Sabelis 1992; Provost *et al.* 2006; Fantinou *et al.* 2009). On smaller preys, the predator spends little effort in subduing them and thus the number of consumed preys would increase during the time. It must be noted that the prey size is not the only factor affecting predators selection (Molles & Pietruszka 1987) because some other factors like the nutritional quality of prey can influence prey choice by predators (Butler & O' Neil 2008). Moreover, morphological and physiological properties of the prey can affect its acceptance by the predators (Nedved & Salvucci 2008).

In the present study, the consumption rate of *C. carnea* increased with exposure to high densities of *A. spiraecola* and *A. gossypii*. Similarly, some other studies have documented that higher availability of prey increases the consumption rate of the predator (Fantinou *et al.* 2012; Mottaghinia *et al.* 2016; Fathipour *et al.* 2018). It seems that predators typically spend less time to find preys at high prey densities and consume more preys than the time when less preys are available.

The response of a predator to varying densities of a prey is important; however, we know that this is not the sole factor for prediction of the success or failure of a biocontrol agent in controlling prey population. Some other factors like numerical response, intrinsic rate of natural increase, competition, and environmental complexities (biotic and abiotic factors) may have a major influence on the efficiency of natural enemies (Pervez & Omkar 2005). Furthermore, continuous production with desirable quality and quantity of natural enemies is necessary for application of them in the field and greenhouse conditions (Hassanpour *et al.* 2021). It can be concluded that both second and third larval instars of *C. carnea* have a good potential to control *A. gossypii* as well as *A. spiraecola* and can be effectively used in IPM programs. Since functional response studies in small arenas like Petri dish may have little resemblance with those measured in natural conditions (O’Neil 1989; Kareiva 1990), further investigations under field conditions are needed to provide more details of the predator-prey interactions.

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