

## Functional Response of *Hippodamia variegata* (Coleoptera: Coccinellidae) to Different Densities of *Aphis gossypii* (Hemiptera: Aphididae) in an Open Patch Design

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### ABSTRACT

Functional response of the female ladybeetle, *Hippodamia variegata* (Goeze) (Col., Coccinellidae) to varying densities (5, 10, 20, 40, 60 and 80) of third instar nymphs of *Aphis gossypii* Glover was assessed in a growth chamber (25°C, 65± 5% RH and a photoperiod of 16L: 8D h), on the black eyed bean, *Vigna unguiculata* (L.), in an open patch design. The logistic regression showed a type II functional response for female ladybeetles with the parameters (using Rogers' model) as follows:  $a = 0.083 \pm 0.011 \text{ h}^{-1}$  and  $T_h = 0.197 \pm 0.040 \text{ h}$ . The mean time for the predator to be settled in a patch was 36.1±9.25, 26.8±5.81, 17.18±4.71, 8.5±2.12, 3.3± 0.88 and 0.8±0.35 minutes at densities of 5, 10, 20, 40, 60 and 80 third instar nymphs of *A. gossypii*, respectively. The settlement time decreased as prey density increased. The maximum theoretical predation ( $T/T_h$ ) for the females was 121.475. The proportion of female *H. variegata* that remained at the end of the experiment and their voracity were dependent on prey density. This may give an indication for the ability of the predator to persevere in the high pest infestations, and thus a high level of control could be expected.

**Keywords:** Cotton aphid, Functional response, *Hippodamia variegata*, Predation, Settlement time.

### INTRODUCTION

The cosmopolitan polyphagous aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae) is widely distributed in tropical, subtropical and temperate regions. This aphid is a pest of cotton, cucurbits, melon and citrus in temperate zones and predominantly attacks vegetables in fields and greenhouses (Leclant and Deguine, 1994). *Hippodamia variegata* (Goeze) is an active aphid predator of plant lice in cereal and oil plants in various countries. This predator has been referred to as the most important predator of aphids on pepper in Bulgaria, on maize in Ukraine, on shrubs in Italy, on grain in India, and on cotton in Turkmenistan (Orr and Obrycki, 1990; Shing and Shing, 1994; El-Hag and Zaitoon,

1996; Obrycki, 1998; Kontodimas and Stathas, 2005; González, 2006). It has been recorded feeding on 19 different aphid species in Turkey (Aslan and Uygun, 2005) and on 12 different aphid species on a variety of crops, weeds, and ornamental plants in Australia (Franzmann, 2002). *H. variegata* is the most abundant coccinellid species in Iran (Radjabi, 1987), and understanding factors that contribute to its success as a predator may be important for enhancing its impact on aphid populations. Because of the increased awareness of the negative side effects of chemical insecticides, using the predators in insect pest management programs has been receiving increased attention for pest control (Atlihan and Bora Kaydan, 2010).

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Functional response is an important behavioral response to reveal different aspects of prey-predator interactions (Jafari and Goldasteh, 2009). The term “functional response” shows the response of individual natural enemy to varying prey density (Solomon, 1949). Statistical methods for estimating the functional response parameters from the experimental data are described in Livdahl and Stiven (1983), Houck and Strauss (1985), Williams and Juliano (1985) and Juliano and Williams (1987). When the number of prey killed is plotted against the number of prey available, a continuum of patterns may emerge from which ecologists delimit three types of functional responses

(Holling 1966; Trexler *et al.*, 1988). Functional response curves may represent linear increase (type I); an increase decelerating to a plateau (type II); and a sigmoid increase (type III).

An investigation on the functional response of the ladybeetle, *H. variegata* at varying density of the mealy plum aphid, *Hyalopterus pruni* Geoffroy showed that the response of each larval stage and adults matched Holling’s type II functional response (Atlihan and Guldal, 2009). The same type of functional response has also been found for all the larval instars and adult males and females of *H. variegata* feeding on varying densities of *A. fabae* (Scolpoli) (Hemiptera: Aphididae) (Farhadi *et al.*, 2010). The functional responses of female and male, third and fourth instar of *Scymnus syriacus* Marseul to varying densities of *A. spiraecola* Patch were also found to be a type II functional response (Emami, 1996). The genus *Scymnus* exhibited different types of functional response from their prey. A type II functional response was determined for *Harmonia axyridis* (Pallas) to its prey, *A. gossypii* at different time intervals (Lee and Kang, 2004).

Recently, Sakaki and Sahragard (2011) found that the kind of patch (open or closed) did not affect the type of functional response (i.e., type II) for female *S. syriacus* to varying densities of *A. gossypii*. The aim of

this study was also to assess the functional response of female *H. variegata* to its prey, *A. gossypii* in an open patch design where the predator had the possibility to leave the experimental arena.

## MATERIALS AND METHODS

### Predator and Prey Stocks

Adults of *H. variegata* and nymphs of *A. gossypii* were collected from *Hibiscus syriacus* L. plants at the College of Agriculture in the University of Guilan (North of Iran). They were reared together on the black eyed bean, *Vigna unguiculata* (L.) infested with *A. gossypii*. The predator was reared for one generation on different nymphs of the cotton aphid, *A. gossypii*, before starting the experiments. The aphid and predator stocks were kept in a growth chamber at  $25\pm 1^{\circ}\text{C}$ ,  $65\pm 5\%$  RH and a photoperiod of 16L: 8Dh.

### Functional Response

To study the effect of open patch design on the type of functional response of *H. variegata*, an individual female of the predator was separately released to varying densities (5, 10, 20, 40, 60 and 80) of third instar *A. gossypii* in a transparent plastic container (15×13×3 cm) on a leaf of black eyed bean. The petiole of the host plant leaf was wrapped with a wet cotton wool. This container was placed in a large plastic container (18×23×5 cm). The bottom of the large one was lined with wet cotton wool in order to prevent the aphids from escaping out, while the female ladybeetles were free and could pass the cotton wool. Individual female lady- beetles (ten-days-old) were starved for 24 hours prior to the experiment to standardize their hunger level. The tested densities of aphids were gently transferred by a fine brush from the host plant to the leaves in experimental arenas at ( $25^{\circ}\text{C}$ ,

65±5% RH and a photoperiod of 16L: 8D h). The mean time required for the female predator to settle in the prey patch was recorded for each treatment of prey density next to its release in the container. In order to allow the predator to leave each experimental arena (container), the cover of the container was removed after settlement of the predator in the patch. Each experiment was replicated ten times. After 24 hours, the number of aphids eaten by an individual female was recorded. The number of female predators that remained in the patch of each treatment at the end of the experiment was also recorded. In order to evaluate the ratio of natural mortality of prey, control treatments were performed in the absence of predators with the above-mentioned prey densities.

### Data Analysis

The type of the functional response was determined by logistic regression analysis [SAS/STAT, CATMOD procedure, version 9.1] using the SAS software (SAS Institute, 2001) for the proportion of prey killed ( $N_e$ ) in relation to initial prey density ( $N_0$ ) (Trexler and Travis, 1993). In the analysis, the regression model was reduced by omitting the cubic term until all remaining coefficients were significant:

$$\frac{N_a}{N_0} = \frac{\exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)}{(1 + \exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3))}$$

Where,  $P_0$ ,  $P_1$ ,  $P_2$ , and  $P_3$  are the intercept of linear, quadratic and cubic coefficients, respectively, estimated using the method of maximum likelihood. If the linear term is not significantly different from 0, a type I functional response was indicated. If the linear parameter  $P_1$  is significantly negative, a type II functional response is evident, whereas a significant positive linear parameter with negative quadratic parameter indicates a density-dependent mortality, i.e., a type III functional response (Juliano, 2001). Since prey were depleted during the

experiment, Rogers' model, which does not assume constant prey density, is appropriate for this experiment (Rogers 1972, Juliano, 2001). Holling's disc equation, in contrast, is based on an assumption of unchanging prey density (Rogers, 1972), and is thus inappropriate for this experiment (Juliano, 2001). Therefore, the random attack equation (Rogers, 1972) was used to estimate handling time ( $T_h$ ) and attack rate ( $a$ ) of the predator as follows:

$$N_a = N_0 \{1 - \exp[-a(T - T_h N_a)]\}$$

Where,  $N_a$  is the number of prey eaten,  $N_0$  is the number of prey offered,  $T$  is the time of exposure,  $a$  is the attack rate and  $T_h$  is the handling time. A nonlinear regression was used (the least square technique with DUD initialization) to estimate predator handling time and attack rate.

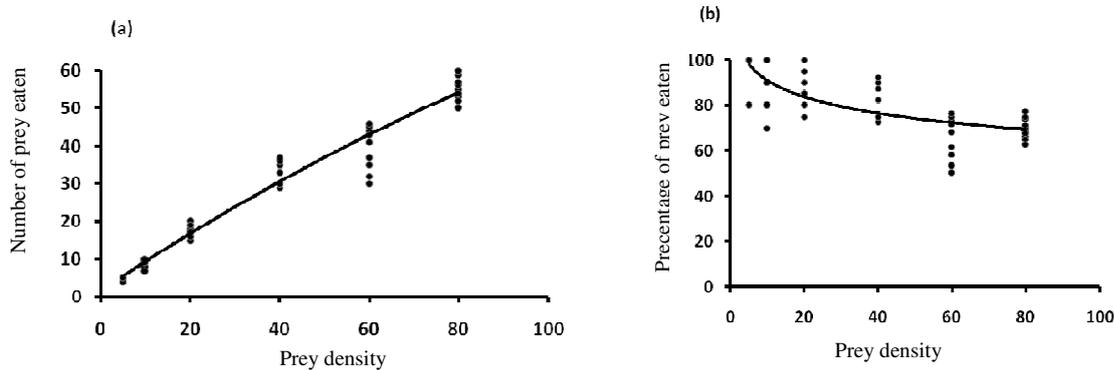
Voracity ( $V_0$ ) of *H. variegata* females in response to the varying densities of *A. gossypii* was determined using the following equation (Soares *et al.*, 2003):

$$V_0 = (A - a_{24})ra_{24}$$

Where,  $V_0$  is the quantification of voracity (the number of prey eaten),  $A$  is the number of prey available,  $a_{24}$  is the number of prey alive after 24 hours and  $ra_{24}$  is the ratio of prey alive after 24 hours exposure time from the initial number of aphids.

### RESULTS

The number of prey eaten increased with increasing the prey density (Figure 1-a). Parameter estimates for logistic regressions of proportion of prey killed ( $N_a/N_0$ ) against number of prey offered ( $N_0$ ) for the female *H. variegata* for 24 hours are presented in Table 1. The logistic regression for female predator had a significant linear parameter (Table 1) and the proportion of prey consumed by female *H. variegata* declined with increasing prey density (Figure 1-b). This suggests that female predator showed a type II functional response. The coefficient



**Figure 1.** Functional responses (a) data fitted to “random predator” equation (Rogers, 1972) and percentage of predation (b) of *Hippodamia variegata* female to varying densities of *Aphis gossypii* in an open batch design.

of attack rates (a) and handling time ( $T_h$ ) (estimated by Rogers’ random attack equation) are presented in Table 2.

Maximum theoretical predation rate ( $T/T_h$ ) estimated for the female *H. variegata* was 121.476 third instar of *A. gossypii*. The Rogers’ model fitted well to the data ( $R^2=0.987$ ). Mean time required for the female predator to settle in a patch was indirectly dependent on the prey density in each experimental arena (Figure 2). The proportion of predators remained in each patch after 24 hours, was directly density dependent ( $R^2=0.867$ ) (Figure 3).

The Voracity (number of prey eaten) of adult females increased significantly with

prey density ( $R^2=0.977$ ), reaching the maximum value when 80 prey were provided (Figure 4).

### DISCUSSION

The functional response of a natural enemy offers a good conceptual framework to understand the action of agents in inundative releases (Waage and Greathead, 1988). Many studies have been devoted to the foraging behavior of insect predators. For example, Dixon (1959) studied the searching behavior of *Adalia decempunctata* (L.), Nakamuta (1982) investigated the searching behavior of *C. septempunctata*

**Table 1.** Maximum likelihood estimates from logistic regression of proportion of prey eaten as a function of initial prey densities by female of *Hippodamia variegata*.

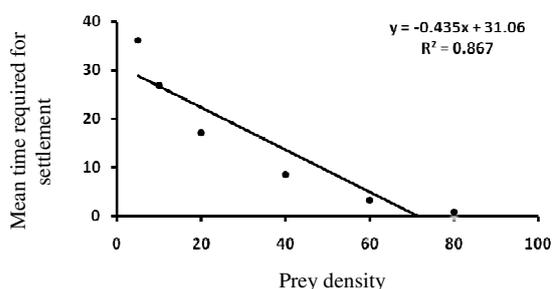
Parameters	Estimate	SE	$X^2$	$P$
Constant	3.422	0.347	96.96	0.0001
Linear	-0.075	0.013	31.28	0.0001
Quadratic	0.0005	0.0001	18.69	0.0001

SE: standard error,  $X^2$ : Chi squared,  $P$ : probability level

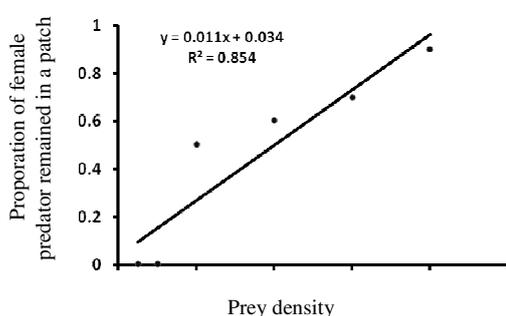
**Table 2.** Coefficient of attack rate (a) ( $0.083\ h^{-1}$ ) and handling time ( $T_h$ ) (0.197 h) (estimated by Rogers’ random attack equation) of female of *Hippodamia variegata* fed on *Aphis gossypii*.

Parameter	Estimate	SE	95% CI		$R^2$
			Lower	Upper	
$a$	0.083	0.011	0.061	0.105	0.987
$T_h$	0.197	0.040	0.115	0.279	

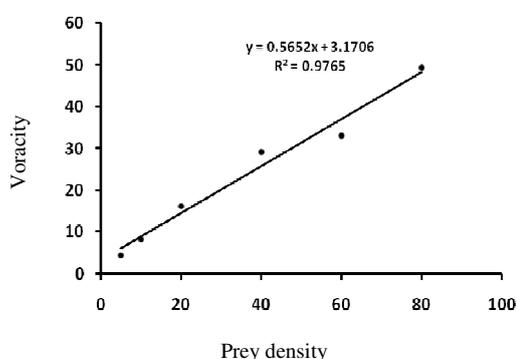
CI: Confidence Interval,  $R^2$ : R squared



**Figure 2.** Mean time (in minutes) required for the female *Hippodamia variegata* to settle in a patch.



**Figure 3.** Proportion of female *Hippodamia variegata* remained in open patches after 24 hours



**Figure 4.** Voracity of female *Hippodamia variegata* on varying density of *Aphis gossypii*.

and Ettifouri and Ferran (1993) studied the effect of larval diet on the searching behavior of *Harmonia axyridis* Pallas.

In this study the declining proportion of prey consumption with the increasing prey density indicated that the functional response data were described well by a Type II asymptotic curve. The negative value

obtained for the linear parameter ( $P_1 < 0$ ) was a good indicator of a Type II response, as well as inverse density dependence relationship between the percentage of prey consumed and the initial prey density for *H. variegata*. This type of functional response has also been found for different life stages of *H. variegata* preying on cotton aphids examined either in two different spatial patterns of cucumber host plants or two leave cucumber seedlings, respectively (Madadi *et al.*, 2011; Mohajeri Parizi *et al.*, 2010). Type II functional response is the most common in insects, as reported for several ladybeetles preying on distinct aphid species, such as *Aphidecta oblitterata* (L.) and *A. bipunctata* (L.) preying on *Elatobium abietinum* (Walker) (Timms *et al.*, 2008), larvae and adults of *Propylea quatuordecimpunctata* (L.) preying on *Diuraphis noxia* (Mordvilko) (Messina and Hanks, 1998), *C. undecimpunctata* (L.) preying on *A. fabae* and *Aleyrodes proletella* (Moura *et al.*, 2006), male adults of *Cheilomenes sulfurea* preying on *A. fabae* (Hodek *et al.*, 1984), adult females of *Cheilomenes sexmaxulata*, *Propylea dissecta*, and *C. transversalis* preying on *Aphis craccivora* (Koch) or *Myzus persicae* (Sulzer) (Pervez and Omkar, 2005), and adults of *Curinus coeruleus* (Mulsant) preying on *Heteropsylla cubana* Crawford (Silva *et al.*, 1992).

Mean time required for the female to settle in a patch was indirectly dependent on the density of *A. gossypii*, as it decreased with the increase of prey density. The result obtained here in an open patch design was in accordance with the studies on functional response of female *H. variegata* in a closed patch (Mohajeri Parizi *et al.*, 2010; Davoodi Dehkordi *et al.*, 2012). Therefore, the results showed that the type of experimental arena did not affect the behavior of the predator. This was also shown by Sakaki and Sahragard (2011) on *S. syriacus* preying on *A. gossypii* in an open patch design. This finding also lowers the concerns expressed by van Alphen and Jervis (1996) on closed patch functional response experiments.



This method proved that although the predator was allowed to leave the patch, the number of female ladybeetles remained in each patch was proportional to prey density.

Results showed that *H. variegata* was able to eat a high number of individuals of *A. gossypii* at the maximum voracity level (i.e., 80 aphids). The female *H. variegata* voracity was directly dependent on prey densities which was a good expression of the proportion of female predators remained in open patches. They may have left patches at lower prey densities because they were not satiated. Similar results have been found by Madadi *et al.* (2011) and much higher voracity levels have been reported for this predator despite exhibiting a type II functional response, since the voracity of all tested stages of *H. variegata* and especially of the fourth instars towards both cotton aphid and pea aphid species hold good promises at least for use in biological control of these aphid species where the release of the late instar larvae or adults would be an obvious choice. The same voracity levels have also been reported for other coccinellids, such as *C. transversalis* (Omkar and James, 2004), *H. convergens*, *C. septempunctata* (Lucas *et al.*, 1997; Omkar and Srivastava, 2003; Katsarou *et al.*, 2005) and *C. undecimpunctata*, preying on *M. persicae* (Cabral *et al.*, 2009).

It can be generally concluded that this behavior of *H. variegata* may present an indication for the ability of this predator to persevere in the high *A. gossypii* infestations, and thus a high level of control could be expected.

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واکنش تابعی کفشدوزک (*Hippodamia variegata* (Col.: Coccinellidae) نسبت به تراکم‌های مختلف شته جالیز (*Aphis gossypii*) در شرایط آزمایشگاه به روش open patch

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چکیده

واکنش تابعی کفشدوزک ماده (*Hippodamia variegata* (Col. Coccinellidae) نسبت به تراکم‌های مختلف (۵، ۱۰، ۲۰، ۴۰، ۶۰ و ۸۰) پوره‌های سن سوم شته جالیز (*Aphis gossypii* (Hem. Aphididae) در یک اتاقک رشد (دمای ۲۵ درجه سانتی‌گراد و رطوبت نسبی  $5 \pm 65$  درصد و دوره نوری ۱۶ ساعت روشنایی و ۸ ساعت تاریکی) به روش open patch روی لویا چشم بلبلی مورد ارزیابی قرار گرفت. رگرسیون logistic واکنش تابعی نوع دوم را برای کفشدوزک ماده با پارامترهای (با استفاده از مدل راجرز) به شرح زیر نشان داد: قدرت جستجو ( $a$ ) و زمان دستیابی ( $T_h$ ) برای کفشدوزک ماده به ترتیب  $h^{-1}$   $0.083 \pm 0.011$  و  $0.197 \pm 0.040$  h برآورد شدند. متوسط زمان برای استقرار شکارگر در یک patch در تراکم‌های مختلف (۵، ۱۰، ۲۰، ۴۰، ۶۰ و ۸۰) طعمه به ترتیب  $9/25 \pm 36/1$ ،  $26/8 \pm 5/81$ ،  $17/18 \pm 4/71$ ،  $8/5 \pm 2/12$ ،  $3/3 \pm 0/88$  و  $0/8 \pm 0/35$  دقیقه بود. زمان استقرار با افزایش تراکم طعمه کاهش پیدا کرد. حداکثر نرخ شکارگری ( $T/T_h$ ) برای کفشدوزک ماده  $121/475$  عدد از پوره‌های سن سوم *A. gossypii* بود. نسبتی از ماده‌های *H. variegata* که در پایان آزمایش در صحنه آزمایش باقی ماندند و پرخوری آن‌ها به تراکم طعمه وابسته بود. این نتایج ممکن است شاخصی از توانایی حضور شکارگر در آلودگی‌های بالای آفت باشد و بنابراین می‌توان سطح بالایی از کنترل را انتظار داشت.