PREDATION POTENTIAL OF A BIOCONTROL AGENT, *HIPPODAMIA VARIEGATA* AGAINST THE APHID, *APHIS GOSSYPII*

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ABSTRACT

We investigated the predation potential of a biocontrol agent, *Hippodamia variegata* (Goeze) (Insecta: Coleoptera: Coccinellidae) in terms of functional response against the increasing density of an aphid-pest, *Aphis gossypii* Glover (Hemiptera: Aphididae). Three predatory stages, *viz*. fourth instar, adult female and adult male of *H. variegata* were tested against the increasing prey density. The prey consumption by the three predatory stages increases curvilinearly with an increase in prey density, which depicts Type II functional response. The fourth instar has the maximum coefficient of attack rate (0.000752 ± 0.000066) with least handling time (0.1766 ± 0.00379 hours = 10.596 min), which shows its potency to be used as an efficient biocontrol agent. However, the adult male has a minimum value of attack rate (0.000476 ± 0.000045) with highest handling time (0.2117 ± 0.00563 hours = 12.702 min), which shows that it has less potential to attack its prey and takes more time to handle, consume and digest its prey. We conclude that fourth instars *H. variegata* could be used as biocontrol agents against the overgrowing populations of aphid, *A. gossypii*.

Keywords: Functional response, Coccinellidae, *Hippodamia variegata,* prey, predator,

No: of Figures:1 **No: of Tables: 2** **No:of References:19**

INTRODUCTION

The analytical approach to determine the effect of prey-density on predation potential of the predator, particularly, the ladybird is referred to as functional response (Holling, 1959; Omkar and Pervez, 2004a; 2011). It is considered is a crucial factor regulating the population dynamics of predator–prey systems. The functional response curves may represent an increasing linear relationship (Type-I), a decelerating curve (Type-II), or a sigmoidal relationship (Type-III). That is, they result in a constant (I), decreasing (II) and increasing (III) rate of prey killing and yield density-dependent, negatively density dependent and positively density-dependent prey mortality, respectively. The functional response curves can be differentiated by evaluating the parameters, viz. coefficient of attack rate and handling time (time spent by the predator in attacking, killing, subduing and digesting the prey) (Pervez and Omkar, 2003). The coefficient of attack rate estimates the steepness of the increase in predation with increasing prey density and handling time helps estimate the satiation threshold.

Predaceous ladybirds (Coleoptera: Coccinellidae) are economically important insects, as they can be manipulated against numerous phytophagous insect pests, *viz*. aphids, diaspids, psyllids, whiteflies along with other soft-bodied insect and acarine pests (Dixon, 2000; Hodek *et al.,* 2012; Omkar and Pervez, 2016). *Hippodamia variegata* (Goeze) is a predaceous ladybird beetle that is distributed in aphid prevalent agro-ecosystems of North India (Omkar and Pervez, 2004b). It feeds on a variety of insect pests, *viz*. psyllids, whiteflies, mealybugs and at least twelve species of aphids infesting numerous crops (Franzmann, 2002; Kontodimas and Stathas, 2005). It is a Palearctic ladybird having a cosmopolitan distribution and can easily be cultured in the laboratory due to its immense mating and reproduction potential (Pervez and Maurice, 2011; Pervez and Singh, 2013). Madadi *et al*. (2011) stated that *H. variegata* has an immense potential to be used against cotton aphid, *Aphis gossypii* Glover and pea aphid, *Acrythosiphon pisum* Harris (Hemiptera: Aphididae). Hence, the experiments were designed to find out the biocontrol potential in terms of functional response of adult male, female and fourth instar larva of *H. variegata* against aphid, *A. gossypii*. We also aimed to quantify the attack rate and the handling time estimates of the above predatory stages of *H. variegata.*

MATERIALS AND METHODS

Stock Culture

Adults of *H. variegata* were collected from the agricultural fields of Kashipur, Uttarakhand, India (30.2937°N, 79.5603°E) and brought to the laboratory. Thereafter, we paired them in Petri dishes (2.0 height x 9.0 cm diameter) containing *ad libitum* aphids, *A. gossypii* infested on the pieces of leaves of *Lagenaria vulgaris* (n=10) and kept in the Environmental Test Chamber (*REMI Instruments*, India) at controlled conditions (27±1°C, 65±5% R.H and 12L: 12D). The F_1 eggs laid by adult female ladybirds were reared from egg hatch to adult emergence on above aphid to obtain fourth instar larvae and the emerging F_2 adults to be used in the experiments.

Experimental Design

We kept a 12-hour starved fourth instar larva in a glass beaker (height 11.0cm and diameter 8.5cm) containing 25 aphids, *A. gossypii* infested on pieces of leaves of *L. vulgaris*. Thereafter, the beaker was covered with a muslin cloth, fastened with a rubber band. This beaker was transferred to an Environmental Test Chamber (*REMI Instruments*, India) maintained at controlled abiotic conditions (27±1°C, 65±5% R.H and 12L: 12D). We removed the beaker from this chamber after 24 hours and counted the unconsumed aphids to quantify the number of aphids consumed. The partially consumed body pieces of dead aphids were considered as consumed (n=10). The experiment was repeated at other aphid densities, *viz*. 50, 100, 150, 200, 300, and 400. Thereafter we repeated the entire experiment using 10-day-old adult male and adult female, *H. variegata* as predators. Ten replicates of the entire experiment were designed (n=10). We transformed the number of aphids consumed to logarithms. Regression analysis was done between the values of log aphid consumption and the log aphid density for the three predatory stages using statistical software, SAS (Version 9.0) on the personal computer.

Data Analysis

We determined the shape of functional response using logistic regression of the proportion of prey eaten (N_a / N_o) as a function of prey offered (N_o) (Juliano, 2001; Pervez and Omkar, 2005). We fitted the data to a polynomial function (Equation 1) which describes the relationship between the proportion of aphid eaten (N_e/N_o) and aphid presented (N_o) using CATMOD Procedure of statistical software SAS (Version 9.0). We fitted the data to a polynomial function that describes the relationship between N_a / N_o and N_o:

$$\frac{N_a}{N_o} = \frac{\exp(P_o + P_1N_o + P_2N_o^2 + P_3N_o^3)}{1 + \exp(P_o + P_1N_o + P_2N_o^2 + P_3N_o^3)} \quad \textbf{[1]}$$

Where, P_o, P_1, P_2 and P_3 are the intercept, linear, quadratic and cubic coefficients, respectively that are quantified using maximum likelihood method. If $P_1 > 0$ and $P_2 < 0$, the proportion of prey consumed is positively density dependent, hence Type III response. If P_1 in negative value shows declination of prey proportion, hence a Type II response. After the determination of the shape, the functional response parameters were estimated using Roger's random predator equation (Rogers, 1972). A Type II response follows Equation (2):

$$N_a = N_o \{1 - \exp [(a [T_hN_a - T]\} \quad \text{-------} \quad \textbf{[2]}$$

Where, N_a and N_o are as above, a = coefficient of attack rate, T = duration of exposure, and T_h = handling time. Data were fitted to equation (2) using the procedure NLIN on SAS (Version 9.0). The attack rate and prey handling time were subjected to Duncan's Multiple Range Test following Atlihan et al. (2010) to determine differences between combinations of predators. All data were tested for homogeneity of variance prior to analysis using Bartlett's Test on SAS (Version 9.0).

RESULTS AND DISCUSSION

Aphid consumption by the fourth instar of *H. variegata* increased from 21.10±0.25 to 130.50±2.33 with an increase in prey density from 25 to 400 individuals of *A. gossypii* (Figure-1). Similarly, the prey consumption by adult male and female ladybirds also increased from 16.00±0.86 to 110.4.30±1.35 and 19.00±0.85 to 114.90±1.52 individuals of *A. gossypii* with an increase in prey density from 25 to 400 aphids, respectively (Figure-1). Our results show that the fourth instar was the most voracious predatory stage followed adult female and male ladybirds. The regression equations for the fourth instar, adult male and adult female were: $\log Y=0.314 + 0.721\log X$; ($r=0.95$; $P<0.001$), $\log Y= 0.402 + 0.564\log X$; ($r=0.96$; $P<0.001$) and $\log Y=0.465 + 0.645\log X$; ($r=0.96$; $P<0.001$), respectively, which indicated positive correlation between the log prey density and log prey consumption. The results revealed that aphid consumption by predatory stages increased with an increase in aphid density. The increased prey consumption with increase in prey density may possibly be ascribed to the simultaneous occurrence of many phenomena, such as rate of searching, duration of prey exposure, handling time and hunger level of the predator.

The functional response exhibited by all the three predatory stages of *H. variegata* exhibited Type II functional response, which was evident from the negative values of linear parameters in the Table-1. The Chi-square values of intercept, linear, quadratic and cubic parameters, where found to be statistically significant. This further confirms the shape of the response to be Type II, *i.e.* a negative density-dependent response of the three predatory stages tested on the prey mortality. Prey encounters were more frequent at higher prey densities and predator continues to feed till satiation or even beyond. An asymptote or a plateau of the prey consumption is found at the higher aphid densities of aphids, which suggests satiation. The mutilated remnants of prey were more at higher prey densities, which accentuate that predator after attaining satiation, did not completely devour the prey and ate only the soft portion leaving the hard body surfaces, such as the appendages. The predator, thus, appeared to extract progressively smaller proportions of the contents from each aphid killed at increased prey density. This aspect of predator-prey interaction seems to be highly supportive of a sustainable biocontrol programme, as predator tends to increase prey mortality even after attaining satiation. The disturbance by another prey possibly aggravates a hungry predator during feeding, which resulted in the killing of more prey individuals than normal. Thus, the greater interferences at higher prey density may partly result in increased prey mortality. Hodek and Evans (2012) have also opined that hungry ladybirds completely devour the first few prey individuals. They encounter and utilize subsequent prey individuals with a gradually reduced predatory drive.

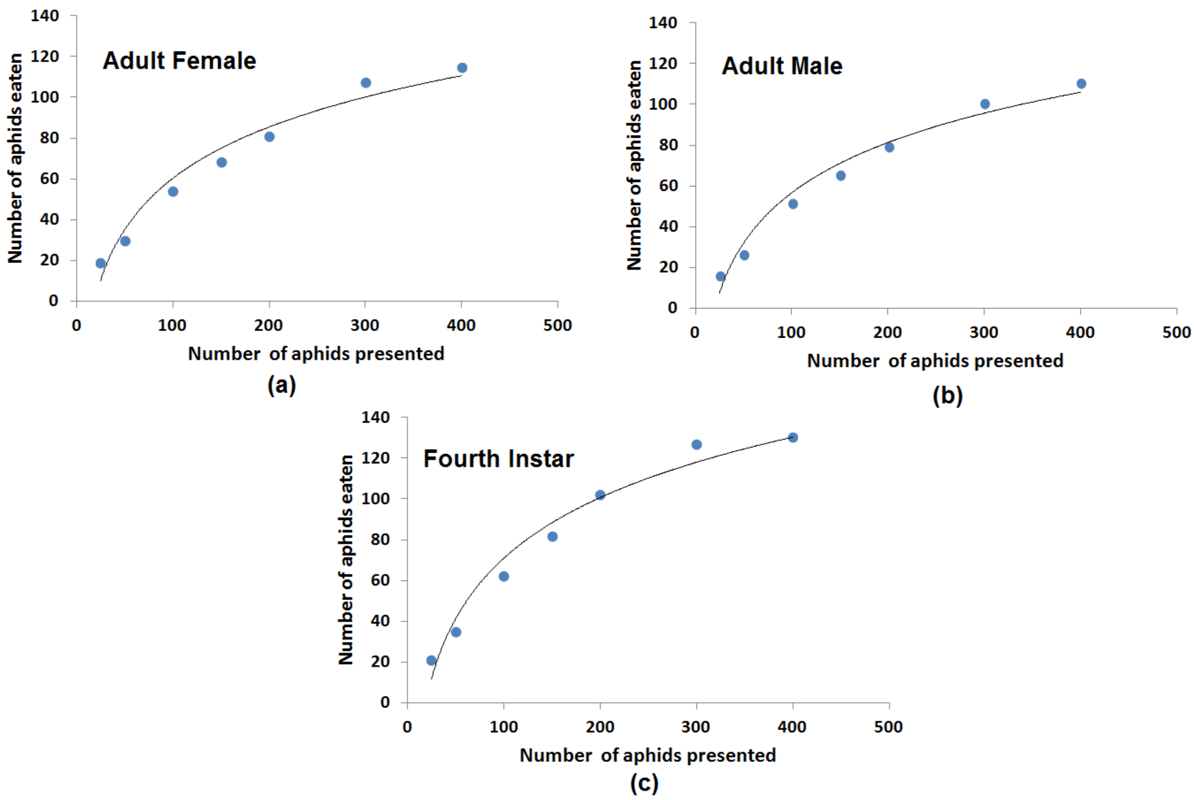

Figure-1: Type II Functional response showing effects of increasing density of aphid, *A. gossypii* on aphid consumption by (a) Adult Female (b) Adult male and (c) Fourth Instar of a ladybird, *H. variegata*.

Table 1. Maximum likelihood estimates from logistic regression (PROC CATMOD) of proportion of A. gossypii consumed as a function of aphid densities by predaceous ladybird, *H. variegata*.

Predator	Parameter	Estimate	Std. Error	Chi Square value	P-value
Fourth Instar	Intercept (P_o)	1.7365	0.1488	136.10	P<0.0001
	Linear (P_1)	-0.0169	0.00239	50.03	P<0.0001
	Quadratic (P_2)	0.000055	0.000011	23.45	P<0.0001
	Cubic (P_3)	-7.08E-8	1.612E-8	19.28	P<0.0001
Adult Female	Intercept (P_o)	1.2808	0.1370	87.45	P<0.0001
	Linear (P_1)	-0.0162	0.00227	51.07	P<0.0001
	Quadratic (P_2)	0.000054	0.000011	23.94	P<0.0001
	Cubic (P_3)	-6.78E-8	1.578E-8	18.44	P<0.0001
Adult Male	Intercept (P_o)	0.3601	0.1302	12.66	P<0.0001
	Linear (P_1)	-0.00247	0.00220	13.55	P<0.0001
	Quadratic (P_2)	-2.49E-6	0.000011	8.56	P<0.001
	Cubic (P_3)	6.72E-10	1.553E-8	6.45	P<0.001

Table 2. Functional response parameter estimate values (SE) of attack rate and handling time (T_h) at 95% confidence limit (CL) for fourth instar, adult male and female ladybird, *H. variegata* combinations obtained by Least Square Method (PROC NLIN).

Predator	Attack rate (a)	Handling Time (T_h)
Fourth Instar	0.000752 ± 0.000066[a]	0.1766 ± 0.00379[a] hour = 10.596 min
Adult Female	0.000523 ± 0.000053[b]	0.2033 ± 0.00569[b] hour = 12.198 min
Adult Male	0.000476 ± 0.000045[c]	0.2117 ± 0.00563[c] hour = 12.702 min

All parameters significant at P<0.0001; df = 2, 48.
Values followed by a different superscript letters within columns denote significant difference

The fourth instar was the most voracious predatory stage followed by the adult female and male ladybirds. The fourth instar has the maximum coefficient of attack rate (0.000752 ± 0.000066) with least handling time (0.1766 ± 0.00379 hours = 10.596 min), which shows its potency to be used as an efficient biocontrol agent (Table-2). The stress of high-energy requirement for the completion of its development *via* attaining critical weight for pupation is a major factor, which tends to make it more voracious than adults. Fourth instar is more efficient in detecting and consuming larger proportions of prey individuals with a relatively better digestive ability than adults. The adult male has a minimum value of attack rate (0.000476 ± 0.000045) with highest handling time (0.2117 ± 0.00563 hours = 12.702 min). The male ladybirds consumed lesser prey, which shows that it has less potential to attack its prey and takes more time to handle, consume and digest its prey. Amongst adults, the female was more voracious than male ladybird, owing to her relatively larger size and high nutrient requirements for reproduction, *i.e*. egg production and oviposition. The relatively smaller size and male's interest in the search for mate could also be the possible reasons for relatively lesser aphid consumption than female ladybird.

We conclude that: (i) prey consumption increases with the increase in prey density, (ii) fourth instar stage was the most voracious in terms of aphid consumption followed by adult female and adult male, (iii) predators exhibited Type II functional response, and (iv) the coefficient of attack rate was maximum with least handling time when fourth instar was foraging. Thus, we can suggest that manipulations of fourth instars of *H. variegata* will give the best results in the biocontrol of *A. gossypii*.

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