Journal of Entomology and Zoology Studies 2019; 7(6): 523-527

Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(6): 523-527

Received: 24-09-2019
Accepted: 28-10-2019

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Functional response of *Cheilomenes sexmaculata* (Fab.) to increasing population densities of cotton aphid, *Aphis gossypii* (Glover) at different time intervals

Arya Krishna and AV Kolhe

Abstract
Laboratory investigations were conducted during the year 2017-2018 to determine the functional response of adult ladybird beetle *Cheilomenes sexmaculata* to increasing prey densities of *Aphis gossypii* on cotton at different time intervals of 12 hrs and 24 hrs after exposure. The results revealed a density dependent, Type II functional response of *C. sexmaculata* to *A. gossypii* at both the time periods. Prey consumption increased curvilinearly with an increase in prey density. Type II functional response was determined by a logistic regression model. The parameters estimated from the random predator equation (The attack rate 'a' and handling time 'T_h') suggest that the predator was more efficient as a biocontrol agent during the first 12 hrs. of exposure to the prey than after that time period.

Keywords: *Aphis gossypii*, *Cheilomenes sexmaculata*, aphididae, coccinellidae, functional response

1. Introduction

Cotton aphid (*Aphis gossypii*) is an important sucking pest of cotton whose population is increasing in last ten years. The high aphid populations may stunt and retard cotton seedling growth and development as a result of its feeding. Late season populations can cause decreased fibre quality as the result of stickiness and the development of sooty mould associated with honeydew dropped onto cotton fibres (Isely, 1946) [1]. Coccinellids, commonly known as ladybird beetles, form an important group of biocontrol agents among insect predators due to their ability to feed on a variety of prey, e.g., aphids, mealy bugs, scale insects, whiteflies, thrips, and many more (Omkar and Pervez, 2004) [2]. The ladybeetle *Cheilomenes sexmaculata* is a widely distributed aphidophagous ladybeetle in India.

There is now an increased awareness of the negative side effects of chemical insecticides on the environment. For this reason, biological control using natural enemies to suppress pests is currently considered an important component in integrated, ecologically sound crop management systems (Hoed and Honek, 1996) [3]. Ecologists have focused on the use of functional and numerical response as a means of assessing the impact of a natural enemy on a prey population.

The number of prey that an individual predator kills (or the number of hosts a parasitoid attacks) is a function of prey density and is known as functional response (Holling, 1966) [4]. In general, the number of prey killed in a fixed time approaches an asymptote as prey density increases (Holling, 1966) [4].

Functional response has been categorized into Type I, Type II, and Type III (Holling, 1959) [5], Type IV (Bressendorff and Toft, 2011) [6] and Type V (Sabelis, 1992) [7]. Most of the studies reveal Holling's Type II functional response in coccinellids with only a few reporting Type III responses (Messina and Hanks, 1998) [8]. Potential biological control agents are often first tested under laboratory conditions to evaluate their potential for success (Kalyebi *et al*. 2005) [9]. The specificity of the biological control agents (i.e., ability to recognize and attack a particular species of host) and their functional response type are important considerations in their use. Holling, 1959 [5] in his seminal work, defined three types of functional response to a range of host (or prey) densities. The functional response type is regarded as crucial to understanding host-parasitoid dynamics (Walde and Murdoch, 1988) [10]. The type III response has the potential to stabilize host-parasitoid dynamics, although this stabilizing ability is relatively weak (Hassell and Comins, 1978) [11]. In classical biological control programs, biocontrol agents that exhibit a type III response are more likely to lead to success in controlling pests in the field than those with a type II response. This is most likely because of host-density-dependent regulation that enhances long-term persistence of both the host and parasitoid populations (Fernandez-Arhex and Corley, 2003) [12].

Thus, the objective of this study was to determine the functional response of adult ladybird beetle, *Cheilomenes sexmaculata* to increasing prey densities of *Aphis gossypii* on cotton at different time intervals of 12 hrs and 24 hrs after exposure and estimation of the parameters of the type of response showed by it.

2. Materials and Methods

2.1 Experimental protocol

The culture of predator, equal sized adults of ladybird beetles, *Cheilomenes sexmaculata*, and prey, cotton leaves infested with cotton aphid *i.e.*, *Aphis gossypii* were collected from the fields of okra and cotton. Other materials required for conducting the experiment *viz.*, equal sized beakers (250 ml), BOD incubator, muslin cloth, plastic trays, magnifying lens, and brush were supplied by the Department of Agricultural Entomology, PGI, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. Adult stages of the predators were collected and kept for 12-hour starvation in the BOD incubator under controlled abiotic conditions *i.e.*, 27±2ºC, 65±5% RH and 14:10 L: D photoperiod. Thereafter, they were kept separately in beakers and provided with *Aphis gossypii* together with host plant leaves at different prey densities, *i.e.*, 10, 20, 40, 80, 160, and 320. Beakers were covered with muslin cloth and kept in the BOD incubator. After 12 and 24 hours the predators were removed from the beakers, and the number of unconsumed aphids were counted. The experiment was replicated six times for each predator at different prey densities, however consumed aphids were not replaced during the experiment.

2.2 Data analysis

Functional response analysis involves determination of the type of functional response and estimation of the parameters of functional response curves (Juliano, 2001) [13]. Since it is difficult to discriminate between types II and III functional responses, therefore, prior to fitting data to a special Rogers Equations, a cubic logistic regression between proportion of host eaten (Ne/No) and host (No) density was performed to determine the shape of functional response (e.g., type II or III) (Juliano, 2001) [13]. The data were fitted to the polynomial function using statistical software SAS (Version 9.4). The equation used was:

$$\frac{N_e}{N_o} = \frac{\exp(P_o + P_1 N_o + P_2 N_o^2 + P_3 N_o^3)}{1 + \exp(P_o + P_1 N_o + P_2 N_o^2 + P_3 N_o^3)}$$

Where, N_e is the prey consumed, N_o is the initial prey density, P_o (intercept), P_1 (linear), P_2 (quadratic), and P_3 (cubic) are the parameters to be estimated. CATMOD procedure was used to estimate these parameters. The data were fitted to the equation below. Positive linear (P_1>0) and negative quadratic (P_2 <0). parameters indicate Type III functional response, while negative linear parameter values indicate Type II functional response (Juliano, 2001) [13]. Thereafter, nonlinear least square regression procedure was used on SAS (Version 9.4) to estimate the Roger's random predator equation (1972) [14] for Type II functional response given below.

$$Ne = No\,\{1 - \exp[a(T_h\,Ne - T)]\}$$

Where, Ne is the number of prey consumed, No is initial prey density, a is the attack constant (or instantaneous search rate) T_h is the handling time per prey, and T is the total time of exposure. This equation overcomes the problem of prey depletion. Statistically different parameter estimates were separated using asymptotic 95% confidence intervals (Juliano, 2001) [13]. The prey consumption at 12 and 24 hrs were analysed in CRD.

Type III functional response follows the equation

$$Ne = No\,\{1- \exp[(d + b\,No)(Th\,Ne - T) / (1+ c\,No)]\}$$

Where b, c, and d are constants.

3. Results

3.1 Mean prey consumption by the predator for different time periods

The mean prey consumption of ladybird beetle *C. sexmaculata* was recorded at varying aphid densities for different time intervals of 12 and 24 hours after exposure and presented in Tables 1 to 2. The prey consumption gradually increased at corresponding prey densities of 10, 20, 40, 80,160, 320 ranging from 8.33 to 32.17, and 9.66 to 41.67/ predator respectively, when recorded at 12 to 24 hrs after exposure. However, the proportion of prey consumed decreased as the density of prey increased irrespective of exposure at different time interval. At 12 hrs after exposure, the highest consumption of 26.83 aphids/ predator was recorded at 320 prey density and it was significantly lower in rest of the prey densities, the data indicate a decrease in prey consumed as prey density decreases (Table 1). At 24 hrs after exposure, the highest consumption of 41.67 aphids/ predator was recorded at 320 prey density and it was significantly lower in rest of the prey densities, the data indicate a decrease in prey consumed as prey density decreases (Table 2).

Table 1: Mean prey consumption by ladybird beetle *C. sexmaculata* on different prey densities of *Aphis gossypii* after 12 hours of exposure

Sr. No	Prey density	Prey consumed (N_e)	Proportion of prey consumed
1	10	6.33 (2. 47)*f	0.633
2	20	9.83 (3. 13)e	0.491
3	40	13.83 (3. 72)d	0.345
4	80	17.17(4. 14)c	0.214
5	160	21.17 (4. 60)b	0.132
6	320	26.83 (5. 18)a	0.083
	SE (m±)	0.06	
	C.D. (P=0.05)	0.17	
	C.V. at 5%	3.76	

* Values in parenthesis are square root transformation values Letters followed by mean values are significantly different from each other by CRD. Values are average of six replications.

Table 2: Mean prey consumption by ladybird beetle *C. sexmaculata* on different prey densities of *Aphis gossypii* after 24 hours of exposure

Sr. No	Prey density	Prey consumed (N_e)	Proportion of prey consumed
1	10	9.83 (3.14)*f	0.983
2	20	14.83 (3.85)e	0.741
3	40	19.33 (4.39)d	0.483
4	80	23.17 (4.81)c	0.289
5	160	32.17 (5.67)b	0.201
6	320	41.67 (6.45)a	0.130
	SE (m±)	0.08	
	C.D. (P=0.05)	0.23	
	C.V. at 5%	4.16	

* Values in parenthesis are square root transformation values Letters followed by mean values are significantly different from each other by CRD. Values are average of six replications.

3.2 Functional response

The prey consumption on different prey densities after 12 hours and cumulative 24 hours after exposure were used for functional response analysis of the predator.

3.2.1 After 12 hours of exposure to its prey cotton aphid

Parameter estimate for logistic regressions of proportion of prey killed (N_e/N_o) against number of prey offered for adults of *C. sexmaculata* for 12 hours are presented in Table 3. The logistic regression for the adults of *C. sexmaculata* had a significant linear parameter $P_1 < 0$ (-0.0398). The negative value of the linear parameter ($P_1<0$) indicated type II response of ladybird beetles (Fig. 1). The type II response is also confirmed as the proportion of prey consumed by the adults of *C. sexmaculata* declined with increasing prey density (Fig. 2). The coefficient of attack rate (a) and handling time (T_h) (estimated by Roger's random predator equation) of adults of *C. sexmaculata* is presented in Table 4. showing handling time and attack rate of 0.3654 and 0.0391 respectively. The data indicated that there was a decline in the consumption rate at higher densities, which resulted in an increase in the functional response curves of *C. sexmaculata*. The maximum predation rate (T/T_h) was found to be 32.84 at 12 hours of exposure period. The value of coefficient of determination (R^2) = 0.975 and standard errors of the estimated parameter indicated that Roger's random predator equation adequately described the functional response of *C. sexmaculata*.

Table 3: Maximum likelihood estimates from logistic regression of proportion of prey (*Aphis gossypii*) eaten as a function of initial prey density by adults of *C. sexmaculata* (n=6) at T= 12 hours by CATMOD procedure

Stage	Parameter	Estimate	SE	X^2	P
Adult	Intercept (P_0)	0.7305	0.2256	10.48	0.0012
	Linear (P_1)	-0.0398	0.0074	29.15	<.0001
	Quadratic (P_2)	0.0002	0.00006	12.06	0.0005
	Cubic P_3	-3.32E-7	1.156E-7	8.24	0.0041

Table 4: Functional response parameter estimate values of attack rate (a) and handling time (T_h) at 95% confidence limit (CL) for *C. sexmaculata* fed on *Aphis gossypii* at T = 12 hour obtained by least square method (NLIN)

Model	Stage	Parameter	Estimate	SE	95% CL		T/T_h
					Upper	Lower	
Roger's random predator equation	Adult	A	0.0391	0.00340	0.0460	0.0322	32.84
		T_h (hrs)	0.3654	0.00999	0.3857	0.3451	

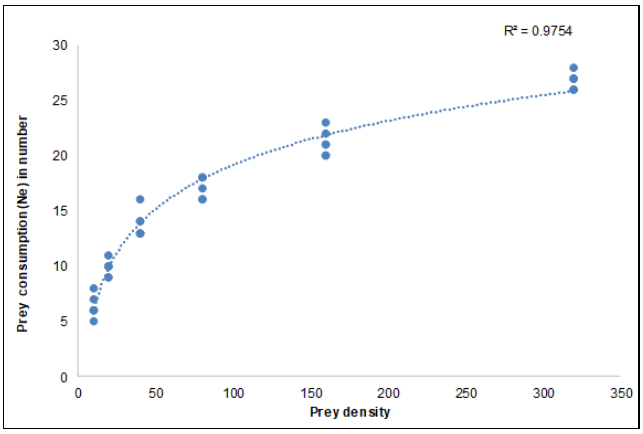

Fig 1: Prey consumption by *C. sexmaculata* adults at various prey densities of *Aphis gossypii* (n= 6 and T= 12 hours)

3.2.2 After 24 hours of exposure to cotton aphid

Parameter estimate for logistic regressions of proportion of prey killed (N_e/N_o) against number of prey offered for adults of *C. sexmaculata* for 24 hours are presented in Table 5. The logistic regression for the adults of *C. sexmaculata* had a significant linear parameter $P_1<0$ (-0.0723). The negative value of the linear parameter ($P_1<0$) indicated type II response of ladybird beetles (Fig. 3). The type II response is also confirmed as the proportion of prey consumed by the adults of *C. sexmaculata* declined with increasing prey density (Fig. 4). The coefficient of attack rate (a) and handling time (T_h) (estimated by Roger's random predator equation) of adults of *C. sexmaculata* is presented in Table 6. Showing handling time and attack rate of 0.4441 and 0.0254, respectively. The data indicated that there was a decline in the consumption rate at higher densities, which resulted in an increase in the functional response curves of *C. sexmaculata*. The maximum predation rate (T/T_h) was found to be 54.04 at 24 hours of exposure period. The value of coefficient of determination (R^2) =0.941 and standard errors of the estimated parameter indicated that Roger's random predator equation adequately described the functional response of *C. sexmaculata*.

Table 5: Maximum likelihood estimates from logistic regression of proportion of prey eaten as a function of initial prey density by adults of *C. sexmaculata* (n=6) at T= 24 hours by CATMOD procedure

Stage	Parameter	Estimate	SE	X^2	P
Adult	Intercept (P_0)	2.4400	0.2620	86.74	<.0001
	Linear (P_1)	-0.0723	0.00791	83.62	<.0001
	Quadratic (P_2)	0.000423	0.000059	50.72	<.0001
	Cubic (P_3)	-7.49E-7	1.17E-7	40.98	<.0001

Table 6: Functional response parameter estimate values of attack rate (a) and handling time (T_h) at 95% confidence limit (CL) for *C. sexmaculata* fed on *Aphis gossypii* at T = 24 hours obtained by least square method (NLIN)

Model	Stage	Parameter	Estimate	SE	95% CL		T/T_h
					Upper	Lower	
Roger's random predator equation	Adult	A	0.0254	0.0027	0.0306	0.0202	54.04
		T_h (hrs)	0.4441	0.0176	0.4799	0.4083	

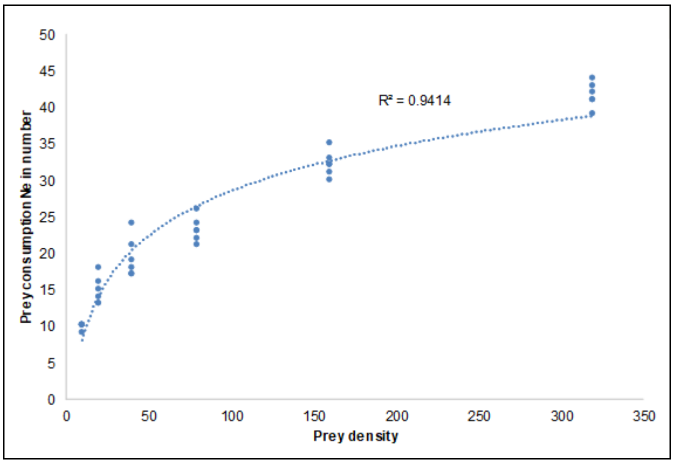

Fig 2: Cumulative prey consumption by *C. sexmaculata* adults at various prey densities of *Aphis gossypii* (n= 6 and T= 24 hours)

4. Discussion

The functional response of a natural enemy offers a good conceptual framework for understanding the action of agents in inundative releases. Many studies have been devoted to the foraging behaviour of insect predators but foraging behaviour across different time periods have received little attention. It is indicated from the data in Table 1 and Table 2 that at higher aphid densities predator had more choice for suitable prey consumption hence higher consumption rate is recorded at higher prey density, irrespective of time of exposure to its prey. However, as the time of exposure to the prey increased from (12 to 24 hrs), there was significant decrease in the prey consumption at decreasing prey densities. The attack rate declines, which may be due to decline in utility of consumption. The present study showed that the functional response described a curvilinear rise to a plateau (Holling type II response). The predator showed a decreasing consumption rate with increasing prey density. These findings coincide with Pervez and Omkar 2003 [15], who found that aphidophagous coccinellid predators did not exhibit a high rate of prey consumption at higher prey densities. The negative values for linear parameters (P_1<0) obtained in this study confirmed type II response for ladybird beetle. Similarly, Pervez and Omkar 2005 [16] showed a type II response of *C. sexmaculata* feeding on aphids.

Type II functional response are evidenced by an initial decrease in the proportion of prey eaten with increasing prey offered. (Trexler *et al.*, 1988) [17]. Although three types of functional response described by Holling may occur in coccinellids, the fact is that type II is the most common in these insects, as reported for several ladybird beetles preying on distinct aphid species., such as *C. septumpuctata* feeding on mustard aphid Pervez, 2004) [2]. *Anegleis cardoni* to varying densities of *Aphis gossypii* (Omkar and Kumar, 2013) [19].

The handling time T_h is a good indication of predation rate. Handling time affects the type of functional response, suggesting that the shorter the handling time the faster the curve reaches the asymptote (Nordlund and Morison 1990) [20]. In the present study the handling time T_h was 0.3654 hrs (21.92 min) after 12 hrs which increased to 0.4441 hrs (26.65 min) in the cumulative observation taken after 24 hours of exposure of predator to the prey species. Low handling time reveals the predator to be a good biocontrol agent. Handling time is proportional to the size of the prey: the larger the prey, the longer the time taken to consume it. The data from the present study provide information as to how C. *sexmaculata* responds to changes in prey density under laboratory conditions. The attack rate 'a' of first 12 hrs. was found to be higher, 0.0391/12 hrs. as against to a lower attack rate at cumulative exposure of next 12 hrs. *i.e.,* 0.0254/24 hrs. The handling time 'T_h' was found 0.3654 hrs. (21.92 min) after 12 hours of cumulative exposure which increased to 0.4441 hrs. (26.65 min) after next 12 hours of cumulative exposure. This shows that the predator was more efficient as a biocontrol agent during the first 12 hrs. of exposure to the prey than after that time period. However, these results are based on a laboratory study. Prey consumption depends upon the habitat complexity and prey-predator densities. Therefore, evaluation of the potential of *C. sexmaculata* together with heterospecifics in the laboratory and the field is needed to fully understand the foraging behaviour and prey-predator interactions.

5. Conclusion

The coccinellid beetle, *C. sexmaculata* showed a type II functional response to increasing prey densities of *Aphis gossypii*. Which represents a decreasing rate of prey killing with increasing prey density. The predator was found to be more active during the first 12 hours than after that period. However, it requires further study of the other aspects of predatory behaviour in order to understand and predict the success of this predator as a successful biocontrol agent.

6. Acknowledgement

The authors are thankful to teachers and staff of Department of Agricultural Entomology for providing facilities for the research work. Authors are also grateful to the teachers of Department of Statistics for their kind help in the completion of this research work. Author is grateful to Dr. V.M. Bhale Vice- Chancellor, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

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