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Functional Response of Four Syrphid Predators Associated with Mealy Cabbage Aphid, *Brevicoryne brassicae* L. on Cruciferous Vegetables

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ABSTRACT

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Functional response of the syrphids *Episyrphus balteatus* deGeer, *Eristalis interruptus* (Poda), *Eristalis cerealis* F. and *Sphaerophoria scripta* (L.), to *Brevicoryne brassicae* L. was studied under laboratory conditions to determine their relative preying potential. The 2nd and 3rd instar larvae of the predators were exposed to increasing densities of similar sized *B. brassicae* nymphs in petri dishes. Third instar of *Ep. balteatus* was found to possess highest consumption (52.8 aphids) closely followed by *Er. Cerealis* (50.7 aphids), *Er. Interruptus* (42.10 aphids) and *S. scripta* (38.7 aphids), All predators and preying stages exhibited type II functional response. The coefficients of attack rate and handling time were found to show significant variation between species and preying stages. The results suggested that third instar larva of *Episyrphus balteatus* is the efficient predator to regulate *B. brassicae* colonies.

Introduction

The functional response and its key parameters vary with several factors, including the relative size of predator and prey (Flinn *et al.*, 1985), hunger (Rotheray, 1983), prey type (Allahyari *et al.*, 2004), predator age and life stage (Farhadi *et al.*, 2010), host plant properties (DeClercq *et al.*, 2000; Sobhani *et al.*, 2013), and environmental conditions such as temperature and relative humidity (Moezipour *et al.*, 2008). Rotheray (1989) suggested that several other factors, such as density of predators, time of day, ability of prey to defend itself, toxicity of prey, and density of prey, may have an influence on searching efficiency and handling time of a predator. As in nature, many pests like aphids aggregate on host

plants as they colonize and reproduce, and damage to plants is usually greatest when aphid densities are high. Therefore, to control these pests, the response of predators to changes in prey density is of considerable interest.

The larvae of hoverflies of the subfamily Syrphinae are specialized aphidophagous predators and have long been recognized as important natural enemies of aphids (Ankersmit *et al.*, 1986; Chambers, 1988). Because of their high reproductive rates and the large number of aphids they consume (Chambers and Adams, 1986) and their elaborate oviposition behavior (Sadeghi and Gilbert, 2000), they can significantly reduce

aphid population growth and abundance (Brewer and Elliott, 2004; Freier *et al.*, 2007; Haenke *et al.*, 2009; Almohammad *et al.*, 2009). Larvae of aphidophagous syrphid flies can be important biocontrol agents in agroecosystems (Chambers, 1988), like vegetables and in protected cultivation (Evenhuis, 1959, 1960; Holdsworth, 1970; Brown and Schmitt, 1994). Many workers evaluated the potential of syrphidfly larvae against various aphid pests of vegetables like *Myzus persicae* (Amoros-Jimenez *et al.*, 2012), *Aphis gossypii* (Sobhani *et al.*, 2013; Putra and Yasuda, 2006), *Acythosiphon pisum* (Putra and Yasuda, 2006), *Lipaphis erysimi* (Singh, 2013; Singh and Singh, 2013; Devi *et al.*, 2011), *Aphis fabae* (Amiri-Jami and Sadeghi-Namaghi 2014), *Nasonovia ribisnigri* (Hopper *et al.*, 2011), *Aphis pomi* (Khan *et al.*, 2016) etc. with encouraging results.

The potential of *Episyrphus balteatus* deGeer as a biological control agent for aphids on several crops has been the subject of several studies, summarised by Amiri-Jami and Sadeghi-Namaghi (2014). However, related syrphid predators mostly are understudied. The present study was carried out to determine the functional response of four syrphidfly predators viz. *Ep. balteatus*, *Er. interruptus*, *Er. cerealis* and *S. scripta*, predominant in cruciferous vegetable fields of Kashmir (India), against *B. brassicae*, a major pest of cruciferous crops (Khan, 2009). The study aims at comparing the preying potential of the four predator species and to ascertain their possible contribution to the suppression of the aphid pest, with the view of their of their potential use as bioagents.

Materials and Methods

Stock cultures

Stock cultures of *Ep. balteatus*, *Er. interruptus*, *Er. cerealis* and *S. scripta* were

established using gravid females captured at the campus of SKUAST-K, Shalimar, Srinagar, India during spring 2014. The stock cultures were maintained in a constant environment of $25\pm 2^{\circ}\text{C}$ under a 14-h photoperiod. The insects were provided with cut flowers of *Brassica napus* as a pollen source; diluted honey (10%), solid crystalline sugar, and water from a soaked pad of cotton wool in a conical flask were placed on the floor of a net-covered cage ($100 \times 70 \times 70$ cm). The cut flowers and water were changed every 2–3 days. To obtain a group of larvae of the same age, females were induced to lay eggs on leaves of apple infested with green apple aphids. For experimental purposes, eggs laid over a period of 6 h were selected and placed in a large Petri dish in an incubator ($25 \pm 1^{\circ}\text{C}$, 60–70% RH and 16-L:8-D photoperiod) to hatch. As the newly emerged larvae are very delicate and difficult to handle, the larvae were left in groups and allowed to feed on green apple aphids for the first three days after hatching. After that, larvae were transferred to experimental Petri dishes, one larva per Petri dish. To rear the predator larvae, aphid colonies were maintained on fresh twigs of apple, in cages ($18 \times 18 \times 18$ cm). The colonies were collected from pesticide free vegetable fields in the University Campus.

Functional response

Functional response of 3rd and 4th instar larvae of the four predator species to various densities of the mealy cabbage aphid, *B. brassicae*, was determined. The predatory larvae were deprived of prey for 12 h prior to the experiment. Individual larvae were then exposed to different densities of the test aphid in petri dishes (9×1.5 cm) on excised leaves of cabbage. Six different densities of similar-sized (generally fourth instars) aphids were provided: 4, 8, 16, 32, 64, and 128 aphids for 2nd instar larvae and 7 aphid densities: 4, 8,

16, 32, 64, 128 and 256 were tested for the 3rd instar predators. At the end of a 24-h period, the syrphid larvae were removed from experimental Petri dishes, and the numbers of unconsumed aphids were counted. All experiments were conducted in an incubator at 25 ± 1 °C, 60–70% RH, and a photoperiod of 16:8 (L:D) h and replicated 10 times for each predator larval stage at each prey density. Control experiments without predators were carried in parallel and natural aphid mortality accounted for.

Statistical analysis

Prior to fitting the data to a particular Hollings' equation (Holling, 1959 and 1966), it's important to know the type of functional response exhibited by a particular instar of a predator to a particular prey species. Logistic regression model is such a tool that is used to determine the shape (type) of functional response by taking into consideration the proportion of prey eaten (N_a/N_0) as a function of prey offered (N_0) (Juliano, 2001). Hence the data were fitted to the following polynomial function that describes the relationship between N_a / N_0 and N_0 :

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

Where,

P_0 = Intercept

P_1 = Linear coefficient

P_2 = Quadratic coefficient

P_3 = Cubic coefficient

N_a = Number of prey eaten

N_0 = Number of prey offered.

The coefficients are estimated using the method of maximum likelihood. If $P_1 > 0$ and $P_2 < 0$, the proportion of prey consumed is positively density dependent, thus describing a type III functional response. If $P_1 < 0$, the

proportion of prey consumed declines monotonically with the initial number of prey offered, thus describing a type II functional response (Juliano, 2001). The coefficients of polynomial logistic regression were determined using the function "glm" in R software (R Development Core Team 2016).

After the determination of type of functional response, the data were analysed by fitting Rogers' Type II Random Predator Equation (Rogers, 1972) with the help of non-linear least square regression to determine the parameters of functional response. Rogers type II Random Predator Equation is given by

$$N_a = N_0 (1 - \exp [a (Th N_a - T)])$$

Where,

N_a = Number of prey eaten

N_0 = Number of prey offered

a = attack rate

Th = handling time

T = time of confinement (24 hours)

To determine the coefficients of attack rate and handling time using non-linear least square regression as suggested by Rogers (1972), the function "nls" provided by the R-software was used (R Development Core Team 2016).

After a and Th were determined for the original data (m_i), the differences among a values, as well as Th values, were tested for significance by estimating the variance using the jackknife technique (Meyer *et al.*, 1986). The Jackknife pseudo-value (m_j) was calculated for the n samples using the following equations:

$$m_{ja} = n.m_{ia} - (n-1)m_{ia}$$

$$m_{jTh} = n.m_{iTh} - (n-1)m_{iTh}$$

The mean values of $(n-1)$ jackknife pseudo-values for a and Th for each prey stage were

subjected to analysis of variance followed by Least Significant Difference Test ($p \leq 0.01$) (R Development Core Team, 2016).

Results and Discussion

The prey consumption rate of the four predatory syrphids differed across species as well as among predatory instars (Figure 1). In general, *Ep. balteatus* consumed highest number of *B. brassicae* nymphs followed by *Er. interruptus*, *S. scripta* and *Er. cerealis*. The 3rd instar larvae of all four predators consumed significantly higher proportion of the *B. brassicae* offered as compared to 2nd instar larvae. Third instar of *Ep. balteatus* was found to possess highest consumption (52.8 aphids) closely followed by *Er. Cerealis* (46.2 aphids), *Er. Interruptus* (38.6 aphids) and *S. scripta* (36.6 aphids).

Logistic regression analysis yield a significant negative linear coefficient ($P_1 < 0$) for both predatory stages of all the predators, suggesting a type II functional response towards *B. brassicae* (Table 1). As expected, proportion of prey consumed was higher at lower densities of prey offered and declined with increasing prey density.

The prey consumption data were fitted to Random Predator equation to determine the coefficient of a and T_h , estimates are presented in table 2 and 3, respectively. Among all predatory stages used, lowest T_h was exhibited by 2nd instar larvae of *Er. cerealis* followed by 3rd instar larvae of *Er. interruptus*. Highest attack rate was possessed by 2nd instar larvae of *Ep. balteatus* followed by 3rd instar larvae of *S. scripta*. However, Jackknife technique revealed insignificant variation in attack rate of the four predators both for 2nd instar ($F=0.44$, d.f.= 3,20, $P=0.727$) and 3rd instar larvae ($F=0.60$, d.f.= 3,24, $P=0.618$). The handling time was also found to show insignificant variation ($F=1.56$,

d.f.= 3,20, $P=0.230$ for 2nd instars, and $F=0.62$, d.f.= 3,24, $P=0.607$ for 3rd instars).

A perusal of the data on prey consumption rates of the predatory stages of *all predator species* indicated that the 3rd instar larvae consumed higher number of aphids. Beddington *et al.*, (1976) pointed out that variation in prey consumption rates could be expected from the between-instar differences that exist with respect to attack rate and handling time (parameters of functional response), and metabolic rate, which increases with development. The variation in prey consumption rates of a predator on various prey species is attributed to various factors such as prey mobility (Dixon, 2000), nutritional status (Thompson, 1999), suitability of the prey for the growth and reproduction of the predator (Shah and Khan, 2014), effect of host plant (Sobhani *et al.*, 2013) etc.

The syrphids associated with *B. brassicae* colonies were found to consume comparatively lower number of aphids as reported by other workers like Amoros-Jimenez *et al.*, (2012), Sobhani *et al.*, (2013), Putra and Yasuda (2006), Amiri-Jami and Sadeghi-Namaghi (2014), Hopper *et al.*, (2011), Dib *et al.*, (2011), Short and Bergh (2004), and Singh and Singh (2013). The variation of prey consumption rates is attributed to several factors such as prey suitability (Shah and Khan 2014); predator and prey size (Putra and Yasuda, 2006; Short and Bergh, 2004); experimental conditions (Farhadi *et al.*, 2010) and geographical variation (Dobzhansky 1933). Amiri-Jami and Sadeghi-Namaghi (2014) and Khan *et al.*, (2016) summarised the daily and complete larval period prey consumption of *Ep. balteatus* and other syrphid predator and reported that syrphids consume higher number of prey as compared to aphidophagous coccinellids, spiders,

anthocorids and *Chrysoperla* sp. The predatory response of various coccinellids to *B. brassicae* also revealed that syrphids have higher aphid consumption potential (Khan, 2009; Shah and Khan, 2013). A type II functional response was displayed on *B.*

brassicae by both instars of all four predatory species. This type of functional response is the most common functional response in insects and has been reported for many insect predators such as coccinellids (Shah and Khan, 2013).

Table.1 Maximum likelihood estimates from logistic regression analysis of the proportion of prey (*B. brassicae*) eaten by different instars of *Episyrphus balteatus*, *Eristalis interruptus*, *Eristalis cerealis* and *Spaerophoria scripta*

Predator	Instar	Parameter	Estimate	Std. Error	z value	Pr(> z)
<i>Episyrphus balteatus</i>	2 nd	Intercept	2.760e+00	1.301e+00	2.121	0.0339
		Linear	-8.047e-02	8.850e-02	-0.909	0.3632
		Quadratic	7.718e-04	1.585e-03	0.487	0.6262
		Cubic	-2.919e-06	7.615e-06	-0.383	0.7014
	3 rd	Intercept	2.534e+00	7.341e-01	3.452	0.000557
		Linear	-5.699e-02	2.588e-02	-2.202	0.027696
		Quadratic	3.287e-04	2.375e-04	1.384	0.166415
		Cubic	-6.697e-07	5.790e-07	-1.156	0.247481
<i>Eristalis interruptus</i>	2 nd	Intercept	1.166e+00	8.968e-01	1.300	0.194
		Linear	-4.945e-02	6.666e-02	-0.742	0.458
		Quadratic	6.794e-04	1.246e-03	0.545	0.586
		Cubic	-3.431e-06	6.115e-06	-0.561	0.575
	3 rd	Intercept	2.225e+00	6.839e-01	3.254	0.00114
		Linear	-5.160e-02	2.461e-02	-2.096	0.03607
		Quadratic	2.996e-04	2.282e-04	1.313	0.18913
		Cubic	-6.237e-07	5.588e-07	-1.116	0.26440
<i>Eristalis cerealis</i>	2 nd	Intercept	9.287e-01	8.891e-01	1.044	0.296
		Linear	-2.019e-02	6.653e-02	-0.303	0.762
		Quadratic	1.299e-04	1.247e-03	0.104	0.917
		Cubic	-8.327e-07	6.124e-06	-0.136	0.892
	3 rd	Intercept	2.440e+00	7.066e-01	3.453	0.000555
		Linear	-6.074e-02	2.525e-02	-2.406	0.016129
		Quadratic	3.794e-04	2.331e-04	1.628	0.103561
		Cubic	-8.066e-07	5.697e-07	-1.416	0.156820
<i>Spaerophoria scripta</i>	2 nd	Intercept	1.919e+00	1.044e+00	1.838	0.0661
		Linear	-6.016e-02	7.454e-02	-0.807	0.4196
		Quadratic	6.083e-04	1.368e-03	0.445	0.6566
		Cubic	-2.624e-06	6.655e-06	-0.394	0.6933
	3 rd	Intercept	2.785e+00	7.636e-01	3.647	0.000265
		Linear	-6.770e-02	2.669e-02	-2.537	0.011184
		Quadratic	4.264e-04	2.436e-04	1.751	0.079966
		Cubic	-8.976e-07	5.921e-07	-1.516	0.129508

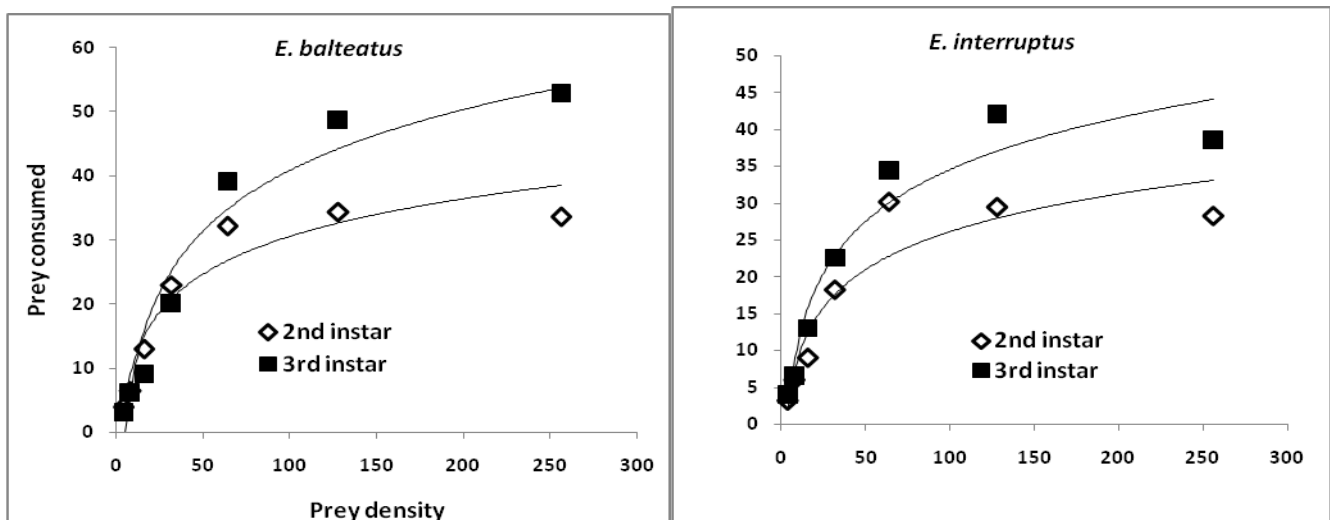
Table.2 Estimates of attack rate (a) for various growth stages of *Episyrphus balteatus*, *Eristalis interruptus*, *Eristalis cerealis* and *Spaerophoria scripta* preying upon *B. brassicae*, for random predator equation

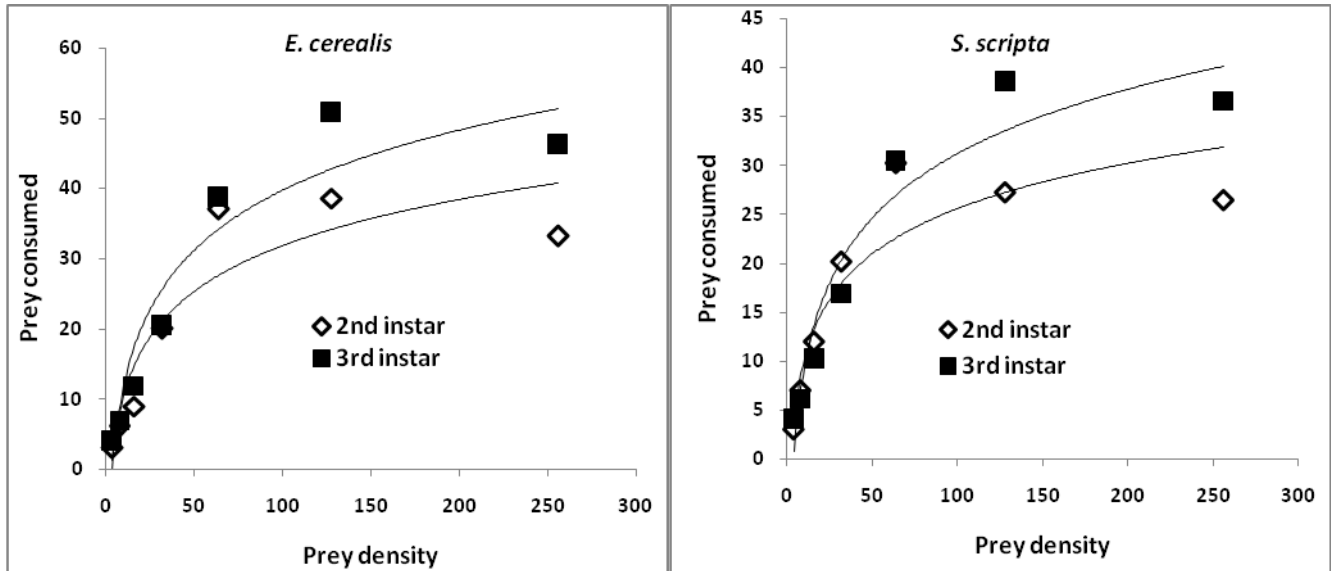
Predator	Instar	Estimate	Std. Error	t value	Pr(> t)
<i>Episyrphus balteatus</i>	2 nd	0.20259	0.04550	4.452	0.0112
	3 rd	0.06156	0.05320	1.157	0.29945
<i>Eristalis interruptus</i>	2 nd	0.04653	0.03739	1.244	0.281
	3 rd	0.02718	0.03771	0.721	0.503
<i>Eristalis cerealis</i>	2 nd	0.02918	0.03834	0.761	0.489
	3 rd	0.05576	0.04390	1.270	0.25990
<i>Spaerophoria scripta</i>	2 nd	0.03425	0.04413	0.776	0.481
	3 rd	0.13387	0.03302	4.055	0.00978

Table.3 Estimates of handling time (Th) for various growth stages of *Episyrphus balteatus*, *Eristalis interruptus*, *Eristalis cerealis* and *Spaerophoria scripta* preying upon *B. brassicae*, for random predator equation

Predator	Instar	Estimate	Std. Error	t value	Pr(> t)
<i>Episyrphus balteatus</i>	2 nd	0.65931	0.01244	52.980	7.6e-07
	3 rd	0.53311	0.08469	6.295	0.00149
<i>Eristalis interruptus</i>	2 nd	0.58034	0.20604	2.817	0.048
	3 rd	0.43526	0.31018	1.403	0.219
<i>Eristalis cerealis</i>	2 nd	0.43286	0.55304	0.783	0.478
	3 rd	0.54172	0.08461	6.402	0.00138
<i>Spaerophoria scripta</i>	2 nd	0.52205	0.46491	1.123	0.324
	3 rd	0.54995	0.01060	51.876	5.03e-08

Fig.1 Functional response of 2nd and 3rd instar larvae of *Episyrphus balteatus*, *Eristalis interruptus*, *Eristalis cerealis* and *Sphaerophoria scripta* to *B.brassicae*





However, in the case of syrphids, a variation in their functional responses has been observed, summarized by Hopper *et al.*, (2011) and Khan *et al.*, (2016). The differences in results from other studies may be due to several abiotic and biotic factors, including the ratio of predator to prey body size (Kalinkat *et al.*, 2013). Despite this, although the four tested predator species vary in size considerably, they exhibited similar type of response to *B. brassicae*, so did the different growth stages of the predators. Van Lenteren and Baker (1976) attributed the higher incidence of type II functional responses to experimental arena sizes that are too small to provide the real encounter rate of predator-prey, especially at low prey densities.

The coefficient of attack rate (a) and handling time (Th) were the parameters used to find out the magnitude of the functional responses exhibited by the predatory stages to *B. brassicae*. Their values differed for various growth stages of each predator and for the four predators, however the variation was found to be insignificant (Table 2, 3). The handling time is considered a good indicator of consumption rate and effectiveness of a predator because it reflects the cumulative

effect of time taken during capturing, killing, subduing and digesting the prey (Shah and Khan, 2013). Thus, the current study revealed that the four predominant syrphid predators associated with *B. brassicae* colonies may contribute equally to regulating the aphid populations, particularly at lower densities owing to the fact that proportionate prey consumption declines with increasing prey density.

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