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Temperature-dependent Prey Consumption and Functional Response of *Episyrphus balteatus* De Geer (Diptera: Syrphidae) to *Macrosiphum rosae* L. (Homoptera: Aphididae) reared on *Rosa alba* L. under Laboratory Conditions

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ABSTRACT

Laboratory experiments were conducted to determine the prey consumption and functional response of the aphidophagous hoverfly *Episyrphus balteatus* DeGeer (Diptera: Syrphidae) to different densities of fourth instar rose aphids, *Macrosiphum rosae* (Homoptera: Aphididae), reared on *Rosa alba* L. (Rosaceae) at 20 °C, 27 °C and 35 °C. The third instar of *E.balteatus* consumed a maximum of 51.3±0.53 *M. rosae* aphids at 27 °C, followed by the third instar with 46.5±0.53 aphid predation at 20 °C. The lowest handling time (T_{μ}) was noted for the third instar larvae of *E. balteatus* (0.407±0.012) at 27°C, and the maximum handling time (T_{μ}) of (0.662±0.024) was noted for 2nd larvae of *E. balteatus* at 35°C. The highest attack rate was noted for 3rd instar larvae (0.201±0.014) at 27°C, while the lowest attack rate was noted for second instar at 35°C. Both instars of *Episyrphus balteatus* exhibited type II functional response at all three temperatures.

Key words : Syrphidae, Aphids, Episyrphus balteatus, Macrosiphum rosae, Rosa alba, Functional response.

Introduction

For centuries, with their magnificent and fragrant blooms, roses have been the world's most popular landscape and ornamental plants (Jaskiewicz, 2006) and hence the most important crop in floriculture. *Rosa alba* L., commonly known as the white rose (sufaid gulab), is a member of the Rosaceae plant family. Due to its ornamental significance, medicinal value and aromatic qualities, this species is widely cultivated in Asia, Europe, North America and North-west Africa (Verma et al., 2020).

Aphids are among the most severe invertebrate pests of crops (Dedryver *et al.*, 2010). The rose aphid, *Macrosiphum rosae* L. (Hemiptera: Aphididae), is the most successful and critical pest of roses and causes huge economic damage (Mehrparvar *et al.*, 2016). This aphid is small, green or pink with long black siphunculi, more or less globular, precarious and exhibits polymorphism (Munib *et al.*, 2015). The most significant damage is to inflorescences, especially at bud burst (Jaskiewicz, 2006), and it is most devastating to one and two-year-old rose shrub sprouts (Margina *et al.*, 1999). Moreover, the aphids secrete honeydew which promotes the growth of sooty mold on leaf surfaces and flowers, reducing the plants' photosynthetic area (Zia *et al.*, 2010) and reducing the flower yields (Karlik and Tjosvold, 2003). In urbanised conditions, this species causes measurable economic losses and affects urban landscapes by lowering the ornamental value of shrubs (Jaskiewicz, 2006).

Conventionally, for the control of aphids, mainly insecticides are used. However, indiscriminate spraying of insecticide chemicals resulted in environmental pollution, insecticide resistance, pest resurgence, residual toxicity on flowers, and killing natural enemies (El-Sherbeni *et al.*, 2019). The insecticide residue on flowers deteriorates their quality and commercial and industrial value. In the presentday scenario, the growing demand for residue-free production of rose flowers can be best achieved using natural enemies to manage rose aphids effectively. The biological control of aphids employing natural enemies has become essential to pesticidefree management strategies (Zehnder *et al.*, 2007).

Episyrphus balteatus (De Geer, 1776) (Diptera: Syrphidae) is a common generalist aphidophagous hoverfly (Sarthou *et al.*, 2005). The predacious larvae of this hoverfly are extremely voracious and can devour large amounts of aphids during the larval phase (Faheem *et al.*, 2019). Functional response is a short behavioural predator-prey interaction that gives insights into the predaceous capacity of natural enemies and enhances the predictability of biological control (Shah and Khan, 2013).

Functional responses depict the rate at which a predator exterminates its prey at various prey densities and determine the predator's efficiency in regulating prey populations (Murdoch and Oaten, 1975). Functional response curves are of four fundamental types viz., type I (linear rise to a plateau), type II (negatively accelerated rise to a plateau), type III (S-shaped rise to a plateau), type IV (domeshaped) (Holling, 1959). Two widely used parameters describe the functional response of a predator feeding on a particular prey species; these are the predator's "attack rate" or search rate (*a*) and its "handling time" (*Th*) (Hassell *et al.*, 1976).

In addition to affecting the functional traits of aphidophagous insects, biological fitness and survivability (Zhang *et al.*, 2014), the increase in temperature can influence aphid consumption (Ferreira *et al.,* 2020). *E. balteatus* larvae have differential feeding behaviour under high temperatures (Hong and Hung, 2010).

Since the information regarding the predatory potential of *Episyrphus balteatus* against *M. rosae* is limited, especially under different temperatures, this study aimed to evaluate the potential of *Episyrphus balteatus* (2nd and 3rd instars) preying on *Macrosiphum rosae* through the study of functional responses at three different temperatures.

Materials and Methods

The studies on the temperature-dependent functional response of *E. balteatus* on *M. rosae* were carried out in the laboratory of the Division of Entomology, SKUAST-K, Shalimar, during the spring of 2019.

Stock Cultures

Stock cultures of Episyrphus balteatus were established by capturing adult flies from the pesticidefree fields of SKUAST-K, Shalimar campus, India, during the spring of 2019. The gravid females were used, and the culture was maintained in constant laboratory conditions of 25±2°C and photoperiod of 16:8 (L:D) hr. As a pollen source, adult flies were provided with flowers of Brassicaacephala, and water, crystalline sugar and diluted honey (10%) from a soaked pad of cotton wool in a conical flask; all provisions were placed on the floor of a net- covered cage (100 by 70 by 70 cm³). To obtain a group of larvae of the same age, gravid females were induced to lay eggs on M. rosae-infested leaves, tender shoots and buds of R. alba. To maintain the health of the adult flies and stock culture, water, nutrient mixtures and cut flowers were changed every 48-72 hours. The eggs laid by flies on the rose aphid-infested plant parts were selected, placed in a petri dish, and shifted in an incubator with a constant environment of 25±2°C, 60-70% relative humidity [RH], and a photoperiod of 16:8 [L:D] hr to hatch. Since newly hatched larvae of E. balteatus are incredibly delicate and cumber some to handle, they were allowed to feed on rose aphids for the first three days after hatching until they moulted into the second instar and then the third instar. The 2nd and 3rd instar larvae were transferred to experimental Petri dishes, one larva per petri dish. Predator larvae were reared by maintaining the M. rosae colonies on the fresh Rosa alba leaves and flower buds placed in cages of 18 by 18 by 18 cm³ dimensions. The rose aphids were obtained from pesticide-free *Rosa alba* shrubs maintained at a private garden for this experiment.

Functional Response

The functional response of the 2nd and 3rd instar larvae of the Episyrphus balteatus to various densities of rose aphids was determined. Before the experiment, chosen larvae were deprived of prey for 12 hours. Individual larvae were placed in Petri dishes (9 ×1.5 cm) and then exposed to different densities of the rose aphids on the excised leaves of rose at varying temperatures of 20°C, 27°C, and 35°C. Eight different densities viz., 2, 4, 8, 16, 32, 64,128 and 256 of similar sized (generally fourth instars) aphids were provided to both 2nd and 3rd instar larvae, respectively. After 24 hours, the larvae were removed from experimental Petri dishes, and the number of unconsumed aphids was counted. The Petri dishes were carefully cleaned and rinsed with 70% ethanol. Aphids still being fed on by the predator were considered dead. A control Petri dish consisting of rose aphids was also set in parallel to note the natural mortality of the aphids in the absence of a predator. All the experiments were conducted in an incubator at 20°C, 27°C and 35°C, 60–70% RH and a photoperiod of 16:8 (L:D) hr; replicated ten times for each predator larval stage at each prey density.

Statistical Analysis

Understanding the type of functional response displayed by a certain instar of a predator to a specific prey species is crucial before fitting the data to a specific Hollings' equation (Holling, 1959 and 1966). The Logistic regression model is a tool that is used to determine the shape (type) of functional response by taking into consideration the proportion of prey consumed (N_a/N_0) as a function of prey offered (N_0). Therefore, 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 $P_1 < 0$, then a type II functional response will be described by a fraction of prey consumed that drops monotonically with the amount of prey initially offered. (Juliano, 2001). The coefficients of polynomial logistic regression were determined using the function "glm" in R software (R Development Core Team, 2020).

After the determination of the type of functional response, the data, i.e., the number of aphids preyed upon by second and third-instar larvae of *Episyrphus balteatus* at different densities, was 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

 $N_a = N_o [1 - \exp \{a (Th N_a - T)\}]$

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, 2020).

Results

The prey consumption rates and per cent consumed by the second and third instar larvae of *E. balteatus* on *M. rosae* at three different temperatures of 20°C, 27°C, and 35°C are given in Table 1. The data indicate that the larvae of *Episyrphus balteatus* consumed rose aphids at all given temperatures. However, the third instar of *E.balteatus* consumed a maximum of $51.3\pm0.53M$. *rosae* aphids at 27°C followed by the third instar with 46.5 ± 0.53 aphid predation at 20°C. At all three temperatures, 3rd instar larvae showed a higher consumption rate than 2nd instar larvae. The prey consumption percentage increased from a prey density of 2 to 16 for both instars at all three temperatures; however, after that, it declined. Regarding the effect of temperature on prey consumption, the highest prey consumption by both the instars was observed at 27°C, followed by 20°C, and the least consumption was observed at 35°C.

The percentage of prey consumed by both the predatory stages decreased as the offered prey density increased. The graphical analysis of the percentage of prey consumed versus offered prey density suggested type II functional response for both the predatory stages of *E. balteatus* at all three temperatures, further confirmed by the Logistic regression

model estimates. The linear coefficient of the Logistic regression model consistently assumed significant negative (<0) values for both the larval instars at all three temperatures, as demonstrated by Fig. 1.

The maximum handling time (T_h) of (0.662 ± 0.024) was noted for 2nd larvae of *E. balteatus* at 35°C followed by 2nd instar larvae with (0.611 ± 0.020) T_h at 20°C and 2nd instar (0.554 ± 0.018) at 27°C respectively. The lowest handling time was noted for 3rd instar larvae of *E. balteatus* (0.407 ± 0.012) at 27°C followed by 3rd instar larvae (0.441 ± 0.012) at 20°C and

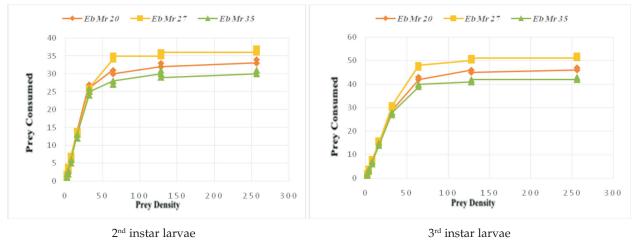


Fig. 1. Functional response of *E. balteatus* predating on different densities of *Macrosiphum rosae* at three different temperatures of 20°C, 27°Cand 35°C. (*Eb* =*Episyrphus balteatus*, *Mr* = *Macrosiphum rosae*)

Predator	Prey	Replications	Macrosiphum rosae					
Stage	density	1	20°C		27°C		35°C	
0	offered		Prey	%	Prey	%	Prey	%
	(N_0)		consumed (N)		consumed (N)		consumed (N)	
2 nd instar	2	10	1.3±0.48	65.0%	1.4 ± 0.52	70.0%	1.1±0.32	55.0%
	4	10	3.0 ± 0.47	75.0%	3.1±0.32	77.5%	2.5±0.53	62.5%
	8	10	6.4±0.52	80.0%	6.5±0.53	81.3%	5.9±0.32	73.8%
	16	10	13.4±0.52	83.8%	13.4±0.52	83.8%	12.6±0.52	78.8%
	32	10	26.3±0.67	82.2%	25.5±0.53	79.7%	24.6±0.70	76.9%
	64	10	30.4±0.52	47.5%	34.6±0.52	54.1%	27.5±0.53	43.0%
	128	10	32.5±0.53	25.4%	35.7±0.48	27.9%	29.6±0.70	23.1%
	256	10	33.6±0.52	13.1%	36.4±0.52	14.2%	30.4±0.52	11.9%
3 rd instar	2	10	1.5±0.53	75.0%	1.8±0.42	90.0%	1.3±0.48	65.0%
	4	10	3.4±0.52	85.0%	3.6±0.52	90.0%	3.1±0.32	77.5%
	8	10	7.0±0.67	87.5%	7.6±0.52	95.0%	6.7±0.48	83.8%
	16	10	14.9±0.57	93.1%	15.4±0.52	96.3%	14.1±0.32	88.1%
	32	10	29.5±0.53	92.2%	30.5±0.53	95.3%	27.5±0.53	85.9%
	64	10	42.4±0.52	66.3%	47.5±0.53	74.2%	39.6±0.52	61.9%
	128	10	45.5±0.53	35.5%	50.5±0.53	39.5%	41.6±0.42	32.5%
	256	10	46.5±0.53	18.2%	51.3±0.53	20.0%	42.5±0.53	16.6%

 Table 1. Predation rates (Mean ± SE) and per cent prey consumption for 2nd and 3rdinstar larvae of *Episyrphus balteatus* on *Macrosiphum rosea* at 20°C, 27°C, 35°C

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Growth stage	Parameters	Estimates	SE	Z- value	Pr (Z)
2 nd instar	Intercept	2.0942234356**	0.2069453120	10.120	< 0.01
	Linear	-0.0395959215**	0.0075055656	-5.276	< 0.01
	Quadratic	0.0001342183	0.0000702900	1.909	>0.05
	Cubic	-0.0000001575	0.000001733	-0.909	>0.05
3 rd instar	Intercept	2.81015662077**	0.2069453120	10.120	< 0.01
	Linear	-0.03521999545	0.0075055656	-5.276	< 0.05
	Quadratic	0.00006084709	0.0000702900	1.909	>0.05
	Cubic	0.0000004257	0.0000001733	-0.909	>0.05

Table 2. Maximum likelihood estimates from logistic regression analysis of the proportion of *Macrosiphum rosae* eaten by second and 3rd instar larvae of Episyrphus balteatus against the initial number of aphids offered at 20°C

**=Significant at 1% level of significance.

Table 3. Maximum likelihood estimates from logistic regression analysis of the proportion of *Macrosiphum rosae* eaten by 2nd and 3rd instar larvae of *Episyrphus balteatus* against the initial number of aphids offered at 27°C

Growth stage	Parameters	Estimates	SE	Z- value	Pr (Z)
2 nd instar	Intercept	1.92624614075**	0.20338269665	9.471	< 0.01
	Linear	-0.02884453019**	0.00740782349	-3.894	< 0.01
	Quadratic	0.00004180689	0.00006945018	0.602	>0.05
	Cubic	0.0000005494	0.00000017117	0.321	>0.05
3 rd instar	Intercept	3.8077992164**	0.3669888263	10.376	< 0.01
	Linear	-0.0510651633**	0.0118082293	-4.325	< 0.05
	Quadratic	0.0001594146	0.0001029915	1.548	>0.05
	Cubic	-0.0000001529	0.0000002445	-0.626	>0.05

**=Significant at 1% level of significance.

Table 4. Maximum likelihood estimates from logistic regression analysis of the proportion of *Macrosiphum rosae* eaten by 2nd and 3rd instar larvae of *Episyrphus balteatus* against the initial number of aphids offered at 35°C

Growth stage Parameters		Estimates	S.E.	Z- value	Pr (Z)	
2 nd instar	Intercept	1.5579816769953**	0.1844032262672	8.449	< 0.01	
	Linear	-0.029714041678**	0.0069452662538	-4.278	< 0.01	
	Quadratic	0.0000616383016	0.0000663583760	0.929	>0.05	
	Cubic	0.000000004147	0.0000001651858	0.003	>0.05	
3 rd instar	Intercept	2.0645014568**	0.2174973616	9.492	< 0.01	
	Linear	0.0212762171**	0.0078123641	-2.723	< 0.05	
	Quadratic	-0.0000378942	0.0000725667	-0.522	>0.05	
	Cubic	0.000002535	0.0000001779	1.425	>0.05	

**=Significant at 1% level of significance.

 3^{rd} instar larvae (0.471± 0.014) at 35° C, respectively (Table 5). The highest attack rate was noted for 3^{rd} instar larvae (0.201± 0.014) at 27° C followed by 3^{rd} instar larvae (0.150± 0.009) at 20° C, and 3^{rd} instar larvae (0.120± 0.007) at 35° C, respectively. The minimum attack rate was noted for 2^{nd} instar at 35° C.

Regarding the effect of temperature on functional response, the temperature of 27°C was found to be the ideal temperature for both the larval instars at all three temperatures employed as the lowest handling time (0.407±0.01)was reported at this tempera-

ture. The search rate (*a*) recorded increased with an increase in temperature and peaked at 27°C. The handling time decreased with an increase in temperature and reached its lowest value at 35°C for both the instars of predator larvae.

Discussion

A perusal of the data on prey consumption rates of 2nd and 3rd instar larvae of *Episyrphus balteatus* indicated that the 3rd instar larvae of *Episyrphus balteatus*

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consumed the highest number of aphids, irrespective of the temperature. The observation that 3rd instar larvae consumed the most significant proportion of prey over 24 hours, at all temperatures, is consistent with the observations of many other workers like those of Jalilian *et al.* (2011) for *E*. balteatus preying upon Myzus persicae; Khan (2017) for E. balteatus preying upon Brevicoryne brassicae; Khan (2015) for E. balteatus preying upon Aphis pomi, Faheem et al. (2019) for E. balteatus preying upon Schizaphis graminum and Baskaran et al. (2009) for E. balteatus preying upon Aphis gossypii. Bedddington et al. (1976) pointed out that variation in prey consumption rates could be expected from the betweeninstar differences concerning attack rate and handling time (parameters of functional response) and metabolic rate, which increases with development.

Among the three temperatures given to *E. balteatus*, the highest prey consumption by both the instars was recorded at 27°C, which is in agreement with a study done by (Noël *et al.*, 2022) on daily consumption of *A. pisum* by *E. balteatus* and found the rate was higher at 26°C. Our results are near to the findings of (Hong *et al.*, 2010), who recorded the highest prey consumption by larval instars of *E. balteatus* at 30 °C. Predator consumption rates on different prey species might vary for a variety of reasons, *viz.*, prey mobility (Dixon, 2000), experimental conditions (Farhadi *et al.*, 2010), the effect of the host plant (Sobhani *et al.*, 2013), and suitability of the prey for the growth and reproduction of the predator (Shah and Khan, 2014).

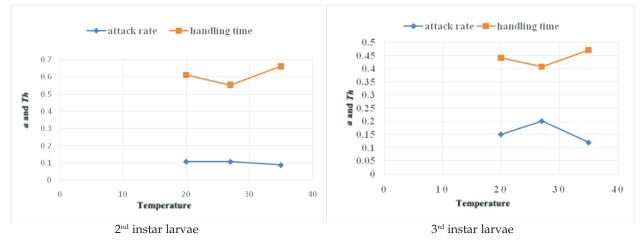


Fig. 2. Attack rate (*a*) and Handling time (*Th*) versus temperature given to 2nd and 3rd instar larvae of *Episyrphus balteatus* preying upon *Macrosiphum rosae* under laboratory conditions.

Table 5. Estimates of attack rate (a) and handling time (T_h) for 2^{nd} and 3^{rd} instar larvae of <i>Episyrphus balteatus</i>	s preying
upon <i>Macrosiphum rosae</i> for random predator equation at 20°C, 27°C &35°C	

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Temperature	Growth stage	Parameters*	Estimates	SE	Z-value	Pr (Z)
20°C	2 nd instar	а	0.1086847**	0.0073233	14.841	< 0.01
		T_h	0.6114045**	0.0202270	30.227	< 0.01
	3 rd instar	a	0.1508874**	0.0094274	16.005	< 0.01
		T_{h}	0.4410788**	0.0124516	35.423	< 0.01
27°C	2 nd instar	a	0.108123**	0.007043	15.352	< 0.01
		T_h	0.553905**	0.018188	30.455	< 0.01
	3 rd instar	a	0.201462**	0.013517	14.905	< 0.01
		T_h	0.407470**	0.010571	38.546	< 0.01
35°C	2 nd instar	a	0.0883686**	0.0060498	14.607	< 0.01
		T_{h}	0.6620566**	0.0236689	27.971	< 0.01
	3 rd instar	a	0.1200021**	0.0073346	16.361	< 0.01
		T_h	0.4711765**	0.0144319	32.648	< 0.01

**a* in hours⁻¹ and T_{μ} in hours.

**=Significant at 1% level of significance

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The Type II functional response was displayed on *M.rosae* by both instars of *E. balteatus*. Amiri-Jami *et al.* (2014) reported the type II functional response of both *E. balteatus* larval sizes fed *on A. fabae*. Jalilian *et al.* (2011) also reported a type II functional response of *E. balteatus* towards *M. persicae*. Type II of functional response has been reported for many insect predators and hence is considered the most common in insects (Shah and Khan, 2013). Van Lenterenand 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.

Low handling time indicates that the predator is a promising biocontrol agent (Rotheray, 1989). Handling time affects the type of functional response, suggesting that the shorter the handling time, the faster the curve reaches the asymptote (Nordlund and Morrison, 1990). Moreover, handling time can affect other components, such as attack rate and searching efficiency (Beddington *et al.*, 1976).

Although obtained under laboratory conditions, the results of this work allow us to conclude that high consumption-ability, high attack rates and low handling times of 3rd instar of *E.balteatus* reflect its efficacy as an important biocontrol agent for the management of *M. rosae* in Rose alba.

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Conflict of interest

The authors declare no conflict of interest.

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