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Functional and Numerical Responses of *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) Parasitizing Cotton Whitefly, *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae)

Nooshin Zandi Sohani, Parviz Shishehbor and Farhan Kocheili
Department of Plant Protection, Faculty of Agriculture, Shahid Chamran University, Ahvaz, Iran

Abstract: Laboratory experiments were conducted to measure the functional and numerical responses of *Eretmocerus mundus* Mercet parasitizing cotton whitefly, *Bemisia tabaci* Gennadius. Parasitoids were isolated singly for 24 h in 5 cm petri dishes with either 5, 10, 20, 40, 60 or 100 second nymphal stages of *B. tabaci*. Results showed a typical type II functional response, with up to 43.2 hosts attacked when 100 hosts were provided. The rate of attack and handling time were 0.0465 and 0.3341 days, respectively. At above mentioned densities, the parasitoids lived an average of $\approx$ 4 days. At host densities of $\leq$20 host/day, the total number of eggs laid was limited by the number of hosts available. Total fecundity was highest with an average of 196 eggs/female at density 100.

Key words: *Eretmocerus mundus*, *Bemisia tabaci*, cotton whitefly, functional response, numerical response

INTRODUCTION

The cotton whitefly, *Bemisia tabaci* (Gennadius), is one of the most important agricultural insect pests in the Middle East, Europe, North and Central America (Mound and Halsey, 1978; McKenzie *et al.*, 2004). In addition to feeding on more than 700 host plant species within 86 botanical families (Greathead, 1986), *B. tabaci* has a high reproductive capacity and destructive life habits that enable it to (1) cause severe damage through plant feeding and (2) transmit more than 90 types of virus diseases in commercial crops (Brunt, 1986; Morales and Jones, 2004).

Because the cotton whitefly has developed resistance to many commonly used insecticides (Omer *et al.*, 1993; Horowitz and Ishaya, 1996), biological control with parasitoids of the family Aphelinidae, is at present one of the control methods available for IPM program. This study deals with the parasitoid, *Eretmocerus mundus* Mercet, an indigenous parasitoid of cotton whitefly in Iran. *E. mundus* is common in fields of cotton, aubergine and cucurbits (Talebi *et al.*, 1988; Shishehbor and Mossadegh, 2003; Kocheili and Mossadegh, 2007).

Any program designed to enhance the effectiveness of parasitoids should consider many factors, including interaction between parasitoid-host. However, review of literature showed that quantitative data on host-parasitoid for *E. mundus* and *B. tabaci* are limited. Level of mortality inflicted on a host population is determined primarily by the response of the parasitoid to host density, while population stability is maintained as a result of both numerical and functional response of parasitoid (Holling, 1959). In order to improve the biological control of whiteflies and to obtain a better understanding of parasitoid-host interaction, laboratory studies are necessary. The objectives of current investigation were to measure the functional response and progeny production of *E. mundus* to varying *B. tabaci* densities.

MATERIALS AND METHODS

Stock culture maintenance: These studies were conducted in Department of Plant Protection, Faculty of Agriculture, Shahid Chamran University, Ahvaz, Iran in 2007. Adult cotton whitefly, *Bemisia tabaci* used in these studies were collected in October 2006 from a cucumber field in Ramin Agricultural and Natural Resources University Campus, Mollasani, Ahvaz, Iran and reared on the foliage of cucumber plants (cultivar Superdominus) grown from seeds in plastic pots (20 cm diameter). Infested plants were kept in wooden-framed rearing cages (120×60×60 cm) covered with white nylon mesh of 210 μm aperture. They were maintained in a laboratory where seasonal temperature ranged from 16-20°C and relative humidity 40-50%. The photoperiod was 14:10 (L : D) h,

Corresponding Author: Parviz Shishehbor, Department of Plant Protection, Faculty of Agriculture,
Shahid Chamran University, Ahvaz, Iran

with illumination (4000 lux) provided from fluorescent lamps. Plants were kept in the cages until they were severely damaged by the whiteflies; new plants being added when needed.

A laboratory culture of *E. mundus*, originally obtained from the above mentioned cucumber field, was established at the Department of Plant Protection, Shahid Chamran University, Ahvaz, Iran. The parasitoids were reared continuously on *B. tabaci* on cucumber plants in wooden cages similar to those described above. Every two weeks new cucumber plants harbouring different nymphal stages of *B. tabaci* were added to the cages. The cages were maintained in a laboratory (16-20°C, 40-50% relative humidity and 14:10 photoperiod).

Group of 30 whiteflies (females and males) were confined in clip cages to lower leaf surface of cucumber plants (3-6 leaf stage) for 24 h. The clip cages were similar to those described by Lewis (1973) with some modifications. After 24 h, the whiteflies and cages were removed and plants harbouring eggs held in the laboratory condition for about 10 days when all immature stages of whiteflies were in the second nymphal instar. Different nymphl stages of *B. tabaci* were designated according to the Nechols and Tauber (1977) as follows: first (crawler), second, third, fourth and pharate adult (pupa). All experiments were carried out in a climate-controlled cabinet at 30±1°C, 40-50% RH and 14:10 photoperiod.

Functional response: Fresh cucumber leaves with 5 cm diameter were examined under a binocular microscope to ascertain that each held 5, 10, 20, 40, 60, or 100 second nymphal stages of *B. tabaci*. Excess nymphs were removed with an insect pin. The leaves were then placed in a petri dish of the same diameter on a fine (ca. 2 mm) layer of agar (2% w/v) as described by Urbaneja *et al.* (2006). A single couple of *E. mundus* (< 24 h old) was introduced into the petri dish which was held in the climate-controlled cabinet. After 24 h, the parasitoids were removed and the leaf with nymphs was returned to a climate-controlled cabinet for whitefly and parasitoid development. After about two weeks the number of parasitized nymphs were recorded. The parasitized nymphs can easily be recognized by their brown colour. Each treatment was replicated five times.

Numerical response: The same exposure techniques were used as described above, except the parasitoids were transferred daily into a new petri dish provided with a fresh leaf and nymphal hosts. Host replacement continued as long as the females survived and males that died were

replaced. Fertility was calculated as the number of parasitized nymphs. There were five replicates for each treatment.

Data analysis: Parasitization and reproduction data were first analyzed by one-way analysis of variance (ANOVA) to test whether the number of host attacked and the number of eggs laid differ for leaves with different initial host density (SAS, 2003).

To simulate functional response, the disc equation (Holling, 1959) and the random attack equation (Rogers, 1972) for parasitoids were used.

$$Na = a'TtNt/1 + a'ThNt \text{ (disc equation)}$$
$$Na = Nt [1 - exp(-(a' Tt Pt/1 + a' Th Nt))] \text{ (random attack equation)}$$

Where:
exp = Base of natural logarithm
Na = No. of host attacked
Pt = No. of parasitoids
Nt = Host density
a' = Instantaneous rate of discovery or searching efficiency
Th = Handling time (expressed as day)
Tt = Total time and with Pt = 1 (one parasitoid)
Tt = 1 (24 h)
Na = Dependent variable
Nt = Independent variable

After determining the type of functional response, parameters Th and a' must be estimated (Juliano, 2001). We used nonlinear least square regression (NLIN procedure in SAS) to estimate the parameters of the Holling's disk equation.

RESULTS AND DISCUSSION

The ANOVA revealed significant effect of host density. The mean number of parasitized whitefly nymphs increased significantly as the number of host increased (Table 1). Results of logistic regression (Table 2) indicated that functional response of *E. mundus* on second nymphal stage of *B. tabaci* is of type II. The functional response of *E. mundus* increased from an average of 5 hosts parasitized per day at host density of 5 second stage nymphs per leaf to an average of 43.2 hosts parasitized per wasp per day at a host density of 100. From this type of response it can be seen that as host density increases there is a proportional increase in host parasitization until an upper limit of about 52 nymphs/day is reached at density of 100 per leaf, indicating

Table 1: Mean numbers and analysis of variance (ANOVA) of second nymphal instar of *Bemisia tabaci* parasitized by *Eretmocerus mundus* on cucumber leaf with different prey density over a 24 h period at 30±1°C

N	Hosts parasitized per leaf at density N		ANOVA source	df	SS	p
	Mean	Range				
5	5.00±0.0 a	5	Density	5	5677.47	<0.0001
10	9.21±1.095a	8-10	Error	24	588.40	
20	18.60±0.748b	16-20				
40	24.80±2.311b	18-32				
60	36.80±2.200c	31-43				
100	43.20±4.294c	31-52				

Means in the column followed by the same letter(s) were not significantly different at the 0.05 level when tested by LSD (1997)
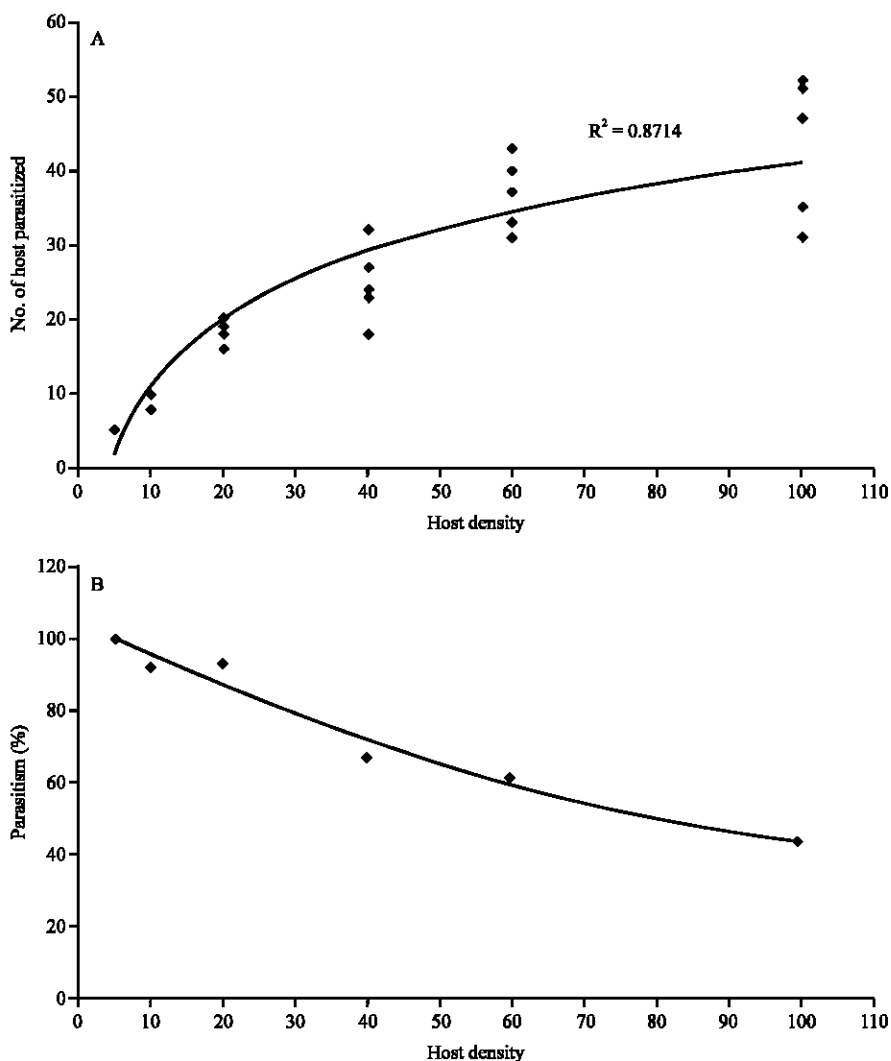

Fig. 1: Mean (SE) number (A) and proportion (B) of second nymphal instars of *Bemisia tabaci* parasitized by *Eretmocrus mundus*. The best fitted type II functional response and logistic regression model are presented in graphs A and B, respectively

parasitization over a 24 h period. Most parasitoids parasitized all or most hosts provided at lower host densities and showed a deceleration in rate of parasitism with considerable individual variation in the number of hosts parasitized at each host density (Table 1, Fig. 1).

Longevity varied with host density. Mean longevity of females was 4.18 days (ranged from 3.8 to 4.8 days). Analysis of variance indicated no significant differences in longevity of female parasitoids between the densities examined (F = 0.63; df = 5, 4; p = 0.68).

Table 2: Results of logistic regression analysis of the second nymphal instar of *B. tabaci* parasitized by *E. mundus* against initial number of nymphs

Parasitoid	Coefficient	Estimate	SE	Chi-squared value	p-value
E. mundus	Constant	5.88390	1.0377	32.15	0.0000
	Linear	-0.29430	0.0669	19.32	0.0000
	Quadratic	0.00490	0.0012	14.65	0.0005
	Cubic	-0.00003	6.9300	13.18	0.0003

Table 3: Parameters estimated by Holling's disc equation for *E. mundud*

Parameters	Estimate	Asymptotic SE	Asymptotic 95% CI	
			Lower	Upper
a'	0.0465	0.00616	0.0339	0.0591
Th	0.3341	0.04530	0.2414	0.4268

Table 4: Mean numbers and analysis of variance (ANOVA) of eggs laid by *Eretmocerus mundus* adult females parasitizing different *Bemisia tabaci* densities at 30±1°C

N	Mean	Range	ANOVA source	df	SS	p
5	22.6±2.16d	19-30	Density	5	190689.9	<0.0001
10	38.6±9.64cd	24-49	Error	24	2074.4	
20	84.4±10.05bc	59-117				
40	107.2±13.7ab	62-140				
60	138.0±8.3a	115-158				
100	196.4±22.02a	125-243				

Means in the column followed by the same letter(s) were not significantly different at the 0.05 level when tested by LSD (1997)
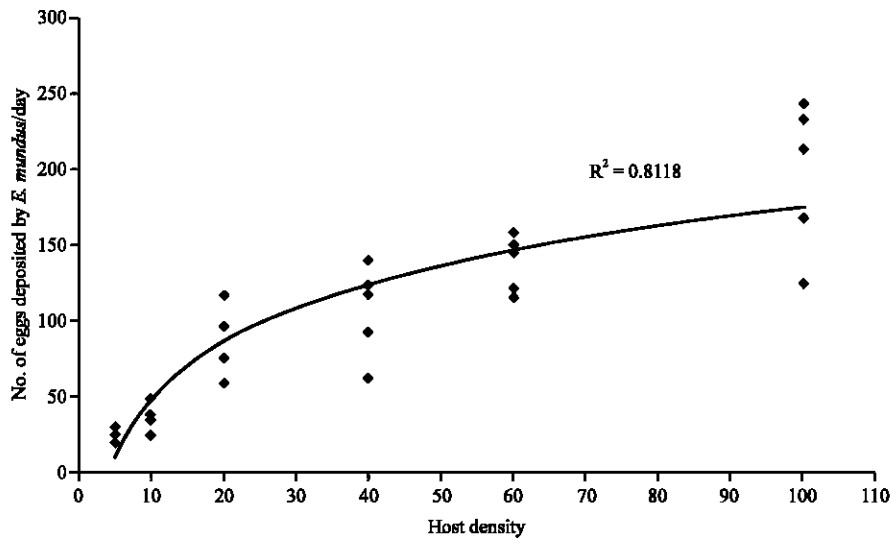

Fig. 2: Total number (Mean±SE) of eggs laid by *Eretmocerus mundus* adult females on different densities of *Bemisia tabaci* second nymphal instar

Numerical response: ANOVA indicated significant overall density effects on mean total fecundity (F = 10.29; df = 5, 4; p = 0.0001) and mean daily fecundity (F = 13.76; df = 5, 4; p = 0.0001). Peak egg production occurred at 100 density (196.4 eggs) (Table 4). At this density, the maximum number of eggs produced in a single day by an individual female was 42.19. Approximately nine times more eggs produced at 100 host density than at 5 host density.

The type II functional response of other aphelinid parasitoids such as *Encarsia formosa* Gahan reported by Yano (1987), Fransen and van Montfort (1987) and Roermund and van Lenteren (1993) on *Trialeurodes*

vaporariorum Westwood, by Lopez-Avilla (1988) and Enkegaard (1994) on *Bemisia tabaci* Gannadius and by Shishehbor and Brennan (1996) on *Trialeurodes ricini* Misra are in accordance with these findings. However, Jones *et al.* (1999) found that *E. mundus* exhibited a type I functional response with *B. tabaci* (strain B). Differences in experimental condition may provide an explanation for discrepancy in functional responses. Jones' treatments contained 5 mated parasitoid females and 5, 25, 50, 100, or 250 whitefly nymphs.

The handling time and the instantaneous rate of discovery or attack coefficient for *E. mundus* parasitizing different densities of second nymphal stage of *B. tabaci*

were 0.3341 and 0.0465 days, respectively (Table 3, estimated by disc equation; random attack equation failed to give positive estimates). Instantaneous rate of discovery (a') is the proportion of the total area searched by a parasitoid/unit of searching time. It determines how rapidly the functional response curve approaches the upper plateau. Moreover, it is a function of (1) maximum distance at which the parasitoid can perceive the host, (2) speed of movement of parasitoid and host and (3) proportion of attacks that are successful (Holling, 1965, 1966). Handling time (Th) is the time a parasitoid spends in identifying, chasing, killing, eating and other related time-consuming activities (Holling, 1959, 1965, 1966). Handling time (Th) and attack rate (a') in this and other similar studies are used mainly for comparative purposes.

O'Neil (1989) and Weidenman and O'Neil (1991) criticized the functional response studies in small laboratory arenas. They argued that under field condition factors such as large searching areas, host plants and weather may influence the effectiveness of natural enemies. Furthermore, natural enemies (predators and parasitoids) seldom occur in nature as single individuals and various developmental stages of prey as well as different prey species may appear simultaneously on a single leaf and/or plant, therefore all these factors may affect the functional response of a natural enemy. Thus it is of interest to know how the responses of natural enemies found in the laboratory reflect their responses in the nature where systems are more complex. However, this kind of experiments is difficult, because not only species composition may both vary spatially and temporally in the nature, but also abiotic factors, such as, temperature may also influence the results.

The longevities of female *E. mundus* determined in the present study may be compared with a study conducted by Tawfic *et al.* (1978) on tobacco. He found that the mean longevity was 3.5 days at 26, 8°C. However, other researchers found a variety of longevity values for this parasitoid. Urbaneja *et al.* (2006) reported longevities of 10.1 and 7.3 days at 25°C on sweet pepper and tomato, respectively. Gerling and Fried (2000) also reported that the longevity of *E. mundus* was 10 days at 25°C on *B. tabaci* on cotton.

Other laboratory studies, with sufficient amount of whitefly nymphs provided, have reported a variety of fecundity values for this species. At 25°C Urbaneja *et al.* (2006) found that *E. mundus* produced 171.1 and 147.8 eggs on *B. tabaci* on sweet pepper and tomato, respectively. Ardeh (2004) observed fecundity of 26.8, 49.4 and 117.5 eggs on *B. tabaci* on gerbera, poinsettia and tomato, respectively. Gerling and Fried (2000)

reported from 81.1 to 247.5 eggs per female during the first 9 days after emergence. However, Sharaf and Batta (1985) reported only 24 eggs/female on tomato and Tawfic *et al.* (1978) 48 eggs at 30°C on cotton.

The results of the present study have improved our understanding of the *E. mundus-B. tabaci* interaction in the laboratory and will be useful for development of a better strategy for the biological control of *B. tabaci* by this parasitoid. Present results suggested that *E. mundus* could be considered for augmentative biological control of *B. tabaci* in cucumber field in southwestern of Iran, however we recommend that additional studies should be conducted in a more natural environment.

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