Pakistan J. Zool., vol. 47(2), pp. 455-460, 2015.

Multiple Effects of Host Density on Egg Density and the Sex Ratio of Progeny of *Bracon hebetor* (Say.) (Hymenoptera: Braconidae)

Anam Jamil,[1] Zain ul Abdin,[1]* Muhammad Arshad,[1] Patrizia Falabella[3], Saqi Kosar Abbas,[1] Muhammad Tahir[1], Amer Jamil,[2] Atif Manzoor[1] and Hoor Shaina[1]

[1]*Department of Entomology, University of Agriculture, Faisalabad, 38040, Pakistan*
[2]*Department of Biochemistry, University of Agriculture, Faisalabad, 38040, Pakistan*
[3]*Department of Science, University of Basilicata, Potenza, Italy*

Abstract.-Insect parasitoid optimises their reproductive potential by exploiting host immune system, varying clutch size and progeny sex ratio with reference to the host density. Egg density and the progeny sex ratio of *Bracon hebetor* Say (Hymenoptera: Braconidae) was studied in relation to the larval density of its host *Galleria mellonella* (Lepidoptera: Pyralidae). Our findings revealed that parasitoid's egg density increased with the increase in host density. A comparison was made between egg laying and egg hatching on different host densities and maximum number of egg laying was observed (134.7 eggs) at the highest host densities (16 larvae) while a minimum number of egg laying (15.6 eggs) was registered at lowest host density (one larvae). The egg hatching was decreased due to crowding of eggs laid on host's larvae and the percentage of adult emergence was also decreased with the increase in crowding of eggs on host's larvae. It was observed that progeny sex ratio (male/total) increased with the increase in host density. Further dispersion pattern of parasitoid eggs on different host densities were estimated by the Green index. This study has generated novel information on egg laying, egg dispersion and sex ratio of *B. hebetor* which may lead to the development of sustainable biocontrol programs for lepidopteron insect pests.

Keywords: Egg density, sex ratio, adult emergence, egg hatching, host density.

INTRODUCTION

Many gregarious insect parasitoids optimise their reproductive potential by regulating the number of eggs (clutch size) on the hosts and the resulting progeny sex ratio (Waage, 1986; Godfray, 1994; Van Alphen and Jervis, 1996). Parasitic hymenoptera shows various strategies to make their hosts optimum for successful parasitization and their progeny development. Host regulation process in insect parasitoids is very complicated. It leads to physiological disorders and nutritional changes in their hosts (Vinson and Iwantsch, 1981; Digilio *et al.*, 2000). Taylor (1988a) reported that parasitoids adjust clutch size to the nutritional value of the host, thereby avoiding larval competition among progeny. Many reports suggest that parasitoids regulate clutch size based on the host size or host quality. For instance, 52 species of *Apanteles* had showed a positive relationship between host size and clutch size (Le Masurier, 1987). *Trichogramma embryophagum* (Hartig) lays more eggs on larger hosts than on smaller ones (Klomp and Teerink, 1967). *Bracon hebetor* parasitizing larvae of *Galleria mellonella* also shows similar ovipositional behaviour related to the host size depositing more eggs on larger hosts than smaller ones (Taylor, 1988b).

Parasitoids also adjust their clutch size based on the host density. *Cephalonomia gallicola* Ashmead), a parasitoid of *Lasioderma serricorne* (F.) lays more eggs on a host when the hosts are rarely encountered (Kearns, 1934). Ullyett (1945) reported that *B. hebetor* regulates allocation of eggs among hosts by decreasing the number of eggs laid on a host as host density increases (Doutt, 1959; Hagstrum and Smittle, 1977).

Sex allocation is an important reproductive decision for parents, deciding the sex of the offspring and how much to invest in them, has received a great deal of theoretical and empirical attention (Charnov, 1982; Hardy, 2005). The study of sex ratios has proven to be the most productive area in evolutionary biology (Charnov, 1982; Frank, 2002). Yu *et al.* (1999) reported that premature death of *B. hebetor* has been registered more when

* Corresponding author: zainunibas@gmail.com
0030-9923/2015/0002-0455 $ 8.00/0

number of the wasp eggs laid on the host (fifth instar larvae of *P. interpunctella*) were increased as compared to less number of eggs laid per female wasp on a host larvae.

B. hebetor is a highly polyphagous gregarious ecto-parasitoid of Pyralid insect pest species of Lepidoptera and they have been used in many biological control programs in different parts of the world. (Fagundes *et al.*, 2005; Yasodha and Natarajan, 2006; Shojaei *et al.*, 2006; Desai *et al.*, 2007; Kyoung *et al.*, 2008; Mohapatra *et al.*, 2008; Inayatullah and Naeem, 2004). It parasitizes a number of important lepidopterous pests of stored products and of field crops (Gupta and Sharma, 2004; Shojaei *et al.*, 2006).

The effects of host quality and quantity in relation to biology and ecology of *B. hebetor* has been reported from different laboratories (Ullyett, 1945; Doutt, 1959; Taylor, 1988b; Yu *et al*., 2003). Elipoulus and Stathas (2008), for instance, investigated the life table parameters of *B. hebetor* parasitising *Anagasta kuehniella* Zeller 1879 (Lepidoptera: Pyralidae) and *Plodia interpunctella* (Hubner 1813) (Lepidoptera: Pyralidae). Their findings were based on the comparison of parasitoid's life duration parameters related to hosts size and various conditions of host density. Yu *et al*. (2003) reported the effect of host density on egg dispersion and the sex ratio of *B. hebetor* and the knowledge of longevity and fecundity of parasitoids are crucial for biological control programs based on augmentative or inoculative parasitoid release (Godfray, 1994; Sahin and Ozkan, 2007).

The present study is focused on the evaluation of multiple effects of host density on egg dispersion and the sex ratio of progeny of *B. hebetor*. This project was designed to generate novel information on egg dispersion and sex ratio of *B. hebetor*.

MATERIALS AND METHODS

Experimental conditions

Experimental insects were reared in the laboratory on 5th instar larvae of greater wax moth *Galleria mellonella* (Lepidoptera: Pyralidae) by following a slightly modified approach as described by Manzoor *et al.* (2011). The adults of the parasitoid, *B. hebetor* were collected directly from the berseem crop, *Trifolium alexandrium* L., located at the main campus of the University of Agriculture, Faisalabad, Pakistan. The larvae, pupae and adults of the host were collected from the infested bee hives located at the main campus of the University. The host and parasitoid cultures were maintained in two separate glass jars, both placed at 27±1^{o}C, 65±5% relative humidity (RH). *B. hebetor* was reared on 12/12 h light and dark photoperiod, while *G. mellonella* larvae were reared in constantly dark conditions. Honey drops containing 50% honey and 50% water were used as a food source for *B. hebetor*, while *G. mellonella* larvae were reared on honey combs and artificial diet.

Experimental procedure

On first day of experiment, newly emerged male and female wasp (five each) were paired and allowed them to mate for 24 h in glass vials (2 cm × 10 cm). On day 2 five groups of two days old, equal weight, 5th instar host larvae were provided in Petri dishes (8.5cm×1.3 cm). All the Petri dishes were numbered (G1-G5) as G1=1 larvae/Petri dish, G2=2 larvae/Petri dish, G3=4 larvae/Petri dish, G4=8 larvae/Petri dish and G5= 16 larvae/Petri dish. Ten gram wax was provided in each Petri dish and the larvae were allowed to settle for 2 h. After that a two days old mated female wasp was provided in each Petri dish and allowed to parasitize the hosts for 24 h. The number of eggs laid on each host were recorded and all parasitized hosts were incubated individually at 27±1^{o}C, 65±5% RH and 12/12 h light/dark period. The number of eggs hatched per host was recorded on daily basis. Adult's emergence and progeny sex ratio per host were recorded daily. Each treatment (group) was repeated 20 times. The dispersion pattern of parasitoids eggs among the hosts was estimated as defined by Green index.

Data analysis

The dispersion pattern of parasitoids eggs among the hosts was estimated by the Green index defined as: $Cx = [(s^2/m) - 1]/(\sum x - 1)$ where s^2; m and $\sum x$ are variance, mean and total number of eggs laid, respectively (Green, 1966). Values of Cx may vary from $-1/(\sum x - 1)$ through 0 to +1 as the dispersion pattern ranges from perfectly uniform through random to perfect clumping.

The relationship between progeny sex ratio and the number of *B. hebetor* eggs per host and related factors were examined by analysis of variance (ANOVA) and the means were separated by using the least significant difference (LSD).

RESULTS

Dispersion pattern of parasitoids eggs among the hosts at different host densities

Green index was reported to be least correlated with the mean data analysis as shown in the Figure 1.

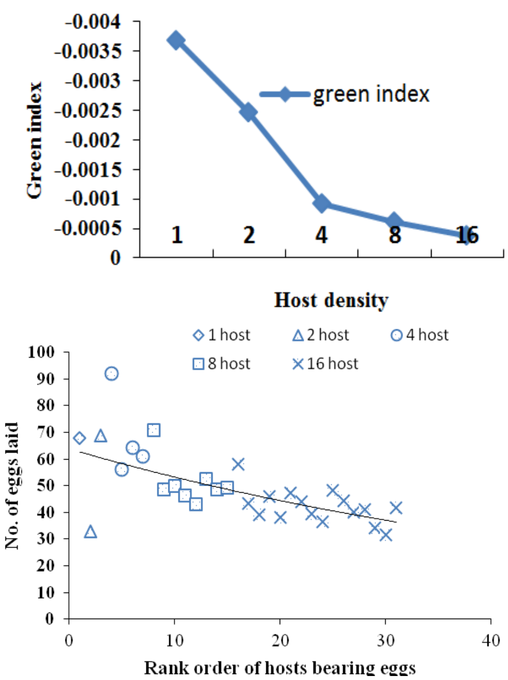

Fig. 1. A, Dispersion of parasitoid eggs on hosts. Dispersion index of egg density of *Bracon hebetor*; B, Relationship between rank order of hosts bearing eggs of *Bracon hebetor* and the number of eggs laid on the host at five different hosts densities.

Egg laying, egg hatching and adult emergence

The analysis of variance of data regarding the mean number of egg laying, egg hatching and adult emergence on different host's density under complete randomized design were given in the Table 1. All the treatments showed statistically significant results on the number of egg laying, egg hatching and adult emergence from various host density.

Table I.- Analysis of variance for egg laying, egg hatching and adult emergence per host at different host density.

Source of variation	DF	SS	MS	F-value	P-value
Egg laying					
Treatment	4	196022	49005.6	560	0.0000
Error	95	8316	87.5		
Total	99	204338			
Egg hatching					
Treatment	4	167479	41869.8	458	0.0000
Error	95	8677	91.3		
Total	99	176156			
Adult emergence					
Treatment	4	117844	29460.9	245	0.0000
Error	95	11407	120.1		
Total	99	129251			

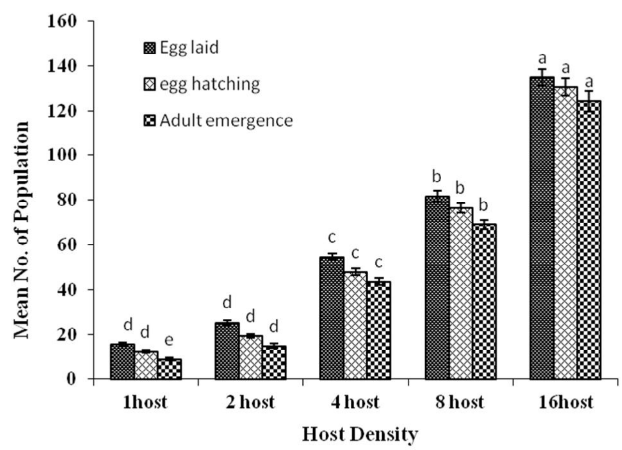

Fig. 2. Mean numbers of egg laid, hatched and adult emergence at different host density.

Mean number of eggs laid, hatched and adults emergence on different hosts densities

Mean number of eggs laid by single female of *B. hebetor* was maximum (134.7 eggs) at highest host density (16 hosts) as compared to single host density where it was recorded minimum (15.6 eggs) as shown in Figure 2. Our data shows egg laying increases as the host density increases and it was noticed that parasitoid optimizes their clutch size on host in such a way that it makes minimum crowding of eggs on the host's body. Maximum egg hatching (130.65 eggs) was recorded at highest host density as compared to single host where hatching was 12.25. It may be speculated that this increase in egg hatching is due to less crowding of eggs on host larvae because of highest host density. It was also observed that percentage of adult emergence is higher at highest host density (16 hosts). This change in percentage of adult emergence is due to that there is less larval competition at highest host density for food, so most of them were developed successfully while parasitic larvae were died at less host density. Finally, it was concluded that both egg hatching and adult emergence increases as the host density increases.

ANOVA for sex ratio of parasitoid emerged from different host densities

The analysis of variance of data given in (Table II) showed the mean percentage of male and female wasp emerged at different host's density under completely randomized design.

Table II.- Analysis of Variance for male and female on different host density.

Source of variation	DF	SS	MS	F-value	P-value
Male					
Treatment	4	105446	26361.5	235	0.0000
Error	95	10642	112.0		
Total	99	116089			
Female					
Treatment	4	1535.74	383.935	23.8	0.0000
Error	95	1531.30	16.119		
Total	99	3067.04			

The data given in the Figure 3 showed the progeny sex ratio, maximum number of male population (88.62) was recorded at 16 hosts (maximum host density) while it was 33.85 at single hosts. It was recorded that progeny sex ratio (male/ total) increases as the host density increase. Female sex ratio initially increases but gradually decreases with the increase in host density.

DISCUSSION

The effects of host density on egg density and sex ratio of *Bracon hebetor* was studied. An average 15.6 eggs were laid when a single host larvae was allowed to be parasitized by the female wasp. It was observed that mortality of *B. hebetor* larvae increases with the increase in egg density per host. Our findings are similar to Yu (1999) who reported that when egg density is higher on the hosts then the mortality of immature progeny is increased. Our findings are in accordance with Waage (1986) who discussed the relationship between the clutch size and frequency of host being encountered based on optimization theory, he demonstrated that clutch size of a parasitoid decreases as host density increases.

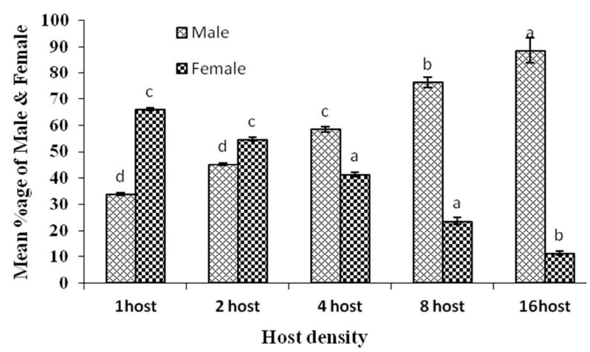

Fig. 3. Mean numbers of male and female sex ratio at different host density.

It is the characteristic behavior of female wasp as it first paralyses all the hosts, then lays eggs randomly. When number of laid eggs increases 7 per host larva, then it relocates them on next host. Similar finding was also reported by Yu *et al.* (2003) who described that egg dispersion is correlated by host density and the probability of egg deposition per host constantly lower down with an increase in the host density. The findings are also in accordance with Ullyett (1945) who reported that *B. hebetor* regulates allocation of eggs among hosts by decreasing the number of eggs laid on a host as host density increases (Doutt, 1959; Hagstrum and Smittle, 1977).

It was found that progeny sex ratio of the wasp is dependent on the number of eggs laid by the wasp and the host density. The egg laying potential of each female parasitoid increases with the increase in host density which results in more male progeny as compared to female.

ACKNOWLEDGEMENTS

We are highly thankful to HEC (Higher education commission, Government of Pakistan (20-1455/R&D/09/223) and IFS (International foundation of Science, Sweden) (Grant No. F/5089-1) for providing financial assistance for this research work.

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(Received 13 August 2014, revised 21 January 2015)