Effect of host’s *Helicoverpa armigera* (Hubner) larval age on the numerical response of the parasitoid *Campoletis chlorideae* Uchida

Maheshwar Singh, Bhuwan Bhaskar Mishra and CPM Tripathi

Abstract
The numerical aspects of interaction between *Campoletis chlorideae* Uchida (Hymenoptera: Ichneumonidae) and different larval stage of the host *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) at different parasitoid density were studied in entomological laboratory, department of Zoology, D. D. U. Gorakhpur University, Gorakhpur, U. P. in summer season of 1999-2000. The host larval age and density of the female parasitoid affect the rate of parasitization. The number of emerging offspring was higher with the second instars larvae (y = 23.9 + 16.279 log x; r = 0.951; P< 0.001), followed by III instar larvae (y = 21.7 + 17.27 log x; r = 0.966; P<0.001), I instar larvae (y = 20.9 + 16.279 log x; r = 0.951; P<0.001) and then IV instar larvae (y = 12.7 + 8.970 log x; r = 0.996; P<0.001) of the host *H. armigera*. With the increase of the parasitoid density, the number of emerging offspring increased nonlinearly with a (F = 48.532; P<0.001) significant correlation. The number of progeny inter-specific competition amongst parasitoid on the 50 hosts in all the larval stages. The higher number of parasitoids have lower rate of multiplication, supports the existence of mutual interference.

Keywords: Numerical response, *Helicoverpa armigera*, *Campoletis chlorideae*, Biological control

1. Introduction
The pod borer *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) is an important pest of chickpea and its causes enormous losses to this crop [1-3]. The endoparasitoid *Campoletis chlorideae* Uchida (Hymenoptera: Ichneumonidae) is an effective bio control agent against the gram pod borer *H. armigera* and parasitises the moths larval stages [4-9]. The parasitoid *C. chlorideae* helps in suppressing the pest population on chickpea [10, 11].

The numerical response, which is a change in the density of the parasitoid in response to change in host density [12, 13]. The impact of a parasitoid on its host population depends upon, among other things, its ability to increase in number and to find and parasitise hosts [14]. The rate of parasitisation, which is directly dependent on host number, is considered as an important condition for both the stability of parasitoid-host population interaction and the success of biological control [15]. Soloman [12] first proposed terms to describe the two-fold nature of the response of a parasitoid to change in the density of the host.

The “Numerical response” is a change in the density of the parasitoid population in response to change in host density. The numerical response is usually of vital population [16] and helps in calculating the number of parasitoid needed to regulate the estimated host population [17]. According to Coppel & Mertins [18], a rapid and strong numerical response characteristic is the most important attribute of a successful agent of pest mortality.

Although numerical response plays a major role in decimating pest population than functional response yet, it has received less attention [19]. This bias towards functional response may lead to erroneous conclusions about their effectiveness in controlling host-population. Several authors have investigated the biology and behaviour of *C. chlorideae* but apparently no attempt has been made to investigate the numerical response of *C. Chloridea* on the different larval age of the host *H. armigera*. Thus, the present work is an attempt to study the effect of host’s *Helicoverpa armigera* (Hubner) larval age on the numerical response of the parasitoid *Campoletis chlorideae* Uchida.
2. Materials and methods

2.1 Culture of host and parasitoid

The parasitoid; *C. Chloridea* and its host, *H. armigera* were reared on *Cicer arietinum* Linn. (Chikpea) in the laboratory at 22±4°C, 70±10% RH and 10h light: 14h dark photoperiod [7] in entomological laboratory, department of Zoology, D.D.U. Gorakhpur University, Gorakhpur, U.P. in summer season of 1999-2000. The different larval stage of the host (1 instar to IV instar) were drawn from the maintained culture and utilized as hosts. One day old, Satiated [20] with a 30% honey solution, mated and experienced female [21, 7] parasitoid were used in the experiment.

2.2 Numerical response

To study the numerical response of the parasitoid *C. Chloridea*, 4 troughs (Ca 2 cm. diameter x 10 cm. height) were arranged and numbered as 1, 2, 3 & 4. 50 hosts of the first larval stage were placed separately on four moistened filter paper and were transferred individually in marked troughs. Troughs were covered with glass plates. One, Two, Four and Eight Parasitoid were introduced in 1st, 2nd, 3rd, 4th troughs respectively and were allowed to attack hosts for 3 hrs. The experiment was replicated five times with new experienced female parasitoid and fresh hosts. The same processes are applied to the second, third & fourth instar larvae of the host *H. armigera*.

After parasitisation the larvae were transferred in the glass tubes (10x3.25 cm) having fresh foliage of *C. arietinum* (chickpea) for further development. The tubes were covered with muslin cloth. After cocoon formation, the cocoons were collected and transferred singly into mark sterilized glass vials (5x1.25 cm) with leaf cuttings of the host plants [22] (to provide moisture to the developing eggs) until emergence. The mouth of the glass vials were kept plugged with absorbent cotton. After emergence the parasitoid were counted and the data so obtained from the different larval ages of the host was subjected to the regression analysis for better understanding of their interaction.

2.3 Statistical analysis

The parasitoid density, developmental period, fecundity, percentage mortality were analyzed by Two way-ANOVA followed by Duncan’s multiple range test (DMRT) at 0.05 level. Regression analysis was performing between different larval age and parasitoid density.

3. Results

The experiments reported here in were intended to evaluate the relationship between four larval stage (1 instar to IV instar) of the host and the amount of parasitisation at varying parasitoid densities. It was observed that with the increase of parasitoid density, the amount of parasitisation increases non-linearly (Table-1; Fig. 1). The amount of parasitisation increases significantly with the increase of parasitoid density and it was maximum in II instar larvae (y =23.9 + 16.279 log x; r = 0.951; P<0.001), followed by III instar larvae (y = 21.7 + 17.27 log x; r = 0.966; P<0.001), I instar larvae (y = 20.9 + 16.279 log x; r = 0.951; P<0.001) and then IV instar larvae (y = 12.7 + 8.970 log x; r = 0.996; P<0.001) of the host *H. armigera* (Table-1; Fig. 1). Analysis of variance shows that the amount of parasitisation was significantly influenced between four host larval age (F = 13.187, P<0.01) and between four parasitoid density (F = 79.801, P<0.001) (Table-4).

The rate of multiplication (number of parasitoid emerged / number of parasitoid put in for parasitisation) was maximum with one parasitoid on 50 hosts (Table-3; Fig. 2). Similar results have also been obtained in the entire four host’s larval age. As the parasitoid density were increases, the rate of multiplication decreases. This decrease was maximum in 4th larval age (y = 12.025 – 11.337 log x; r = -0.996, P<0.001) followed by 1st larval age (y = 18.85 – 16.694 log x; r = -0.972, P<0.001) then 3rd larval age (y = 19.725 – 17.525 log x; r = -0.995, P<0.001) and 2nd larval age (y = 21.55 – 19.559 log x; r = -0.994, P<0.001) of the host (Fig. 2). Analysis of variance also revealed that amount of parasitisation was also significant between four host larval age (F = 13.187, P<0.01) and between four parasitoid density (F = 79.801, P<0.001) (Table-4).

### Table 1: Number of offspring emerged at different initial number of parasitoid *C. Chloridea* put with 50 hosts of different larval age of *H. armigera* for 3 hrs. Each entry is the mean of five replicates (mean±SD).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Sum of squares</th>
<th>Mean Squares</th>
<th>F-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between 4 host age</td>
<td>3</td>
<td>518.687</td>
<td>172.879</td>
<td>48.532*</td>
</tr>
<tr>
<td>Between 4 parasitoid density</td>
<td>3</td>
<td>415.187</td>
<td>138.396</td>
<td>38.848*</td>
</tr>
<tr>
<td>Between interaction</td>
<td>9</td>
<td>32.062</td>
<td>3.562</td>
<td>*P&lt;0.001</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>965.937</td>
<td>64.3958</td>
<td></td>
</tr>
</tbody>
</table>

*Significant at 99% probability level. *F* values were significant at all probability levels (90, 95 and 99%), *df=3, 20; *df=2,20.

### Table 2: Two-way ANOVA data of number of offspring emerged at different initial number of parasitoid *C. Chloridea* put with 50 hosts of different larval age of *H. armigera*.

<table>
<thead>
<tr>
<th>Initial parasitoid density</th>
<th>1st instar</th>
<th>2nd instar</th>
<th>3rd instar</th>
<th>4th instar</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19±2.82</td>
<td>22±3.28</td>
<td>20±3.75</td>
<td>13±2.82</td>
</tr>
<tr>
<td>2</td>
<td>28±2.44</td>
<td>31±2.82</td>
<td>29±3.16</td>
<td>15±3.68</td>
</tr>
<tr>
<td>4</td>
<td>32±1.41</td>
<td>35±2.28</td>
<td>33±1.78</td>
<td>18±2.28</td>
</tr>
<tr>
<td>8</td>
<td>34±2.32</td>
<td>37±2.00</td>
<td>36±1.41</td>
<td>21±3.46</td>
</tr>
<tr>
<td>Pooled SE</td>
<td>1.399</td>
<td>1.417</td>
<td>1.470</td>
<td>0.972</td>
</tr>
</tbody>
</table>

Regression: y = a+b log x

<table>
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<th>F-Value</th>
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<tr>
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<td>Total</td>
<td>15</td>
<td>965.937</td>
<td>64.3958</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3: Number of offspring emerged per parasitoid at different initial number of parasitoid *Campeolites chlorideae* put with 50 hosts of different larval age of *Helicoverpa armigera* for 3 hrs. Each entry is the mean of 5 replicates (mean±SD).

<table>
<thead>
<tr>
<th>Initial parasitoid density</th>
<th>1st instar</th>
<th>2nd instar</th>
<th>3rd instar</th>
<th>4th instar</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19±2.82</td>
<td>22±3.28</td>
<td>20±3.75</td>
<td>13±2.82</td>
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<td>31±2.82</td>
<td>29±3.16</td>
<td>15±3.68</td>
</tr>
<tr>
<td>4</td>
<td>32±1.41</td>
<td>35±2.28</td>
<td>33±1.78</td>
<td>18±2.28</td>
</tr>
<tr>
<td>8</td>
<td>34±2.32</td>
<td>37±2.00</td>
<td>36±1.41</td>
<td>21±3.46</td>
</tr>
<tr>
<td>Pooled SE</td>
<td>1.399</td>
<td>1.417</td>
<td>1.470</td>
<td>0.972</td>
</tr>
</tbody>
</table>

Regression: y = a+b log x

The experiments reported here in were intended to evaluate the relationship between four larval stage (1 instar to IV instar) of the host and the amount of parasitisation at varying parasitoid densities. It was observed that with the increase of parasitoid density, the amount of parasitisation increases non-linearly (Table-1; Fig. 1). The amount of parasitisation increases significantly with the increase of parasitoid density and it was maximum in II instar larvae (y =23.9 + 16.279 log x; r = 0.951; P<0.001), followed by III instar larvae (y = 21.7 + 17.27 log x; r = 0.966; P<0.001), I instar larvae (y = 20.9 + 16.279 log x; r = 0.951; P<0.001) and then IV instar larvae (y = 12.7 + 8.970 log x; r = 0.996; P<0.001) of the host *H. armigera* (Table-1; Fig. 1). Analysis of variance shows that the amount of parasitisation was significantly influenced between four host larval age (F = 13.187, P<0.01) as well as between the four parasitoid density (F = 38.848; P<0.001) (Table-2).
Table 4: Two-way ANOVA data of number of offspring emerged per parasitoid at different initial number of parasitoid *C. chlorideae* put with 50 hosts of different larval age of *H. armigera*.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Sum of squares</th>
<th>Mean Squares</th>
<th>F-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between 4 host age</td>
<td>3</td>
<td>80.320</td>
<td>26.773</td>
<td>13.187*</td>
</tr>
<tr>
<td>Between 4 parasitoid density</td>
<td>3</td>
<td>486.062</td>
<td>162.020</td>
<td>79.801*</td>
</tr>
<tr>
<td>Between interaction</td>
<td>9</td>
<td>18.273</td>
<td>2.030</td>
<td>P&lt;0.01*</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>584.656</td>
<td>38.977</td>
<td></td>
</tr>
</tbody>
</table>

*Significant at 99% probability level. *F values were significant at all probability levels (90, 95 and 99%), *df=3, 20; **df=2,20

4. Discussion
The host age significantly affects the number of parasitisation at varied parasitoid densities. The endolaryngeal parasitoid *C. chlorideae* parasitises the different larval age of the host *H. armigera*. The female parasitoid when come near the host is attracted towards it. This attraction of the parasitoid to the host is mainly due to odour of the host [23, 24]. Having reached the region in which the host is located, the female parasitoids tries to find out the exact spot by vibrating its antennae. When once the parasitoid is within the reach of the host, it is probably gives to final stimulus to oviposition [25].

With the increase of parasitoid density, the number of emerging offspring increases up to four parasitoids then shows a tendency towards stabilization. This indicates that in order to achieve a considerable number of parasitoid more parasitoid might be required [26, 27]. Table-1 and Fig. 1 showed that when double the numbers of parasitoid were put in for parasitisation, a doubling of parasitisation ratio in any case was not observed. This might be due to limited oviposition time [21, 28] and the reduction of individual’s efficiency because of mutual interference [29,30]. Under field conditions, a strong mutual interference might be taking place due to an increase in the parasitoid density, which may result in the dispersal of the parasitoid [32].

The amount of parasitisation increases significantly with the increase of parasitoid density in the entire four host’s larval stage and in maximum in 2nd instars larvae due to chemical stimuli (kairomones) and the other different factors. The kairomones stimulate the host seeking response of the parasitoid, thus play a significant role in host location, and host acceptance by the parasitoid [33, 34]. The parasitoid *C. chlorideae* preferred 2nd instars larvae of the host *H. armigera* [7, 8] because this stage, in addition to having more food than first instars and quality of food resources kairomones, emanates more host- seeking stimulant. The large size of the host, hardness of the cuticle and defense mechanism of 3rd and 4th instars of the host *H. armigera* was play an important role in the host stage preference by the parasitoid [35].

The rate of multiplication has an inverse relationship with the parasitoid density. This is the most significant trend exhibited during this study. More parasitoid has a lower rate of multiplication which shows the intraspecific competition amongst the parasitoid [36, 27, 37]. The number of offspring emerged per parasitoid (Table-3; Fig. 2) was maximum in 1 parasitoid density and then decreases to 8 parasitoid density. The number of offspring was maximum in 2nd larval instars and is followed by 3rd instars, 1st instars and 4th instars of the host *H. armigera*.

During the experiments, different types of interferences amongst the parasitoid were recorded: (i) Direct interference amongst the parasitoid (mutual interference of intraspecific competition). Interference may insure due to intraspecific or due to physical or chemical nature of the host plant [38] on which the hosts are found. In their attempt to approach the host or in trying to overcome the obstacle in approaching the host many encounters amongst them occur. This is a probable course of interference. Direct interference might be in the form of heads on (antennal encounter), or head of one and tail of the other (Cephalo-Caudal encounter), or in the form of rubbing of the sides when one is trying to overtake the other during movement (lateral encounter), or even only the caudal ends of the parasitoid may be touch each other (Caudal encounter). This type of interference decreases their searching sufficiency [39]. (ii) Avoidance of contaminated hosts and (iii) Loss of parasitoid egg due to mutilation [40]. These factors might be acting singly or in conjunction. At higher densities, the ovipositing females were brought in close proximity to each other because of less oviposition area and as a result,
they probe in the location on the sting unit \[41\]. Mutual interference can also cause fewer eggs to be laid \[22\] and increase the production of male progeny \[14, 42\].

The results obtained showed that the parasitoid density increases the mutual interference increases, which cause a reduction in individuals’ searching efficiency \[43, 30\]. The effect of this interference explored theoretically by \[44, 45\]. They demonstrated that the greater the value of mutual interference constant the greater is the tendency for the interaction to become stable. However, Stinner and Lucas \[46\] consider that no such interference exists and decrease in the area of discovery with increase in parasitoid density is the results of a non-Poisson distribution of its eggs.

5. Conclusion
The present investigation furnished an insight into the number of parasitoid needed to regulate the estimated host population. The results discussed so far reveal that \(C.\ chloridae\) is a potential bio control agent against \(H.\ armigera\). The number of emerging offspring was maximum in 2nd instars than other instars. It is therefore, recommended that less number of parasitoid may be released at any recommended site for control purposes because the rate of multiplication is maximum with one parasitoid on 50 hosts.

6. References
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