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Muhammad Adnan
Faculty of Agriculture, Department of Entomology, Gomal University, Dera Ismail Khan

Ashraf Khan
Agricultural Research Institute (ARI), Entomology Section. Ratta Kulachi, Dera Ismail Khan

Correspondence
Muhammad Adnan
Faculty of Agriculture, Department of E ntomology, Gomal University, Dera Ismail Khan

Effect of prey density the biology and functional response of *Chrysoperla carnea*

Muhammad Adnan and Ashraf Khan

Abstract
Effect of prey density on biology and functional response of green lacewing, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) was studied in the laboratory of Entomology Section of Agricultural Research Institute, Dera Ismail Khan at 25±1 ºC, 65±5% RH and 10:14 light : dark regime. Newly emerged larvae of *C. carnea* were fed 20, 30, 40, 50, 60, 70, 80, 90 and 100 fresh eggs of *Sitotroga cerealella* (Lepidoptera: Gelechiidae) in a small plastic bottles. It was observed that the prey density had a significant effect on positive consumption rate, development and fecundity of *C. carnea*. In general maximum consumption with shortest developmental time, maximum fecundity and longest adult longevity were observed as prey density increased. In all treatments, the predatory potential was high when the prey density was raised. Daily predation rate of *C. carnea* increased slowly during the first two instars and reached to its peak in the third larval instars. Although, *C. carnea* completed its development at all prey densities, the increase in prey densities reduced developmental time and mortality. Lacewing larvae provided with an overabundance of *S. cerealella* eggs developed faster than the larvae provided with fewer eggs. Lacewing fed during larval stage with 20 eggs/day showed the lowest fecundity with the increase in prey density. A smaller intrinsic rate of increase was due to the fact that the population fed at a low prey density had prolonged developmental time, the higher mortality rate in immature stages as well as a low daily rate of progeny.

Keywords: *Chrysoperla carnea*, *Sitotroga cerealella*, prey density, functional response

1. Introduction
The Green lacewing, *Chrysoperla carnea* (Stephens) belongs to order Neuroptera, family Chrysopidae and genus *Chrysoperla.* This order consists of a group of insects with rather soft bodies, biting mouthparts and two pairs of very similar membranous wings which are usually held roof-like along the abdomen at rest. It has long been assumed to be a single morphologically identical species with a holarctic distribution [1]. They are pale green, about 12-20 mm long with long antennae and bright, golden or copper-coloured eyes. These adults are active fliers, particularly during the evening and night and have a characteristic, fluttering flight [2]. The eggs of green lacewing are oval in shape and secured under the leaves in field condition and under the surface of the cage in laboratory condition by long slender stalks. Oval shaped eggs are protectively laid singly at the end/ tips of long silken stalks, resembling miniature cattails growing from the plant foliage, these are pale green, turning gray in 2-3 days. After 6-7 days eggs hatch out, the larvae which are very active, have three instars, and are gray or brownish, alligator-like with well-developed legs and large pincers with which they suck the body fluids of the prey. The larvae of *C. carnea* are brownish in colour. Mature third instar larvae spin round, parchment-like silken cocoons usually in hidden places in plants and pupate inside cocoons. Larvae grow from <1 mm to 6-8 mm. The emergence of adults occurs in 8-10 days. There may be two to several generations per year [3].
It is rare in the tropics to find a large colony of Aphis without some neuropterans larvae feeding on them [4]. One larva may devour as many as five hundred Aphids in its life and there is no doubt that they play an important part in the natural control of many small homopterous pests [5]. *Chrysoperla spp.,* especially *C. carnea* an *C. rufilabris,* are sold commercially by numerous producers and suppliers [6, 7] to control insect pests. Green lacewing is an example of one of these species that is not predacious in the adult stage; larval stage is predatory stage while in some species adults are also predators [5, 8]. It also feed on slow moving, soft-bodied arthropods such as aphids, jassids, thrips, whitefly, scales, mealy bugs and mites [9].

The more recent evidence suggests that it is not a single species but instead a complex of several to many biological species characterized by different male courtship songs [10].

Different experiments were conducted by many entomologists on *Chrysoperla sp*. using artificial diets and natural food (insect host) for their rearing in the laboratory and tried to suggest simple, effective and cheaper diet for further experiments. The proper and correct proportion of each food component is very much important. Both larval and adult food compositions were tested and evaluated. Ulhaq tested three larval diets to find out cheaper and better diet for their mass rearing. The diets were compared with the standard diet containing yeast extract, casein, honey, sugar and distilled water to evaluate fecundity, larval period, pupal period and adult longevity. Their results revealed that the mixture of egg yolk, milk and honey was better than all other diets [15]. Two entomologist tested three larval diets i.e a mixture of honey, yeast, and water and semi artificial diet was a mixture of honey, essential amino acid, yolk egg powder, extracting of flour moth's body, a, d, e and b vitamin groups, yeast, and water to evaluate developmental time, weight, longevity and mortality percentage of larvae and pupae and fecundity, fertility and sex ratio of female. The best artificial diet out of three was semi-artificial diet which showed the highest weights of larvae and pupae [11].

The diets contained artificial ingredients significantly influenced oviposition and post-oviposition periods and fecundity of females [12, 13]. However, when *C. carnea* larvae were reared on nymphs of *Schizaphis graminum, Bemisia tabaci* as well as artificial diets, significant impact was determined on fecundity, larval duration, hatchability and sex ratio (female/male). During the scarcity of natural foods the artificial diet is the best source for mass rearing of *C. carnea* (Joker and Zarabi, 2012) [14].

For female fecundity and expanded adult life span researchers conducted experiments suing different artificial as well as natural diets and their concentrations. Using several artificial diets suggested that the diet containing egg yolk, milk and honey in the ratio of 5ml: 10ml: 5ml proved to be the best resulting in significantly higher egg laying by the female *C. carnea* as compared to the other diets tested under the same laboratory conditions [15]. This diet consists of three components and each component has the promoting effect on egg production. [16] The sugar is a very important component in adult diet for the insects that has pronounced effect on the egg production. Similarly it had recommended yeast and sugar for maximum egg production. Honey is also a very important component regarding fecundity, [17, 18] analyzed that a mixture of honey and yeast autolysate is a suitable adult diet for production of fertile eggs. Last but not the least component is yolk that is the most important one. [12] reared adults of *C. carnea* on adult diet consisting of milk, eggs, fruits sugars and yeast and found a favorable effect on fecundity. Higher fecundity observed in diet containing egg yolk is because as egg yolk is rich in protein (amino acids) [19]. There are 15.5% amino acids as compared to egg white and mixed egg which contain 9.8% and 11.95% respectively [19]. Similarly folic acid, which is particularly more important for egg productions is much higher (117 µ g) in egg yolk than in mixed egg (73 µ g) and an egg white (3 µg) [20].

2. Materials and Methods

An experiment on the "biological parameters and functional attributes of *C. carnea* reared on natural and artificial diets" was conducted at Agricultural research institute, Dera Ismail Khan under laboratory conditions.

2.1 Experimental materials

The materials used in this in this experiment were *Sitotroga cerealella* rearing chambers, wheat, camel brush, needle pin, black paper sheets, egg sieving mesh, small size plastic bottles, honey, yeast, distilled water, C. carnea adults rearing cages, magnifying glass and simple microscope.

2.2 Methodology

The experiment was conducted in Completely Randomized Design (CRD) with 9 treatments and three replications. Newly hatched larvae of *C. carnea* were used in this experiments. The predatory potential of the predator was tested by providing different concentration of host (*Sitotroga cerealella)* eggs as treatments with the increase of 10 eggs per treatment starting from 20 eggs/treatment up to 100 eggs/treatment. Eggs were properly counted with the help of camel hair brush and magnifying glass on the black sheet which helped in the visibility of eggs, and put in small size plastic bottles of the same size. 1st larval instars of *C. carnea* were then introduced into that vary bottles.

2.3 Rearing of the host, *Sitotroga cerealella*

Sterilized whole wheat grains were used to maintain *S. cerealella* culture. Wheat then dried, spread in trays in the refrigerator then eggs of *S. cereallala* were introduced in these trays. Larvae of *S. cerealella* hatched out after about a week which was fed on the wheat grain for 25 days, the adults were then collected in glass jars with the help of aspirator and transferred in to the adult jars with a fine mesh (size 40) on one side to facilitate the egg laying. The jars containing adults were placed on its side with mesh down on trays containing laundry starch to receive eggs, which were collected by sieving the starch in 100 mesh sieve. The freshly collected eggs were used for the experiment and recycle of the culture.

2.4 Rearing of *Chrysoperla carnea*

The adult of *Chrysoperla carnea* were collected from the cotton field of ARI, and kept in glass rearing cages provided with artificial diet (honey, yeast and distilled water) There eggs were detached from the black sheet with the help of sharp razor blade and then one egg of *C. carnea* transferred into each treatment. Freezed eggs of *S. cerealella* were provided on daily basis after hatching larva.

2.5 Data recording

All biological parameters including a larval and pupal period (days), percent adult emergence, adult survival, longevity and time of egg handling were recorded on daily basis. From hatching till spinning of the cocoon was designated the larval period and from cocoon formation and coming out from the pupal case as a pupal period. During larval period fresh eggs were given on daily basis and old/consumed eggs were removed. Larval stages were differentiated by watching the exuae in each treatment.

2.6 Handling of eggs

Egg searching, catching and consumption time was recorded for each larval instars. Handling time was noted to be started after eating on the egg. The search for new egg till its consumption was recorded through stop watch. Two larval instars were observed each day under the simple microscope in each treatment.

2.7 Percent adult emergence

Percent adult emergence was observed in all the treatments in all the replications. The time from egg hatching till adult emergence was considered as a reproductive period. Adults emerged from the treatments were then converted into a percentage using the following formula.

$$\text{Percent adult emergence} = \frac{\text{Emerged adults x100}}{\text{Total eggs}}$$

2.8 Statistical analysis

The data recorded for each parameter was analyzed statically by using Statistix 8.1 software and means were separated by using Fisher Protected Least Significance Difference Test at 5% level of significance [21].

3. Results and Discussion

3.1 Effect of food density on different life stages and total longevity (in days) of *C. carnea*

Results regarding duration of different life stages duration are shown in Table 1. It indicated that duration of different stages decreased with the increase in food density. Duration recorded for first larval instar fed with various food densities was statistically significant. The minimum (1.33days) duration was recorded for T6 (70 eggs/day) which was followed (1.67 days) by both T5 (60 eggs/day) and T8 (90 eggs/day). Duration recorded for T7, T9, T4, T3 and T2 was 2.33, 2.33, 2.67, 3.33 and 3.67 days, respectively. The maximum duration was observed for 20 eggs/day treatment (T1) which was 4.67 days. A significant difference was recorded among duration spent by larvae in the 2nd instar feeding on different densities of food. The minimum duration was recorded at T9 (100 eggs/day) which was followed (4.33 days) by T6 (70 eggs/day), T7 (80 eggs/day) and T8 (90 eggs/day). Duration recorded at T1, T3, T4, and T5 were 7.33, 7.33, 6.67, and 5.33 days respectively. The instars provided with 30 eggs/day (T2) took maximum time (7.67 days) to emerge into 3rd larval instar. - Results of the 3rd larval instar also noted to be different significantly. The minimum duration was recorded for T4, T8, and T9 which was 2.33 days for each, followed by T6, and T7 with 2.67 days each. The larvae in in T2, T3, and T5 took 4.00, 3.33, 3.33days, respectively, before spinning cocoons around them. The maximum time (4.33 days) was required for the larvae in T1 (20 eggs/day) to enter into it next stage. Duration spent in the pupal stage was not significantly different, however maximum and minimum days were recorded for T8 (8.33 days) and T5 (5.33 days)In case of adults longevity, significant minimum duration (5.33 days) was observed in the treatment having 20 eggs/days (T1) while maximum duration was noticed in treatment containing 70 eggs/day (T6) which was 10.67 days. The minimum duration was followed by T3 (6.67 days). Longest total longevity (29.33 days) was commenced in 20 and 30 eggs/day treatments followed by 40 eggs/day treatment (27.00 days) while shortest adult longevity (23.67 days and 24.33 days) was noticed in 60 eggs/day and 100 eggs/day treatments, respectively.

It is evident from the results that increased number of host eggs decreased reproductive period in all the three larval instars. However it did not influence pupal duration. The adults with less food lived for a shorter period as compared to sufficient food treatments. Total time spent by C. carnea in reproductive as well as the adult stage was maximum for that treatment which were containing lower food density.

Table 1: Effect of food density on different life stages and total longevity (in days) of *C. carnea*

Treatments (No. eggs)	1st Instar	2nd Instar	3rd Instar	Pupa	Adult Longevity	Total Longevity
T1-20	4.67a	7.33a	4.33a	7.67	5.33e	29.33a
T2-30	3.67ab	7.67a	4.00ab	7.00	7.00cde	29.33a
T3-40	3.33ab	7.33a	3.33abc	6.33	6.67de	27.00ab
T4-50	2.67bc	6.67ab	2.33c	6.67	8.33bcd	26.67ab
T5-60	1.67c	5.33bc	3.33abc	5.33	8.00bcd	23.67b
T6-70	1.33c	4.33cd	2.67bc	6.67	10.67a	25.67ab
T7-80	2.33bc	4.33cd	2.67bc	7.67	9.33ab	26.33ab
T8-90	1.67c	4.33cd	2.33c	8.33	8.33bcd	25.00b
T9-100	2.33bc	2.67d	2.33c	8.00	9.00abc	24.33b
LSD	1.51	1.72	1.36	ns	2.29	3.97

Means in columns followed by a different letter(s) are significantly different at 5% level of probability (LSD-TEST).
*ns = Non significant

3.2 Percent adult emergence of *C. carnea* feeding on different densities of host eggs

Fig. 1 indicated results regarding adult emergence provided different food density. It revealed that emergence of adult increased with the increase in the concentration of eggs/food density. Minimum emergence was observed when provided 20 eggs. Adult emergence was noted to be increased as the provision of more eggs/food was insured. There were fewer variations in adult emergence when they were provided 40-70 eggs. However, it seemed to be maximum when the number of eggs/food increased to 90. No much difference was observed when provided 80 and 100 eggs as well.

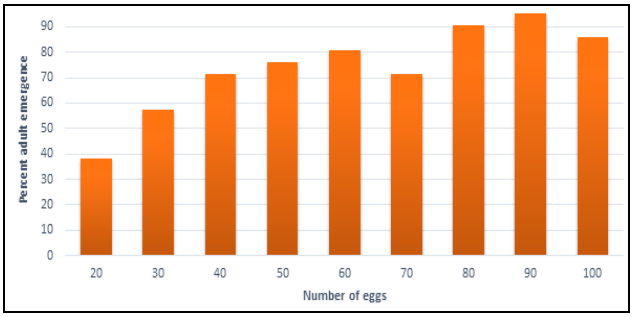

Fig 1: Percent adult emergence of *C. carnea* feeding on a different number of host eggs

3.3 Time of egg handling for instars of *C. carnea* provided with a different number of host eggs

Fig. 2 revealed the time taken by 1st, 2nd and 3rd larval instars of *C. carnea* to search out and consume egg. It was observed to be decreased with the increase in a number of eggs in the small plastic bottles, however no much variation in handling time was noted beyond 40 eggs/ bottle. Maximum time was recorded for 1st instar provided with 20 eggs followed by 40 eggs. Time spent by instars having 50-100 eggs revealed less difference among themselves. More time needed to handle the egg was recorded for 2nd instar provided with 30 eggs/day, followed by the treatment having 20 eggs/day. No much evident variation in the handling time was noticed in the rest of the treatments. In the case of 3rd instar, maximum time was recorded for instar having 30 eggs/day which was followed by 20 and 100 eggs/day treatments. The rest of the treatment revealed less difference among themselves However, convincingly less time was taken by instars in bottle having access to 70 eggs/day.

It is evident from the Fig. 2 that handling time variation decrease with the increase in eggs density per treatment for about all the larval instars. No effect was noted in handling time beyond 50 eggs/ treatment. However, it was also observed that when the number of eggs was lesser in the defined treatments, it compelled the larval instars to spend more time to search and consume egg.

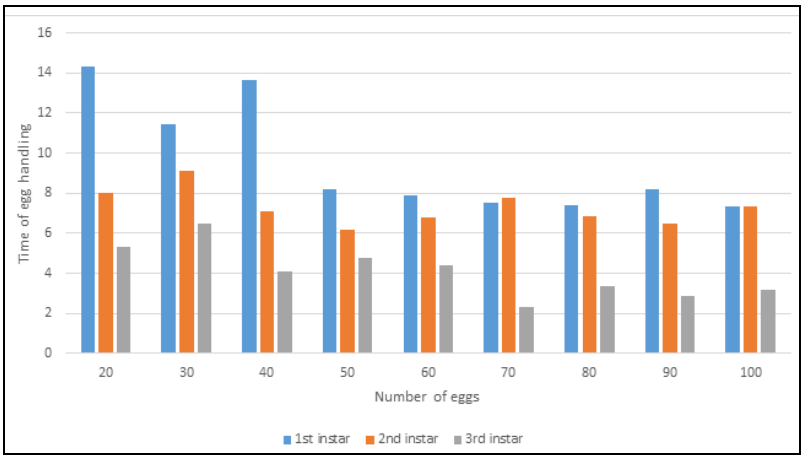

Fig 2: Time of egg handling for instars of *C. carnea* provided with a different number of host eggs.

4. Conclusions and Recommendations

The results of the present experiments are concluded and recommendations made as following:

Reproductive stages, as well as adult longevity, were affected by different densities of *S. cerealella* egg. The Food density (host eggs) also influenced percent of adult emergence which showed as increase as the density of host eggs increased. Although handling time of larval instars were not much affected by food density, however, variations were commenced in lower egg densities. The results recommend that density in between 50-70 eggs/ larvae per day should be provided in laboratory conditions for the smooth rearing of *C. carnea.*

These findings could be useful in defining more optimum conditions for the mass rearing of *C.carnea.*

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