

Host deprivation effects on the reproduction and survival of the parasitoid *Campoletis chlorideae* Uchida (Hymenoptera: Ichneumonidae)

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Abstract

The ichneumonid *Campoletis chlorideae* Uchida is an arrhenotokous idiobiont parasitoid that infests second instar larvae of the pod borer, *Helicoverpa armigera* (Hübner), a severe pest of pulse crops in India. For successful biocontrol practices, abundant hosts must be present in fields at the time of the mass release of parasitoids. Host deprivation, in general, influences the reproduction and survival of parasitoids. However, how host deprivation affects the reproduction and survival of *C. chlorideae* is still unknown. In the present study, we attempted to evaluate the longevity, fecundity and progeny sex ratio of *C. chlorideae* in response to host deprivation. The second instar larvae of the *H. armigera* were reared on an artificial diet under laboratory conditions [22±4°C, 70±10% RH and 10:14 h (L:D) photo-period] and exposed as host to newly emerged mated female *C. chlorideae* deprived of hosts for 0, 1, 2, 3, 4 and 5 days and entire life (for longevity only).

Keywords: progeny sex ratio; host deprivation; pre-oviposition period; fecundity; sex ratio; biological control

Introduction

The Chickpea (*Cicer arietinum* L.) has been one of the most important constituents of Indian pulse cropping and consumption patterns and has long considered as 'king of pulses' [8], being a cheap source of proteins [26]. During 2020-21, in India, it was grown on 109.44 lakh ha area, with a total production of 119.10 lakh tonnes and average productivity of 1088.3 kg/ha [14], which is about 72.9 percent of harvested area with 75 per cent production of chickpea at the global level. Madhya Pradesh, Rajasthan, Maharashtra, Uttar Pradesh, Andhra Pradesh, Karnataka, Chhattisgarh, Bihar and Jharkhand are central chickpea-producing states of India [11]. The Uttar Pradesh state alone contributes about 7 per cent of country's chickpea production [46].

The American bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is a polyphagous pest and damages a wide variety of crops worldwide [33]. In India, its incidence is reported throughout the year, completing up to seven generations by feeding on 182 plant species, including pulse crops; the chickpea being the major one [2]. The yield loss in chickpeas due to this dreaded pest was reported as 10-60 percent under normal weather conditions, while it was said to be 50-100 percent in favorable weather conditions [24, 40]. The direct attack on fruiting structures, voracious feeding, high fecundity, multi-voltinism, overlapping generations, and ability to adapt and develop resistance against all common groups of insecticides applied to its management have exacerbated its severe pest status [6].

In Eastern Uttar Pradesh, India, *H. armigera* is one of the significant biotic constraints for chickpea cultivation [30, 31]. The most commonly used method against it, in this region is the chemical pesticide application. Still, it cannot wipe *Helicoverpa* out since it quickly develops resistance to applied

chemicals, including pyrethroids [32]. The potential of used chemicals is also screened off as the grown-up *Helicoverpa* larvae feed upon developing grains inside the pods. In addition, large quantities of persistent insecticides raise concerns about applicator safety, environmental contamination and possibly harmful effects on non-target animals and humans.

In such a situation, the use of biopesticides and genetically modified (GM) crops that express insecticidal genes, such as those derived from the soil bacterium *Bacillus thuringiensis* (Bt), opened new endeavours to control *Helicoverpa* on crops [9, 34, 38]. However, the use of biopesticides on a small scale in developing countries such as India (Mishra et al., 2020) and the development of resistance against Bt transgenic cotton and chickpeas in several places, including India [3, 10, 42], have drawn attention to the need for alternative control measures that can be used to control this severe pest in an economically and environmentally acceptable manner.

The biological control of insect pests has been considered the cornerstone of integrated pest management (IPM) and uses living organisms (parasitoids, predators, or herbivorous arthropods) to suppress a pest's density to lower levels [45]. Unlike other control measures, its effect is permanent, ecologically non-disruptive, and self-sustaining, and after the initial costs involving investigations and release, the recurrent costs are nominal [5, 21].

The abundance of parasitoids and their hosts in fields must be synchronized for successful biological control practices [27, 48], i.e., a sufficient number of hosts must be present in the field at the time of the mass release of parasitoids [43]. A delay in the availability of hosts for the parasitoids may strongly affect their reproduction and survival. A host shortage is likely to occur in nature, reducing the efficiency of parasitoid species. None of the parasitoids can manage their fecundity and sex ratio if they

are deprived of hosts for a long time. However, a parasitoid that can tolerate more extended host deprivation would be considered reasonable. These aspects are crucial for implementing an efficient mass-rearing program and the mass release of parasitoid species in fields.

The ichneumonid *Campoletis chlorideae* Uchida is a common parasitoid of the pod borer, *Helicoverpa armigera* (Hübner) on chickpea crops in India [37,44]. It is an arrhenotokous, idiobiont parasitoid species that effectively parasitizes the second instar larvae of *H. armigera*, both at the vegetative and fruiting stages of the chickpea crop [1, 28]. Previous studies revealed that *C. chlorideae* might be considered a promising alternative to the exploitative and disruptive chemical control measures against *H. armigera* on chickpeas in eastern Uttar Pradesh, India [30] [31]. However, before any attempt is made to mass rear and release this parasitoid, the factors that may affect its reproduction and survival must be understood. Host deprivation is one of the crucial factors to consider [19, 20, 23].

Methods

Collection of parasitoids and hosts

Cocoons of the parasitoid *C. chlorideae* and larvae of the host *H. armigera* were collected separately from the chickpea crops grown near Gorakhpur City. The collected cocoons of the parasitoid and larvae of the host were reared in the laboratory [22±4°C, 70±10% RH and 10:14 h (L:D) photoperiod] at Department of Zoology, D.A.V. Post Graduate College, Gorakhpur -273001, Uttar Pradesh, India.

Culture of the hosts

The field-collected larvae were transferred singly into glass vials (ca. 10 x 3.35 cm) with moistened filter paper at the bottoms using a small camel hair brush. The mouths of the glass vials were plugged with absorbent cotton. Fresh and green leaves and pods of chickpea were provided as food for the host larvae, which were reared until pupation. After pupation, the pupae were transferred to the fresh, sterilised glass vials with moistened filter paper at their bottoms. Emerging adults were provided a 30% honey solution as food.

For the culture of *H. armigera*, a couple of adults were kept together in a beaker (1000 ml) until mating was observed. Moistened filter paper was kept at the bottom of the beaker to provide humidity inside it. When the flying moth needed to rest, a strip of muslin cloth was hung inside. The mouth of the beaker was covered by a muslin cloth. The mated females were then removed from the beaker and introduced into the small, marked wooden cages (ca. 45 x 50 x 60 cm) containing potted young plants of chickpea. A piece of sponge soaked in a 30% honey solution was kept in each cage as food and was changed daily. The eggs deposited each day on the leaves and pods of the host plant, were transferred to the marked beakers (ca. 250 ml) and kept until hatching. The larvae were then collected in glass tubes (ca. 10 x 3.25 cm). For the culture of hosts of a known age, only newly hatched first instar larvae were allowed to remain in the beaker, and the rest were removed. Second instar larvae, which are most preferred by the parasitoid [31], were collected from the maintained culture and used as hosts for the experiments.

Culture of the parasitoid

The field-collected cocoons of the parasitoid were transferred

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singly with a small camel hair brush into glass vials (ca. 10 x 3.35 cm), each having moistened filter paper at its bottom. Adults emerging from the cocoons were then fed a 30% honey solution *ad libitum* for 2-4 hours. After that, the female and male parasitoids were put together in a glass tube (ca. 10 x 3.25 cm) until mating was observed (2–6 hours). The males were then taken out of the glass tubes. The mated females were introduced into the small, marked wooden cages (ca. 45 x 50 x 60 cm) with potted young plants of chickpea and about 100 healthy second instar host larvae. A small piece of sponge soaked in a 30% honey solution was placed into each wooden cage as food for the parasitoids. After parasitizations, the parasitoids were removed, and the host plants were placed into cages (ca. 30 x 30 x 40 cm) for further development. The potted plants were examined daily for cocoon formation. The cocoons were collected and transferred singly into the marked, sterilized glass vials. After adult emergence, the number of each sex was determined.

Laboratory experiments

A couple of adult virgin male and female *C. chlorideae* were obtained from culture and kept together in separate tubes (ca. 1 x 10 cm) until mating was observed. Now, mated females, deprived of hosts for 0, 1, 2, 3, 4, and 5 days, were introduced into four separate wooden cages (ca. 45 x 50 x 60 cm), each having potted young plants of chickpea and about 100 healthy second instar host larvae for parasitization. After every 24 hours, the females were removed from their respective cages and re-introduced in other similar cages with potted young chickpea plants and about 100 healthy second instar host larvae after proper feeding and resting throughout their lives. The susceptible host larvae were placed in other cages and examined daily. As soon as the parasitized larvae transformed into cocoons, they were counted and transferred separately with a part of the leaf to glass vials (ca. 1 x 5 cm) with moist filter paper at the bottom. Upon adult emergence, the number of each sex was determined [31].

Analysis of the data

The survival of the female parasitoids was tabulated each day when oviposition was observed. Fecundity was measured as the number of cocoons formed from the eggs laid by females throughout their lives. The progeny sex ratio was estimated as the proportion of males in the total progeny. The data were tested using one-way analysis of variance (ANOVA) and regression. The ANOVA followed by Duncan Multiple range test (DMRT) at 0.05 level. The data on the percent immature mortality and progeny sex ratio were arcsin-square-root transformed before the ANOVA.

Results

Increasing host deprivation shortened the preoviposition period. Average preoviposition times for the females deprived of hosts for 5 days was 6.4 ± 2.6 minutes, in contrast to the 26.5 ± 5.2 minutes for those supplied with hosts soon after mating (Figure 1). A reciprocal relation was observed between progeny yield and host deprivation of *C. chlorideae* females (Table 1). However, the progeny yield of females deprived of hosts for up to three days did not significantly differ from that of those supplied to hosts daily (F = 187.32, P<0.05) (Table 1). The progeny production reduced by approximately 56 and 65

percent respectively, when host supply were delayed for four and five days (Table 1). The trends in daily progeny yield revealed an increased urge to lay eggs by host-deprived females. Females deprived of hosts laid most of their eggs during the first three days of oviposition, in contrast to a consistent oviposition pattern by the females supplied with hosts since mating. The percent mortality of cocoons increased with increasing host deprivation and was highest (48.8 ± 7.2) when the host's supply was delayed by five days (Table 1). The females produced a female-biased progeny throughout and maintained a sex ratio close to 0.5, even when deprived of hosts for five days ($F = 36.65$, $P < 0.001$) (Table 1). A direct and positive relationship existed between the longevity of a parental female and the length of time she was withheld from hosts (Figure 1). As the host deprivation period increased, a female's life span increased. Those deprived of hosts for their entire lives, lived significantly longer than their counterparts exposed to hosts since emergence (Figure 1).

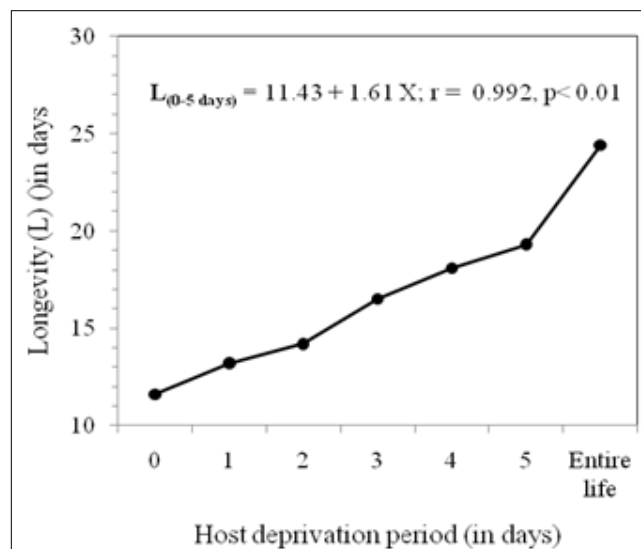


Fig 1: Effect of host deprivation on the longevity of the parasitoid, *C. chloridae*

Table 1: Preoviposition period, total progeny production, progeny sex ratio, and per cent pupal mortality of *C. chloridae* females deprived of hosts (*H. armigera* larvae) for different periods of time

Days without hosts	Preoviposition period (mins.)	Life-time progeny yield/Female	Progeny sex ratio (PSR)	Per cent pupal mortality
0	26.5 ± 5.2 ^a	137.3 ± 14.7 ^a	0.341 ± 0.057 ^a	17.0 ± 2.2 ^a
1	18.4 ± 4.2 ^b	136.2 ± 12.3 ^a	0.375 ± 0.063 ^a	22.3 ± 2.8 ^b
2	17.4 ± 3.8 ^b	134.4 ± 13.7 ^a	0.423 ± 0.068 ^a	23.3 ± 3.4 ^b
3	11.6 ± 4.3 ^c	133.6 ± 14.8 ^a	0.461 ± 0.073 ^b	27.6 ± 5.7 ^c
4	10.2 ± 3.2 ^c	73.5 ± 7.6 ^b	0.471 ± 0.092 ^b	38.8 ± 6.4 ^d
5	6.4 ± 2.6 ^d	48.3 ± 5.3 ^c	0.492 ± 0.092 ^b	48.8 ± 7.2 ^e
Entire life				

*All data are given as mean ± SD. Within each column, means followed by the different letters are significantly different at $p < 0.05$ (DMRT). The data of progeny sex ratio and per cent pupal mortality were arcsin-square-root transformed before the test; however untransformed data are presented in table.

Discussion

The ability of females to adjust their egg production according to host availability is one of the most critical parameters for the reproductive success of a parasitoid [17, 41]. To maximize her lifetime reproduction, parasitoids must balance time limitations, egg limitations, and the costs of oviposition [35]. In this study, we observed reduced progeny yield and increased longevity of maternal females under host deprivation. Results revealed similar trends to those from previous studies on *Trichogramma brassicae* [17], *T. Kaykai* [19], *Mastrus ridibundus* [20], *Microplitis rufiventris* [18], *Aphidius gifuensis* [29], *Trichopriya anastephae* [23] and *Habrobracon hebator* [27]. Female parasitoids deprived of hosts either resorb their eggs as a source of nutrition or can retain their eggs and keep their parasitism rate low [20]. In the present study, we observed insignificant variations in the progeny yield of females deprived of hosts for three days to those females who received hosts without any delay. It suggests that in female *C. chloridae*, egg resorption did not occur for up to three days of host deprivation. Increasing host deprivation lowered the number of progeny she produced. The more drastic reduction in progeny production by *C. chloridae* probably resulted from a lower capacity by these females to create and store eggs for long periods. The results are in close agreement with [17], who reported a drastic effect of temporary host deprivation on the parasitization rates of *Trichogramma brassicae* Bezdenko parasitizing *E. kuehniella* eggs when females were deprived of

hosts for more than three days. A similar influence of host deprivation has also been reported for the egg parasitoid *Gryon pennsylvanicum* on squash bug eggs [47]. These workers attributed this effect to the wasp's low ability to store eggs for more than three days.

Progeny sex ratio is an essential factor affecting parasitoid species' population performance. Insect populations generally have equal investments in both sexes, resulting in almost equal proportions of sons and daughters [15]. However, deviation in sex ratio is also reported to be influenced by ecological, physiological, and behavioral factors [16, 22, 31]. In this study, we discovered that *C. chloridae* females produced female-biased progeny regardless of host deprivation period variation. It is consistent with the results from previous studies on *Mastrus ridibundus* (Hymenoptera: Ichneumonidae) [20], *Trichopriya anastephae* (Hymenoptera: Diapriidae) [23], *Habrobracon hebator* (Hymenoptera: Braconidae) and *Hadronotus pennsylvanicus* (Hymenoptera: Scelionidae) [41].

Being arrhenotokous, the parasitoid species of the order Hymenoptera tend to produce a more male-biased progeny with an increase in maternal age [23]. This switch, however, is most likely caused by sperm depletion or spermathecal gland secretion depletion [36]. According to [16], as eggs remain longer in the ovarioles of host-deprived females, they have a better chance of being fertilized and thus producing females.

High reproductive efforts early in the lives of the females compromised their longevity. The longer the period of host

deprivation, the longer the wasps lived. Studies with different taxa have shown that organisms that invest more in reproductive effort may reduce their life expectancy because of a somatic-gametic trade-off [7, 13, 20]. Increasing longevity may occur at the expense of egg production. Female wasps that provided unlimited hosts, on the other hand, lived longer than those that provided limited hosts or those that maintained colonies in the absence of hosts [4, 36].

Conclusion

Our conclusion indicates that *C. chlorideae* females can manage the progeny yield, particularly concerning daughters, deprived of the host even up to three days. The results of the present study can be used to understand how to manipulate host availability during the mass rearing and pre-release periods to increase the reproductive potential of parasitoids. We recommend a mass release of *C. chlorideae* within four days of emergence to improve reproductive suitability and establishment potential against *H. armigera* on chickpeas.

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