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Reproductive performance of *Chouioia cunea* Yang (Hym.: Eulophidae) parasitizing fall webworm, *Hyphantria cunea* Drury (Lep.: Arctiidae)

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Abstract
The fall webworm, *Hyphantria cunea* Drury (Lep.: Arctiidae), is an important pest of forest and cultivated plants in Guilan Province, Iran. The reproductive performance of *Chouioia cunea* Yang (Hym.: Eulophidae), a gregarious pupal parasitoid of *H. cunea* was studied at 24±1°C, 70±5% (RH), and a photoperiod of 14:10 (L:D) hours. The pupal hosts were exposed to 1, 2, 4, 8, 12, 16 newly emerged adult parasitoids. The parasitoids remained in contact with host pupae for 24 hours in Petri-dishes (10x1 cm) until the death of all parasitoids. The results showed that parasitoid density influenced offspring production, as the higher parasitoid densities resulted in the lowest mean number of offspring per female (179.06± 6.29). The sex ratio was not influenced by parasitoid density, but the age of parasitoid affected sex ratio as a higher sex ratio (0.92±0.001♀) was observed in the progeny produced by younger parents. Rate of parasitism was higher at density of 4 wasps (33.33%). The mean percent parasitism by 1, 2 and 3 day-old female parasitoids were 21, 13 and 9, respectively ($P<0.05$). Maximum number of offspring produced per female was obtained at host/parasitoid ratio of 15 to 4. The female parasitoids survived 1-3 days after oviposition. The searching efficiency of the parasitoid decreased from 0.18 to 0.009h^{-1} with increasing its density. The survival rate for *C. cunea* was not significantly different at all densities of male or females, but a statistical difference was observed with increasing parasitoid age. It was concluded that the performance of *C. cunea* was mainly affected by its density and age.
Key words: foraging behavior, parasitism, reproduction, *Hyphantria cunea*, *Chouioia cunea*

عملکرد تولیدمثلی *Chouioia cunea* Yang (Hym.: Eulophidae) زنبور پارازیتویید پروانه ابریشم‌باف پاییزی *Hyphantria cunea* Drury (Lep.: Arctiidae)

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چکیده
پروانه ابریشم‌باف پاییزی *Hyphantria cunea* (Drury) (Lepidoptera: Arctiidae) آفت پروانه آفت مهم گیاهان جنگلی باغی و محصولات زراعی در استان گیلان است. در این پژوهش، عملکرد تولید مثلی زنبور *Chouioia cunea* Yang (Hym.: Eulophidae)، پارازیتوئید تجمعی شفیره *H. cunea* در دمای ۱±۲٤°C، رطوبت ٪۵±۷۰ و دوره نوری ۱٤:۱۰ ساعت روشنایی، به تاریکی بررسی شد. شفیره‌های میزبان در معرض تراکم‌های ۱، ۲، ٤، ۸، ۱۲ و ۱٦ حشرات کامل پارازیتویید تازه خارج شده قرار داده شدند. زنبورهای پارازیتویید داخل ظروف پتری (۱x ۱۰ سانتی‌متر) به مدت ۲٤ ساعت در تماس با شفیره‌های میزبان باقی ماندند. این آزمایش تا پایان عمر تمام زنبورهای پارازیتویید ادامه یافت. نتایج نشان داد که تراکم پارازیتویید در تولید نتاج موثر بود، به‌طوری‌که بالاترین تراکم منجر به تولید کم‌ترین نتاج شد (٦/۲۹±۱۷۹/۰٦). تراکم پارازیتویید روی نسبت جنسی تاثیری نداشت، اما سن پارازیتویید بر نسبت جنسی تاثیر داشت و بالاترین نسبت جنسی در نتاج تولید شده توسط زنبورهای یک روزه مشاهده شد (♀۰/۰۰۱±۰/۹۲). بالاترین نرخ پارازیتیسم در تراکم ٤ عدد زنبور پارازیتویید به‌دست آمد (٪۳۳/۳۳). متوسط درصد پارازیتیسم زنبورهای ماده با طول عمر ۱، ۲ و ۳ روزه به ترتیب ٪۲۱، ٪۱۳ و ٪۹ تعیین شد (p <۰/۰۵). بیشینه تعداد نتاج تولید شده به ازای هر ماده در نسبت ٤ عدد پارازیتویید به ۱۵ شفیره میزبان به‌دست آمد. پارازیتوییدهای ماده ۱ تا ۳ روز پس از تخم‌ریزی زنده ماندند. قدرت جستجوی پارازیتویید با افزایش تراکم آن، از ۰/۱۸ به ۰/۰۰۹ بر ساعت کاهش یافت. نرخ بقای نتاج زنبور پارازیتویید

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C. cunea از لحاظ آماری در همه تراکم‌ها در هر دو جنس نر و ماده دارای تفاوت معنی‌دار نبود، اما با افزایش سن پارازیتویید تفاوت آماری مشاهده شد. نتیجه‌گیری شد که تراکم و سن زنبور پارازیتویید *C. cunea* به‌طور اساسی عملکرد تولیدمثلی آن را تحت تاثیر قرار می‌دهد.

واژگان کلیدی: رفتار کاوشگری، پارازیتیسم، تولید مثل، *Hyphantria cunea*، *Chouioia cunea*

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Introduction

The Fall webworm, *Hyphantria cunea* Drury (Lepidoptera: Arctiidae), is a polyphagous defoliating pest native to the US, Canada (Warren and Tadic, 1970) and New Zealand (Kean and Kumarasinghe, 2007). It is presently invaded many world areas such as Europe and Asia (Li *et al*., 2001). It was first spotted in Iran in 2002. The eggs of the pest hatch within one to two weeks, and emerging larvae immediately begin spinning their silk tent. Full-grown larvae leave the web to pupate in leaf litter or bark crevices. It overwinters in the pupal stage. Pupation takes place in a thin cocoon. There are two generations per year in Guilan Province (Rezaei *et al*., 2003).

A native pupal endoparasitoid, *Chouioia cunea* Yang (Hym.: Eulophidae, Tetrastichinae) causes considerable mortality on *H. cunea* pupae in some areas of China (Yang, 1989). *C. cunea* was found to be the dominant parasitoid of fall webworm pupae in Sangachin and Lashtenesha in Guilan Province during 2004-2005 as the rate of pupal parasitism was observed to be higher in the second generation (Ejlali, 2005). It completes its egg, larval, pupal and pre-egg-laying adult stages in the host pupae. The emerging adults also mate inside the host pupa where serves as an empty shell after the content was eaten by the parasitoid's larvae. Female, then bites a hole to come out, and all the other wasps usually follow her way through the hole. The females lay eggs soon after emergence by pricking host pupa with the ovipositor. The parasitoid larvae feed on haemolymph and organs in the host pupa. Once the larvae mature, the materials inside the host pupa are all consumed (Yang and Xie, 1998).

The reproductive potential of a parasitoid is one of the factors to be considered in evaluating its performance as biological control agent. It determines the population growth, and efficiency of the parasitoid. The reproductive potential of the both parasitoid and the insect pest are essential in evaluating the parasitoid’s capability as a viable control agent (van Lenteren, 1986).

The mass rearing represents an important stage of control programs (Parra *et al*., 2002; Pastori *et al*., 2008; Pereira *et al*., 2009), and the nutritional quality, size, age, mechanical resistance and capacity of immunological response of parasitoids should be considered to select alternative hosts (Godfray, 1994).

The parasitoids density per host affects the parasitoid offspring (Thomazini and BertiFilho, 2000; Matos Neto *et al*., 2004), the sex ratio (Choi *et al*., 2001), the parasitism capacity (Sampaio *et al*., 2001), the duration of the life cycle, the body size, and the longevity of the adults (Silva-Torres and Matthews, 2003).

To maximize the mass rearing of parasitoids, it is necessary to study the relation of parasitoids density to the host (Sagarra *et al*., 2000).

The aim of this study was to investigate reproductive performance of the parasitoid *C. cunea* on the fall webworm *H. cunea* Drury in laboratory conditions.

Material and methods

Host culture

Overwintering pupae of fall webworm were collected from various locations in Guilan Province, especially Shaft and Somae-Sara areas, under barks of old or dead forest trees, among leaf litters, ornamental trees, shrubs and hedgerows to establish a rearing stock of *C. cunea* wasps. The larvae of the pest were collected from the infested trees and transferred to transparent plastic trays (15×10×8 cm) for pupation in a growth chamber at 24±1°C, 70±5% of related humidity (RH), and a photoperiod of 14:10 (L:D) hours.

Parasitoid culture

Once adult parasitoids of *C. cunea* emerged from hosts' pupae, they were kept in glass petri dishes (10×2 cm) and fed with honey. The 48 to 72 hour-old pupae of *H. cunea*, after removal from their cocoons, were exposed to the parasitoid females for 24 hours in a growth chamber with identical condition.

Effect of parasitoid density on reproduction

To study the effect of parasitoid, different densities of progeny produced per female. A total of 15 pupae of the host were presented to a one day-old female parasitoid in a Petri-dish (10 x1cm) for 24 hours. The parasitoid densities of 1, 2, 4, 8, 12, 16 were used and each density level was replicated 15 times. Host pupae were replaced daily until the death of adult parasitoids. Parasitized pupae were kept under the same conditions until progeny emerged. The Percentage of parasitized host, mean number of progeny in each pupa and sex ratio of the parasitoid were recorded.

Effect of parasitoid age on offspring production and survival

The effect of parasitoid age on the rate of parasitism, offspring production, and sex ratio, female parasitoids aged 1, 2, and 3 days were individually exposed to 15 host pupae in a Petri-dish (10 x1cm) for 24 hours at the same condition. Host pupae were replaced daily until the death of adult parasitoids.

Effect of parasitoid density on searching efficiency

The data obtained from the the section 3 were used to find out the effect of parasitoid density on its searching efficiency. For data analysis, the method used by Hassell and Varley's (1969) was applied. The related equation is:

$$\log \alpha = \log Q - m \log Pt$$

α is the searching efficiency, Q is the quest constant, P_t is the parasitoid density and m is the coefficient of mutual interference.

The value of α at each parasitoid density was estimated utilizing the following formula, which derived from Nicholson's model by Hassell (1978):

$$\alpha = \frac{1}{Pt} \log_e \frac{Nt}{Nt - N\alpha}$$

N_t is the initial host density and N_a is the number of parasitized hosts.

Experiments were done in a completely randomized design and the means were separated by Tukey's test at the 5% level. The data analyses were performed through SAS software and figures drawn by Excel.

Results

Effect of parasitoid density on reproduction

The density of female *C. cunea* affected percentage of parasitism significantly (df=5, 89, F=7.03, P<0.0001) (Table 1).

Table 1. Effect of different densities of *Chouioia cunea* on parasitism of *Hyphantria cunea*.

Parasitoid density	No. of hosts	No. of hosts parasitized	Mean No. of pupae parasitized (Mean±SE)	Percent parasitism
1	225	27	1.8±0.03	12 c
2	225	51	3.4±0.07	22.66 abc
4	225	75	5±0.10	33.33 a
8	225	56	3.73±0.17	24.88 ab
12	225	48	3.2±0.11	21.33 bc
16	225	32	2.13±0.76	14.22 bc

Means within a column followed by the same letter do not differ significantly (Tukey's test, P < 0.05).

The parasitoid density also influenced the number of progeny produced per *H. cunea* pupa significantly (df=5, 89, F=47.76, P<0.0001). The quantity of offspring per pupa ranged from 0 to 996 (Table 2). There was no significant difference in sex ratios of progeny produced by the parasitoid in all different densities tested (df = 5, 89, F = 2.18; P=0.063).

This study also showed that there was no significant difference in survival rate of male and female offspring in all parasitoid densities (Table 3).

Table 2. Effect of the parasitoid density on offspring produced by *Chouioia cunea*, reared on *Hyphantria cunea*.

Parasitoid density	Mean No. offspring (Mean±SE)	Range	
		Min	Max
1	268.5± 2.58 cd	139	289
2	545.75± 3.52 b	339	634
4	828.62± 9.52 a	425	996
8	373.86± 12.81 c	72	701
12	256.66± 10.80 cd	9	548
16	179.06± 6.29 d	0	365

Means within a column followed by the same letter do not differ significantly (Tukey's test, $P < 0.05$).

Effect of parasitoid age on offspring production and survival

The mean percentage of parasitism by 1, 2 and 3 day-old females were significantly different (df=2,44, F=26.30, P<0.05). The age of *C. cunea* affected its sex ratio significantly (df =2, 44, F = 27.30; P < 0.0001). The mean sex ratios (female proportion) of 1 to 3 day-old females decreased with age from 0.92 to 0.53, respectively (Table 4). The number of offspring per host pupa decreased with increasing parasitoid age (Figure 1). The parasitoid offspring survival decreased significantly with increasing parasitoid age. It was also shown that the age of ovipositing females influenced female (df=2, 44, F=17.52, P<0.0001), and male (df=2, 44, F=10.66, P<0.0001) survival rate significantly (Table 5).

Table 3. Effect of *Chouioia cunea* density on its progeny survival rate when reared on *Hyphantria cunea*.

Parasitoid density	Mean female survival rate (Mean±SE)	Mean male survival rate (Mean±SE)
1	0.75±0.006 a	0.24±0.001 a
2	0.72±0.004 a	0.27±0.0005 a
4	0.63±0.007 a	0.36±0.0003 a
8	0.69±0.008 a	0.30±0.001 a
12	0.67±0.014 a	0.32±0.004 a
16	0.66±1.65 a	0.40±1.66 a

Means within a column followed by the same letter do not differ significantly (Tukey's test, $P < 0.05$).

Effect of parasitoid density on searching efficiency

Figure 2 shows the relationship between the searching efficiency (α) and the parasitoid density (P_t). The results indicated that with increasing parasitoid density, the searching efficiency of individual parasitoids reduced. This suggested that there was a mutual interference among the searching female parasitoids. The coefficient of mutual interference (m) and the model were estimated as -0.9268 and log a = −0.9268 log P_t− 0.7044.

Table 4. Effect of *Chouioia cunea* age on the percentage of parasitism and sex ratio on *Hyphantria cunea*.

Parasitoid age (day)	Parasitism percentage (%) (Mean±SE)	Sex ratio (Mean±SE)
1	21±0.007 a	0.92±0.001 a
2	13±0.005 b	0.70±0.004 b
3	9±0.005 b	0.53±0.005 c

Means within a column followed by the same letter do not differ significantly (Tukey's test, $P < 0.05$).

Interaction between age and parasitoid density

The number of progeny per female *C. cunea* in densities of 1, 2 and 4 parasitoids increased, and decreased at densities of 8, 12, 16 parasitoids (Figure 3). The rate of parasitism increased up to density of 4 parasitoids and later decreased. However, the number of progeny decreased as parasitoids grew older (Figure 4).

Table 5. Effect of *Chouioia cunea* age on its adult survival rates reared on *Hyphantria cunea*.

Parasitoid age	Mean female survival rate (Mean±SE)	Mean male survival rate (Mean±SE)
1	0.85±0.002a	0.30±0.001a
2	0.79±0.002 b	0.20±0.002 a
3	0.69±0.001c	0.14±0.002 b

Means within a column followed by the same letter do not differ significantly (Tukey's test, $P < 0.05$).

Discussion

Data analysis showed that the mean number of offspring and percentage of parasitism significantly decreased with parasitoid density. Higher densities of adult parasitoids produced lower number of offspring, probably because of the fixed number of hosts. Lu (1992) also found that an increase in parasitoid density reduced the number of parasitized eggs per female.

Statistical analysis showed that parasitoid densities did not affect the sex ratio in all densities. This relationship was also stated by other researchers (e.g., Murdoch *et al*., 2003; Ode and Hardy, 2008; Irvin and Hoddle, 2006).

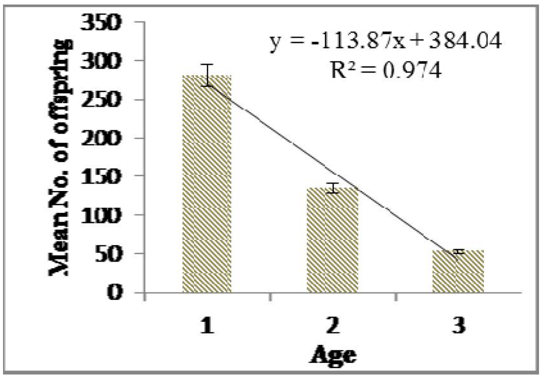

Fig. 1. Effect of *Chouioia cunea* age on offspring production.

It was found that the number of offspring and percentage of parasitism increased in the first days of parasitoid age in all densities and then reduced. Gonzalez-Zamora *et al*., (2015) obtained the same results when studied the influence of food source, host and parasitoid densities on the biology of *Aphytis melinus* DeBach to optimize its mass production.

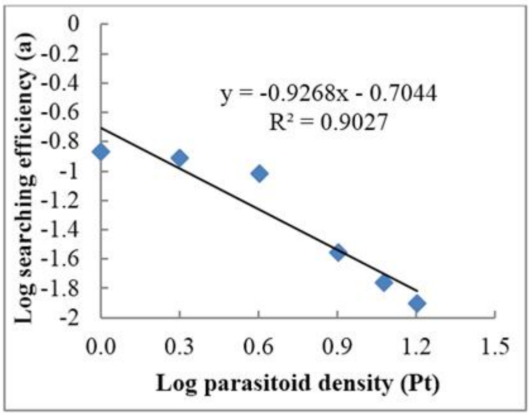

Fig. 2. Relationship between searching efficiency and searching parasitoid density (P_t) of *Chouioia cunea* parasitizing pupae of *Hyphantria cunea*.

The rate of progeny production, parasitism and sex ratio were effectively influenced by the parasitoid age, as the progeny production decreased the age of parasitoid increased. Amalin *et al*. (2005) demonstrated that the rate of parasitism in young females of

Ceratogramma etiennei was higher than their old counterparts. The rate of parasitism by *Glyptapanteles flavicoxis* also decreased as the parasitoid age increased (Hu *et al*., 1986). It has also been noted for *C. curvimaculatus* (Hentz, 1998) and *C. grandis* (Greenberg *et al*., 1995). The rate of parasitism by *C. cunea* is consistent with those documented by Hu *et al*. (1986) and Amalin *et al*. (2005). Kumar *et al*. (1990) and Singh *et al*. (1997) worked on *Nesolynx thymus* Girault and *Trichomelopsis apanteloctena*, respectively and recorded no significant effect of parasitoid age on the rate of parasitism.

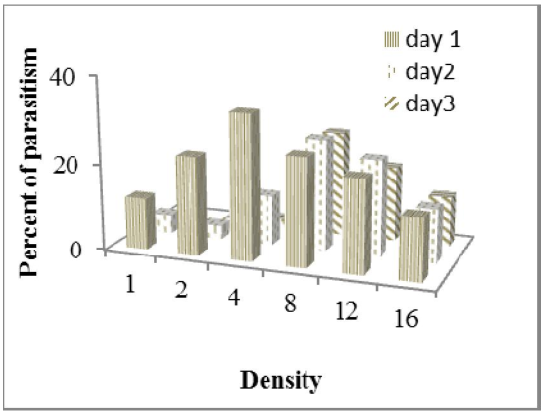

Fig. 3. The interaction between age and density of parasitoid on the percentage of parasitized host.

The rate of progeny production, parasitism and sex ratio were effectively influenced by the parasitoid age (Hirashima *et al*.; 1990; Greenberg *et al*.,1995; Leatemia *et al*., 1995; Hentz, 1998; Honda, 1998; Amalin *et al*., 2005). The sex ratio of *Chouioia cunea* is disproportionate to its age likely due to the production of relatively high number of female progeny by the younger female parasitoids. Significantly high sex ratio has been found in the progeny produced by younger parent females of *Trichogramma chilonis*, *T. ostriniae* (Hirashima *et al*., 1990) and *T. minutum* (Leatemia *et al*., 1995).

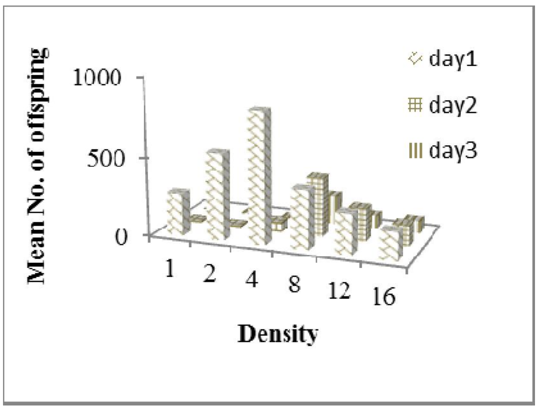

Fig. 4. The interaction between age and density of parasitoid on the offspring

Effectiveness of searching parasitoids decreases as parasitoid density increases due to "interference" of searching parasitoid (Farhad *et al*., 2011).

The negative value of coefficient of interference in regression line shows an inverse relationship between parasitoid density and its per capita searching efficiency. This relationship was also shown for *Diaeretiella rapae* (McIntosh) parasitizing *Lipaphis erysimi* Kaltenbach (Shukla *et al*., 1997) and for *D. rapae* on *B. brassicae* (Fathipour *et al*., 2004) that reflects intraspecific competition in parasitoids. In addition, high parasitoid density causes a higher proportion of male progeny, probably because females lay unfertilized eggs (Jones *et al*., 1999). The significant reduction of host parasitization per parasitoid with increasing parasitoid density suggests that interference amongst parasitoids also increases at higher parasitoid density. This is probably due to a closed experimental arena and limited time for parasitization and a high probability of mutual interference (Tahriri *et al.*, 2007; Farhad *et al*., 2011). Results in this study showed that when parasitoid density increases from 1 to 16, the per capita searching efficiency decreases from 0.18 to $0.009h^{-1}$.

Our results indicated that age and density of a parasitoid significantly affect the progeny production, rate of parasitism and sex ratio of *C. cunea*. It was concluded that the performance of *C. cunea* caused by density and age.

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