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Size of Interacting Resource-Host-Parasitoid Populations Influences Mass Rearing of *Cotesia vestalis*

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ABSTRACT

The importance of *Cotesia vestalis* (Haliday) has been well documented as one of the most significant biocontrol agents for the diamondback moth, *Plutella xylostella* (L.). The aim of the present study was to optimize the procedure of mass rearing of *C. vestalis*. The effects of space and plant-herbivore-parasitoid biomass on the quality of produced wasps were investigated. In particular, the effects of food and space resources of *P. xylostella* and *C. vestalis* on mass-reared *C. vestalis* were investigated. The results indicated that there was no significant difference between treatments for percentage parasitism, survival rate and larval developmental period of the produced wasps. However, developmental period of pupa, the ratio of female offsprings and sex ratio of the mass-reared wasps were significantly affected by the given treatments. Based on these findings, it can be concluded that the extent of available resources plays a crucial role in mass rearing of *C. vestalis*.

Key words: Mass rearing, optimization, space, density, *Plutella xylostella*.

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INTRODUCTION

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera, Plutellidae), is a serious pest of cruciferous plants. To control *P. xylostella* farmers use a large amount of insecticides, which has been created several issues, such as pesticide resistance and eliminations of natural enemies from fields (Talekar and Shelton, 1993; Sarfraz *et al.*, 2005; Afiunizadeh and Karimzadeh, 2015). This overuse of pesticides, which has been resulted in pest resurgence, has led researchers to develop alternative and more sustainable pest management strategies, as an exemplification, biological control (Karimzadeh and Sayyed, 2011; Gulzar *et al.*, 2012; Jafary *et al.*, 2016).

Cotesia vestalis (Haliday) (Hymenoptera: Braconidae), is one of the primary larval endoparasitoid of *P. xylostella* (Sarfraz *et al.*, 2005). The percentage parasitism of *P. xylostella* larvae by *C. vestalis* in the field conditions ranged between 40.0-83.3% in Japan, 3.6-73.2% in Hawaii, 30-50% in South Africa and 5.4-88.7% in Jamaica. In the field experiments and without application of pesticide, parasitism percentage of *P. xylostella* was 90-95% by *C. vestalis* (Kawazu *et al.*, 2011). Afiunizadeh and Karimzadeh (2015) used a more precise method of assessment of percentage parasitism (i.e., recruitment method) to indicate that about 21.0% of field populations of *P. xylostella* were parasitized by *C. vestalis*. In comparison with other larval parasitoids to illustrate, *Diadegma semiclausum* (Hellen) (Hymenoptera, Ichneumonidae), *C. vestalis* has shown the ability to act and establish well at higher temperature (20-30ºC). In Taiwan, the integration of *Bacillus thuringiensis* and release of *C. vestalis* has led to a great reduction of diamondback moth populations (Talekar and Shelton, 1993). *C. vestalis* has been subjected to more than 20 classical biological control introductions, of which many have been successful (Sarfraz *et al.*, 2005). In addition, this parasitoid appears to have a wider range of natural distribution compared to *D. semiclausum*. *C. vestalis* has been reported to attack the diamondback moth in many regions to exemplify, Malaysia, Taiwan, Vietnam, China, Japan and Iran with no records of introductions (Afiunizadeh *et al.*, 2011; Furlong *et al.*, 2013; Afiunizadeh and Karimzadeh 2015).

C. vestalis has been reported from Iran on *Simyra dentinosa* Freyer (Lepidoptera, Noctuidae) (Karimpour *et al.*, 2005), and on *P. xylostella* (Golizadeh*et al.*, 2007; Afiunizadeh and Karimzadeh 2010). Afiunizadeh and Karimzadeh (2015) reported *C. vestalis* had the highest distribution and percentage parasitism (21%) in comparison with others larval and pupal parasitoid of *P. xylostella* in central Iran. It has been concluded that the most effective larval parasitoid of *P. xylostella* is *C. vestalis* (Afiunizadeh and Karimzadeh, 2015). The mass rearing and release of this parasitoid, therefore, may establish an ecological and more sustainable strategy for *P. xylostella* management (Afiunizadeh and Karimzadeh, 2010; Afiunizadeh and Karimzadeh, 2015). However, there is little information regarding the mass rearing of *C. vestalis*. Different aspects of rearing of beneficial insects have been discussed by Singh (1982). For most parasitoids there are only limited reports about optimization of mass-rearing conditions. Ramalho *et al.* (2012) evaluated the criteria of optimizing the mass rearing of *Cotesia flavipes* (Cam.) in laboratory. They investigated the optimal density of the host larvae (*Diatraea saccharalis* Fub.) parasitism capacity and adult parasitoid diets.

In another experiment, Vacari *et al.* (2012) investigated the quality of *C. flavipes* reared on different host densities, and the cost of commercial production. The effects of colour and height of artificial feeding site for *C. vestalis* have been reported by Mitsunaga *et al.* (2004), who found *C. vestalis* was more attracted to yellow compared with others colours. In addition, the parasitoid chooses a feeding site hung 50 cm above ground over the one with 200 cm above ground.

It has been well documented that Chinese cabbage (*Brassica pekinensis*) is the most suitable host plant for rearing of *C. vestalis*, as the greatest parasitism success of *C. vestalis* has been achieved by feeding on *B. pekinensis* (Talekar and Yang, 1991; Liu and Jiang, 2003; Jafary *et al.*, 2012; Karimzadeh *et al.*, 2013; Heidary and Karimzadeh, 2014). Effect of food resource for adults of *C. vestalis* has been investigated in laboratory (Mitsunaga *et al.*, 2004); the highest survival and parasitism rates were obtained when adults of *C. vestalis* fed on a 50% honeybee solution. Also, honey-bee bread has shown a great potential food for adults of *C. vestalis* (Soyelu, 2013). In order to develop a mass rearing program, it would be necessary to optimize the space and resource (food plant and host) density and initial parasitoid density. In a previous work (Rezaei *et al.*, 2014b), we have already indicated that herbivore overcrowding and space limitation have negative effects on mass rearing of *C. vestalis*. The present study aimed to investigate to what extent the enlargement of optimal space and resource-host-parasitoid densities can influence the success of mass rearing of *C. vestalis*.

MATERIALS AND METHODS

Plant and insect rearing

Chinese cabbage, *Brassica pekinensis* (Lour.), was grown in plastic pots (10 cm diameter and 11 cm height) under glasshouse condition (25±5ºC, 70±5% RH and L:D 16:8 h) without the application of any pesticide. The cultures of *P. xylostella* and *C. vestalis* were maintained by collection from common cabbage and cauliflower fields in PirBakran region (32°28′ 8″ N and 51°33′ 28″ E, at 1,610 m altitude) of Isfahan province (central Iran). The colonies of *P. xylostella* were maintained on 5-week-old Chinese cabbage in ventilated cages (40×40×40 cm). The cultures of *C. vestalis* were then maintained on *P. xylostella* larvae in same cages. All cultures and rearing were kept at standard constant environmental condition (25 ± 2ºC, 70 ± 5% RH and L:D 16:8 h). Aqueous honey solutions (40%) (Mitsunaga *et al.*, 2004) were placed in the rearing cages for feeding adults of *P. xylostella* and *C. vestalis* (Karimzadeh *et al.*, 2004; Rezaei *et al.*, 2014a, b).

Experimental design

Experiments were conducted to investigate the effects of enlargement of optimal space and resource-host-parasitoid (RHP) densities on life-history parameters (percentage parasitism, survival rate, sex ratio, larval and pupal developmental periods, and percentage of survived females) of *C. vestalis*. Treatments include 1) one unit of optimal space and RHP densities (a small cage (40×40×40 cm), two plants (5-6-week-old Chinese cabbage), 40 hosts (early 3rd instar larvae of *P. xylostella*) and

5 parasitoid wasps (3-day-old, mated females of *C. vestalis*), 2) three units of optimal space and RHP densities (a median cage (70×55×50 cm), six plants, 120 hosts and 15 parasitoid wasps) and 3) six units of optimal space and RHP densities (a big cage (100×70×55 cm), twelve plants, 240 hosts and 30 parasitoid wasps. To start the experiment, the late 2nd instar larvae of *P. xylostella* were established on 5-6-week-old Chinese cabbages. After 24 h (when the larval population was established), 3-day-old mated females of *C. vestalis* were released in each cage for 24 h. The larvae were then maintained on the host plants until the hosts were pupated or wasp cocoons were formed. Each treatment was daily monitored and the numbers of formed host pupae and wasp cocoons were recorded. The parasitoid cocoons were further monitored for adult emergence and sex ratio. The treatments were replicated four times in a randomized complete block design with four replications and maintained under constant environmental conditions (25 ± 2 ºC, 70 ± 5% and L:D 16:8 h).

Statistical analyses

The percentage parasitism was evaluated as a ratio of the wasp to the sum of host and wasp. The data on percentage parasitism and survival rate were analysed using logistic analysis of deviance. Data on sex ratio was analysed using logistic analysis of deviance (for the difference between the treatments) and exact binomial test (for difference of each treatment with the sex ratio of 1:1). Data on developmental periods were analysed using nested ANOVA. Pair comparisons were done using Tukey's honest significant difference. All statistical analyses were completed in *R. 2.10.0* software (Crawley, 2014).

RESULTS

Percentage parasitism of *P. xylostella* larvae by *C. vestalis*

When percentage parasitism was investigated based on the wasp cocoons, there was no significant (df=9, t-value=1.374, P=0.203) difference between treatments (Table 1). The mean of percentage parasitism based on the wasp cocoons, varied between treatments from 83.3% (treatment 2) to 90.2% (treatment 1). Also, When percentage parasitism was calculated based on the emerged adult wasps, there was no significant (df=9, t-value=1.313, P =0.222) difference between treatments (Table 1). The mean of percentage parasitism based on the emerged adult wasps, varied between treatments from 82.1% (treatment 2) to 89.3% (treatment 1).

Survival rate of *C. vestalis*

When survival rate was analysed based on the wasp cocoons, there was no significant (df=12, t-value=-0.867, P=0.402) difference between treatments (Table 1). The mean of survival rate based on the wasp cocoons, varied between treatments from 68.8% (treatment 1 and 2) to 73.8% (treatment 3). However, when survival rate was evaluated based on the emerged adult wasps, there was no significant (df=12, t-value=0.911, P=0.380) difference between treatments (Table 1). The mean of survival rate based on the emerged adult wasps, varied between treatments from 62.5% (treatment 1) to 66.5% (treatment 3).

Table 1.The effects of enlargement of optimal space and resource-host-parasitoid (RHP) densities on parasitism (by *C. vestalis*) and survival of *Plutella xylostella* larvae.

	Percentage parasitism		Percentage survival	
Treatment	Cocoon based	Adult based	Cocoon based	Adult based
1 (one unit of optimal space[1] and RHP densities[2])	90.2 ± 3.0 a[3]	89.3 ± 2.7 a	68.8±3.6 a	62.5±7.2 a
2 (three unit of optimal space and RHP densities)	83.3 ± 2.8 a	82.1 ± 2.9 a	68.8±5.5 a	62.9±4.6 a
3 (six unit of optimal space and RHP densities)	83.7 ± 1.7 a	82.2 ± 2.4 a	73.8±2.4 a	66.5±4.7 a

1 One unit of optimal space denotes a cage of 40×40×40 cm. 2 One unit RHP density denotes two plants (5-6-week-old Chinese cabbage), 40 *P. xylostella* larvae and 5 parasitoid (3-d-old, mated females of C. vestalis). 3 Means with same letter in each column are not significantly (P > 0.05) different (Tukey).

Developmental period of larvae and cocoons

When developmental period of larvae was evaluated, there was no significant ($F_{2,9}$=0.966, P=0.417) difference between treatments (Table 2). The mean of developmental period of larvae varied among treatments from 7.45 days (treatment 1) to 8.02 days (treatment 3). The analysed developmental period of produced wasps cocoons showed significant ($F_{2,9}$ = 6.922, P<0.015) difference (Table 2) and the mean of developmental period of cocoon varied among treatments from 3.77 days (treatment 1) to 4.00 days (treatment 3). The first treatment has significantly lower developmental period of cocoons in comparison with other treatments.

Table 2.The effects of enlargement of optimal space and resource-host-parasitoid (RHP) densities on pupal and larval developmental period of produced parasitoids wasps, *C. vestalis*.

	Developmental period	
Treatment	Larval	Pupal
1 (one unit of optimal space[1] and RHP densities[2])	7.45 ± 0.24 a[3]	3.77 ± 0.04 b
2 (three unit of optimal space and RHP densities)	7.76 ± 0.18 a	3.96 ± 0.04 a
3 (six unit of optimal space and RHP densities)	8.02 ± 0.41 a	4.00 ± 0.06 a

1 One unit of optimal space denotes a cage of 40×40×40 cm. 2 One unit RHP density denotes two plants (5-6-week-old Chinese cabbage), 40 *P. xylostella* larvae and 5 parasitoid (3-d-old, mated females of *C. vestalis*) 3 Means with same letter in each column are not significantly (P > 0.05) different (Tukey)

Sex ratio (the ratio of produced female wasps)

When logistic analysis of deviance was used there were significant (df=9, t-value=-3.524, P<0.001) differences between treatments (Table 3). The mean of production of female wasps varied between treatments from 0.26 (treatment 1) to 0.45 (treatment 2 and 3). The first treatment produced a lower female sex ratio in comparison to the other treatments. Exact binomial test, however, showed a significant (P<0.01) difference between treatments. The first treatment (0.26) has significantly lower sex ratio compare to second and third treatment (0.45).

Percentage of survived female wasps

When the number of surviving female wasps was calculated, there was a significant (df = 9, t-value = -3.513, P<0.001) difference between treatments (Table 3). The first treatment (16.2%) produced a significantly lower percentage of surviving female wasps in comparison to the second (28.3%) and third (30.0%) treatments.

Table 3. The effects of enlargement of optimal space and resource-host-parasitoid (RHP) densities on sex ratio and percentage of survived female wasps of *C. vestalis* (success in biological control).

Treatment	Sex ratio	Survived female wasps
1 (one unit of optimal space[1] and RHP densities[2])	0.26 ± 0.12 a[3*]	16.2 ± 5.1 a
2 (three unit of optimal space and RHP densities)	0.45 ± 0.08 b	28.3 ± 7.2 b
3 (six unit of optimal space and RHP densities)	0.45 ± 0.02 b[*]	30.0 ± 1.0 b

1 One unit of optimal space denotes a cage of 40×40×40 cm. 2 One unit RHP density denotes two plants (5-6-week-old Chinese cabbage), 40 *P. xylostella* larvae and 5 parasitoid (3-d-old, mated females of *C. vestalis*). 3 Means with same letter in each column are not significantly (P > 0.05) different (Tukey). *A significant difference was obtained when the sex ratio was compared with the sex ratio of 1:1.

CONCLUSIONS AND DISCUSSIONS

The success of biological control programs depend upon releasing huge numbers of biocontrol agents in target areas to reduce pest populations to a lower level, possibly below the economic injury threshold. The main purpose of mass rearing programs is optimal production of beneficial insects, which would be released at target areas. The production of high quality insects has been always received attention (Singh, 1982; Parker, 2005; Watt *et al.*, 2015). The current research attempted to optimize the enlargement of space and resource-host-parasitoid (RHP) biomass for mass rearing of *C. vestalis*. This parasitoid has been subjected to release in many areas for biological control program of *P. xylostella* but there is little information on the optimization of its mass rearing (Talekar and Shelton, 1993; Furlong *et al.*, 2013).

Previous experiments in our laboratory also showed that the different density of *P. xylostella* and *C. vestalis* had influenced the life-history parameters of produced wasps, such as sex ratio, developmental period, percentage parasitism and survival rate; the best results were obtained when a host plant (5-6-week-old Chinese cabbage) containing twenty larvae of the herbivore (the 2nd instar *P. xylostella* larvae) was exposed to five female parasitoid wasp (*C. vestalis* females) in a standard-sized cage (40×40×40 cm) for 24 h (M. Rezaei and J. Karimzadeh, unpublished data). This optimized herbivore density reduced intraspecific competition for food, and resulted in more herbivore survival. In addition, due to low number of *P. xylostella* larvae on each plant there was no need for food plant renewal.

Here, we extend the space and resource-host-parasitoid (RHP) biomass to consider their possible effects on the wasp production. This has clearly a significant influence on the offspring sex ratio, and hence, on the produced female wasps. According to the optimal host and parasitoid densities, for achievement to the high number production of parasitoid wasps we extend the optimal RHP biomass three and six times greater. These cage sizes of second treatment (70×55×50 cm) and third treatment (100×70×55 cm) are not used in any study for rearing of *C. vestalis*. Using the optimal density of parasitoid (5 parasitoids for 20 host larvae), superparasitism by *C. vestalis* was prevented. Superparasitism has negative effects on mass rearing system. Li *et al.* (2001) examined the effect of superparasitism on the bionomics *C. vestalis* in which they found that superparasitism can cause physical combat between parasitoid larvae. In comparison to normal single-oviposition parasitism, reduced survival, body size, female sex ratio and parasitic capacity caused by superparasitism, however it stretches out developmental time and adult longevity. Moreover, the negative effects increased with the number of supernumerary parasitoid larvae in the host. Female larvae of parasitoids are mainly better competitors than males under superparasitism conditions (Montoya *et al.*, 2011). In some parasitoids the superparasitism has others effects that it could be impressed some biological factors especially more produced females sex ratio as an exemplification, *D. longicaudata* (Cancino and Montoya, 2008). The negative effects of superparasitism could be reduced by management of exposure time (Montoya *et al.*, 2012; Suarez *et al.*, 2012; Zhang *et al.*, 2016). In the current study, we assumed 24 h for exposure time of *C. vestalis* to host larvae. It is suggested that different exposure time of *C. vestalis* to host larvae would have investigated in further studies. According to the results, durations of egg incubation and larval period varied between treatments from 7.45 to 8.02 days, which are similar to the results of Alizadeh *et al.* (2011). The present study showed that the best outcomes (*i.e.*, the lowest pupal period) resulted from the 1st treatment, which indicates a better suitability of the used space and RHP biomass (or rearing condition).

Parasitoid sex ratio can vary due to various factors as illustration, photoperiod effects, inbreeding, virginity, host size, host quality, superparasitism, numbers of mated females in a foraging patch and rearing conditions (Godfray, 1994; Heimpel and Lundgren, 2000; Montoya *et al.*, 2011). Parasitoid wasps change their produced progeny according to environmental condition. Female parasitoids are of prime importance because females are responsible for population increase via growth rate, and because only females contribute to host (here means pest) mortality (Silva *et al.*, 2014). Many parasitoid species are not commercially available on account of many reasons, one of which might be male-biased sex ratios in mass rearing. Sex ratio, therefore, is considered as an important limiting factor for a number of parasitoid species (Godfray, 1994; Heimpel and Lundgren, 2000). To have a successful mass rearing of parasitoids for field release, it is paramount to determine the factors influence sex ratio (Montoya *et al.*, 2011). The sex ratio of offsprings of *C. vestalis* parasitizing the 2nd, 3rd and 4th instar of *P. xylostella* larvae has shown no significant difference (Kawaguchi and Tanaka, 1999). However, in another key parasitoid of the diamondback moth, *D. semiclausum*, sex ratio of produced wasps in the fourth host instar was significantly different from the first three instars (Yang *et al.*, 1993). The ratio of female progenies and the parasitism rate of *C. vestalis* have shown a decrease with the age of the female wasps (Kawaguchi and Tanaka, 1999). In the current study, a much better sex ratio of offspring wasps was obtained when the space and RHP biomass was extended. According to the local mate competition (LMC) model female parents of parasitoid produce more female offspring when have less contact with other females in the foraging patch (Asante and Danthanarayana, 1993; González-Zamora *et al.*, 2015). It is obvious that the results of this study make scene with this model. This idea has been supported by another study on a related wasp species, *Cotesia flavipes* (Vacari *et al.*, 2012) and it showed by other studies on different parasitoid species as an exemplification, *Spathius galinae* Belokobylskij and Strazenac (Hymenoptera: Braconidae) (Watt *et al.*, 2015). Ghimire and Phillips (2010) were reported different rearing container had any impact on the sex ratio of *Habrobracon hebetor* Say (Hymenoptera: Braconidae) in mass rearing condition. In contrast to the current study, they used of the same host-parasitoid densities for different containers. This variation may be owing to differences in the experimental design. Heimpel and Lundgren (2000) suggested that there was some potential for increasing the female-biased sex ratio of *C. vestalis* in commercial settings. Asante and Danthanarayana (1993) are on this assumption that there are several evolutionary models to predict sex ratio in parasitic wasp as illustration, Local Mate Competition (LMC), host quality models and other factors cases in point, superparasitism, host and parasitoid densities. On the basis of the current study, we reported enlargement of rearing space (with the same proportion of RHP) as another factor that impresses sex ratio of produced wasps. Thus, it is recommended that this factor be investigated for others parasitoids in mass rearing condition. In conclusion, the present study indicated that the second (cage of 70×55×50 cm, with 6 plants, 120 host larvae and 15 parasitoid wasps) and third (cage of 100×70×55 cm, with 12 plants, 240 host larvae and 30 parasitoid wasps) treatments are both optimum for mass rearing of *C. vestalis.* This research has initiated many questions, which need further investigations. It is necessary to weight up the result of this study from economical aspects.

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