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Mehran Rezaei
Department of Entomology, College of Agriculture, Khorramabad University, Lorestan, Iran

Javad Karimzadeh
Department of Plant Protection, Isfahan Research Center for Agriculture and Natural Resources, PO Box 199, Isfahan, 81785, Iran

Jahanshir Shakarami
Department of Entomology, College of Agriculture, Khorramabad University, Lorestan, Iran

Shahriar Jafary
Department of Entomology, College of Agriculture, Khorramabad University, Lorestan, Iran

Correspondence:
Mehran Rezaei
Department of Entomology, College of Agriculture, Khorramabad University, Lorestan, Iran

The influences of space and plant-host biomass on some biological key factors of *Cotesia vestalis* in mass-rearing conditions

Mehran Rezaei, Javad Karimzadeh, Jahanshir Shakarami and Shahriar Jafary

Abstract
Cotesia vestalis, one of the most important biocontrol agents of the diamondback moth, *Plutella xylostella*. The present study aimed to explore some simple ways to optimize the mass rearing of *C. vestalis*. Here, the effects of space and plant-host biomass on the quality of produced wasps were investigated. Experiment was conducted in four replication and each one of them concludes four treatments such as: 1) a small cage (40×20×40 cm), a plant and 20 *P. xylostella* larvae, 2) a small cage, two plants and 40 *P. xylostella* larvae, 3) a big cage (40×40×40 cm), two plants and 40 *P. xylostella* larvae, and 4) a big cage, four plants and 80 *P. xylostella* larvae. There was a significant effect of space and plant-host biomass on the sex ratio of produced wasps. The third treatment was the best in this regard. A mass rearing of *C. vestalis* can be recommended with the cage size of 40×40×40 cm and 40 host larvae established on two plants exposed to 5 female wasps for 24 h.

Keywords: *Plutella*, *Cotesia*, mass rearing, optimization, space, density

1. Introduction
The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera, Plutellidae), is the most destructive cosmopolitan insect pests of cruciferous plants. To control *P. xylostella*, farmers use large quantities of insecticides [16, 20]. The overuse of chemical pesticides against this pest has resulted in resistance to all groups of insecticides, including insect growth regulators [3, 2, 15] and *Bacillus thuringiensis* Berliner [13, 8]. In addition, the intensive and indiscriminate use of pesticides has destroyed the *P. xylostella* natural enemies, especially the parasitoids and predators, in *Brassica* crops agroecosystem. Biological control is widely recognized as a major component of *P. xylostella* management strategies particularly where control with chemicals has failed [1]. Larval parasitoids are the most effective natural enemies of *P. xylostella*. More than 40 hymenopterous parasitoids are known to be associated with *P. xylostella* larvae [20]. *Cotesia vestalis* (Haliday) (Hymenoptera, Braconidae), is a solitary and *P. xylostella*-specific larval endoparasitoid, has been introduced into many countries and helps control *P. xylostella*. Results of various studies in other countries showed that the parasitoid disrupts the population of *P. xylostella* in the field and releases of *C. vestalis* apparently exert an effective check of this pest [4]. *Cotesia vestalis* is a koinobiont parasitoid, develop in hosts that continue to feed and grow during the initial stages of parasitism. *C. vestalis* has three instars. The first two instars molted inside the host, whereas the third instar exited the host to spin a cocoon and pupate [1]. A thorough understanding of the biological characteristics of a parasitoid is required to achieve successful mass rearing and pest control. Mass production methods usually produce large numbers of entomophages, free from contaminants, which can be released immediately in the field [18]. However, there are virtually no general rules that can be laid down as to optimum conditions of cage-size, environment, feeding and illumination for breeding these. Each problem demands different treatment, depending on the requirements of individual species and number to be produced, and each scheme are usually based on technique developed in the laboratory and then modified for cheap, large-scale production [17]. The rearing process involves 3 steps: raising of cabbage plants, mass rearing of *P. xylostella* and mass production of *C. vestalis*. In order to develop a rearing program it is necessary to study the biology of the beneficial species and its insect's host, and to seek information on plant hosts or artificial diets. The influences of space and plant-host biomass are paramount factors

involved to the production of beneficial and pest insects. The present study aimed to examine the effects of space and plant-host biomass on eight biological factors (percentage parasitism based on cocoons, percentage parasitism based on emerged adults, survival rate based on cocoons, survival rate based on emerged adults, sex ratio, larvae developmental period, cocoons developmental period and percentage of survived female) of *C. vestalis*.

2. Materials and methods

2.1 Plant and insect rearing protocol

Chinese cabbage (*Brassica pekinensis*) was grown organically in plastic pots (10 cm diameter) under greenhouse condition (25±5ºC, 70±5% RH and L:D 16:8 h) without the application of any pesticide or fertilizer. These plants were used to rear *P. xylostella*. To start a culture of *P. xylostella*, its pupa and larvae were collected from common cabbage and cauliflower fields in Isfahan province (central Iran). *Cotesia vestalis* culture was started with the parasitized *P. xylostella* larvae collected from the same fields. Populations of *P. xylostella* were kept on 5-week-old Chinese cabbage in ventilated cages (40×40×40 cm). Similarly, the cultures of *C. vestalis*, in turn, were maintained on *P. xylostella* larvae in ventilated oviposition cages (40×40×40 cm). Both cultures were reared at standard constant environment (25 ± 2 ºC, 70 ± 5% RH and L:D 16:8 h). Aqueous honey solutions (35%) were placed in each cage as a source of carbohydrate for adult of *P. xylostella* and wasps. The adult food supply (honey solution) was replaced every 48 h [9, 11].

2.2 Experimental design

To explore the effects of space and plant-host biomass on life-history parameters of *C. vestalis*, an experiment was conducted as a randomized complete block design with four replications. Each replication includes the following treatments: 1) a small cage (40×20×40 cm), a plant (5-6-week-old Chinese cabbage) and 20 *P. xylostella* larvae, 2) a small cage, two plants and 40 *P. xylostella* larvae, 3) a big cage (40×40×40 cm), two plants and 40 *P. xylostella* larvae, and 4) a big cage, four plants and 80 *P. xylostella* larvae. To start the experiment, the 2nd instar *P. xylostella* larvae were established on the mentioned Chinese cabbages. After 24 h, five 3-d-old, mated females of *C. vestalis* 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. The parasitoid cocoons were further monitored for adult emergence. The experiment was conducted in constant environmental condition (25 ± 2 ºC, 70 ± 5% and L:D 16:8 h) [14].

2.3 Statistical analyses

The percentage parasitism was calculated as a ratio of the wasp to the sum of host and wasp. The data on percentage parasitism and survival rate were analyzed using logistic analysis of deviance. Data on sex ratio was analyzed 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 analyzed using nested ANOVA. Pair comparisons were done using Tukey's honest significance test. All statistical analyses were completed in *R. 2.10.0* [5, 6].

3. Results

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

When percentage parasitism was calculated based on the wasp cocoons, there was no significant (df = 12, t-value = 0.983, P = 0.345) difference between treatments (Table 1). The mean of percentage parasitism based on the wasp cocoons, varied between treatments from 76.6% (treatment 2) to 88.2% (treatment 1).

When percentage parasitism was calculated based on the emerged adult wasps, there was also no significant (df = 12, t-value = 1.040, P = 0.319) difference between treatments (Table 1). The mean of percentage parasitism based on the emerged adult wasps, varied between treatments from 73.9% (treatment 2) to 87.3% (treatment 1).

3.2 Survival rate of *C. vestalis*

When survival rate was calculated 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 59.4% (treatment 2) to 75.0% (treatment 1).

When survival rate was calculated 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 51.2% (treatment 2) to 68.8% (treatment 1).

Table 1: The effects of space and plant-host biomass on parasitism (by *C. vestalis*) and survival of *P. xylostella* larvae

	Percentage parasitism		Percentage survival	
Treatment	**Cocoon based**	**Adult based**	**Cocoon based**	**Adult based**
1(small cage, a plant, 20 larvae)	88.2±4.9 a[1]	87.3±5.7 a	75.0±10.2 a	68.8±12.3 a
2 (small cage, two plants, 40 larvae)	76.6±10.8 a	73.9±11.8 a	59.4±4.3 a	51.2±3.8 a
3 (big cage, two plants, 40 larvae)	77.1±5.1 a	74.4±5.9 a	67.5±5.1 a	58.1±6.8 a
4 (big cage, four plants, 80 larvae)	77.3±4.1 a	75.5±4.1 a	69.1±5.5 a	62.5±4.4 a

[1] Means with same letter in each column are not significantly (P > 0.05) different (Tukey)

3.3 Sex ratio (the ratio of produced female wasps)

When logistic analysis of deviance was used there was no significant (df = 12, t-value = -1.873, P = 0.086) difference between treatments (Table 2). The mean of production of female wasps varied between treatments from 0.70 (treatment 3) to 0.45 (treatment 2). Exact binomial test, however, showed a significant (P < 0.001) difference between the sex ratio resulted from the third treatment (0.70) compared with the sex ratio of 0.50.

3.4 Developmental period of larvae and cocoons

When developmental period of larvae was calculated, there was no significant ($F_{3,12}$ = 0.609, P = 0.622) difference between treatments (Table 2). The mean of developmental period of larvae varied among treatments from 7.69 days (treatment 3) to 8.72 days (treatment 1). In addition, the analyzed developmental period of produced wasps cocoons showed no significant ($F_{3,12}$ = 0.743, P = 0.547) difference (Table 2) and the mean of developmental period of cocoon

varied among treatments from 4.10 days (treatment 3) to 4.53 days (treatment 4).

Table 2: The effects of space and plant-host biomass on sex ratio and larvae and cocoons developmental period of *C. vestalis*.

Treatment	Sex ratio	Developmental period	
		Larval period	Pupal period
1(small cage, a plant, 20 larvae)	0.60±0.08 a[1]	8.72±0.51 a	4.50±0.13 a
2 (small cage, two plants, 40 larvae)	0.45±0.11 a	8.55±0.79 a	4.36±0.22 a
3 (big cage, two plants, 40 larvae)	0.70±0.08 a*	7.69±0.36 a	4.10±0.30 a
4 (big cage, four plants, 80 larvae)	0.46±0.07 a	8.12±0.63 a	4.53±0.24 a

[1] 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

Table 3: The effects of space and plant-host biomass on percentage of survived female wasps of *C. vestalis* (success in biological control).

Treatment	Percentage of survived female wasps
1(small cage, a plant, 20 larvae)	41.2±11.4 b[1]
2 (small cage, two plants, 40 larvae)	23.1±3.9 a
3 (big cage, two plants, 40 larvae)	40.6±7.4 b
4 (big cage, four plants, 80 larvae)	29.1±4.2 ab

[1] Means with same letter in each column are not significantly (P > 0.05) different (Tukey)

3.5 Percentage of survived female wasps

When the number of survived female wasps was calculated, there was a significant (df=12, z-value = -3.325, P < 0.001) difference between treatments (Table 3). The first (41.2%) and third (40.6%) treatments have significant greater percentage production of survived female wasps in contrast with other treatments.

4. Discussion

One of the factors involved in mass rearing of the beneficial species for biocontrol programs of the insect pests is the optimization of the host and parasitoid densities. Little is known about the ways of optimizing the mass production of *C. vestalis*. Here, we established a basic and model condition for the mass rearing of *C. vestalis* based on the life-history parameters of the produced wasps. Previous studies have shown that *C. vestalis* respond to volatiles derived from the host, the host plant and from plant-host complexes [12, 19]. Chinese cabbage is the most susceptible host plant to attack by *P. xylostella*. When larvae of *P. xylostella* were fed by Chinese cabbage they had the lowest larval and pupal periods, more pupal weight and highest survival rate compared with the other host plants [10, 21]. It has been also shown that greater percentage parasitism by *C. vestalis* can be achieved on *P. xylostella* larvae fed on Chinese cabbage compared with common cabbage, cauliflower or broccoli [12, 21]. However, at the time of performing the present study there was no information in the literature about the optimized space and density of plant and *P. xylostella* larvae for the mass rearing of *C. vestalis*. Previous experiments in our lab showed that initial densities of *P. xylostella* and *C. vestalis* influenced biological factors, such as percentage parasitism, survival rate, sex ratio and developmental period (M. Rezaei, J. Karimzadeh, J. Shakarami and S. Jafari, unpublished data); such that when the standard cage size (40×40×40 cm) was used the best results was obtained with 20 host larvae per plant and a 24-h-exposure to 5 female wasps. In the present study, more attempts have been done to optimize the cage size and plant-host biomass for mass production of *C. vestalis*. Here, it was shown that the cage size and plant-host biomass are both influential in success of a mass rearing of *C. vestalis*. The results showed that the first (small cage, a plant, 20 larvae) and third (big cage, two plants, 40 larvae) treatments are suitable for both the future trials and the mass rearing of *C. vestalis*; as they produced more female wasps. However, the third treatment may be more applicable due to use of fewer numbers if initial wasps and a significant greater sex ratio. Vacari *et al*. (2012) stated that a release of parasitoids with the sex ratio of 60% females appeared to be satisfactory for the establishment and eventual control of the pest in field. Below this value, low efficiency in parasitism occurs. However, a high proportion of females compared to males may cause arrhenotokous parthenogenesis, which in, unmated female produce male offsprings only. This is undesirable for the maintenance of the parasitoid population in field and even in biocontrol producing facilities. The present study indicated that extending the space for the same host-plant biomass may results in greater produced females (as seen in treatment 1 and 3). In an experiment with different host density of *Diatraea saccharalis* (3, 4 and 5 larvae per dish) exposed to *Cotesia flavipes*, Vacari *et al*. (2012) also found that host density affected the parasitoid sex ratio [22]. A host density of three larvae per dish resulted in a better sex ratio (0.77). It is well known that parasitoid wasps change their offspring in response to changes in the environment and that this adaptation might explain the differences in the sex ratio of wasps [7].

5. Conclusion

In total, the third treatment had significantly higher number of produced female wasps. The third treatment was the best in this regard. A mass rearing of *C. vestalis* can be recommended with the cage size of 40×40×40 cm and 40 host larvae established on two plants exposed to 5 female wasps for 24 h.

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