Prey Responses of *Propylaea japonica* in Different Instars to *Acyrthosiphon pisum* under Growth Chamber Condition

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Abstract: The functional response of *Propylaea japonica* Thunberg (Coleoptera: Coccinellidae) to five densities of *Acyrthosiphon pisum* Harris (Homoptera: Aphididae) was investigated by disc method under growth chamber condition, in order to understand the controlling capability of *P. japonica* to *A. pisum*. Plotting prey density against prey killed by four larval instars, adult males and females of *Propylaea japonica* fit well with the type Ⅱ model of Holling's disc equation. Adult females consumed the highest number of the prey, followed by fourth instars and adult males. Based on the functional response data, the model predicts a maximum of 6.5, 20.4, 32.3, 71.4, 58.8, and 80.0 nymphs to be consumed per day by individual first, second, third instar, adult male, fourth instar, and adult female, respectively. The results indicated the predation of *P. japonica* on *A. pisum* was greater.

Key words: *Propylaea japonica*; *Acyrthosiphon pisum*; Functonal response; Predation

不同龄期龟纹瓢虫对豌豆蚜的捕食功能反应

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摘要：为了解龟纹瓢虫(*Propylaea japonica*)控制豌豆蚜(*Acyrthosiphon pisum*)的能力，在实验室内采用圆盘法测定了不同龄期龟纹瓢虫对豌豆蚜的捕食功能反应，以期掌握龟纹瓢虫控制豌豆蚜的能力。结果表明：龟纹瓢虫 4 个龄期的幼虫和雌雄成虫捕食豌豆蚜的数量与豌豆蚜密度呈负加速曲线关系，符合 Honing-Ⅱ型功能反应模型。龟纹瓢虫雌成虫的日捕食量最大，其次是第 4 龄幼虫和雄虫。模型预测了龟纹瓢虫 1,2,3,4 龄幼虫及雄、雌成虫对豌豆蚜的日最大捕食量分别是 6.5,20.4,32.3,71.4,58.8,80.0 头。综上所述，龟纹瓢虫对豌豆蚜有较强的控制能力。

关键词：龟纹瓢虫；豌豆蚜；功能反应；捕食
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Pea aphid, *Acyrthosiphon pisum* Harris (Homoptera: Aphididae), is one of the most devastating insect pests of leguminous forage all over the world including China[1-3]. It prefers clustering at the delicate foliage and buds, sucking juice and excreting honeydew, which affect the quality and quantity of herbage production. More seriously, pea aphid is a vector of alfalfa mosaic virus (AMV), a disease that makes black stem disease of lucerne more severe and cause much more damage than the aphid itself[4]. Ladybird beetle *Propylaea japonica* Thunberg (Coleoptera: Coccinellidae) is a native and widespread aphidophagous coccinellid predator in China. It has been reported that *Propylaea japonica* preyed on *Sitobion avenae*[5], *Aphis gossypii*[6], *Rhopalosiphum maidis*[7], *Macrosiphum*

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sanborni[8], *Myzus persicae*[9]. Moreover, the predatory functional responses of *P. japonica* adults which were in satiation and hunger for 24 h on *A. pisum* in alfalfa (*Medicago sativa* L.) were determined[10]. Nevertheless, no any paper has covered all stages of *P. japonica* preying on *A. pisum* in alfalfa in China. Therefore, the prey responses and effects of all predaceous stages of this ladybird on the different densities of *A. pisum* were investigated here. The capacity of controlling pea aphids was analyzed to provide a reference for the use of pest biological control in alfalfa.

1 Materials and Methods

1.1 Insect cultures

Adults of *Propylaea japonica* originally collected from the alfalfa fields at Gansu Agricultural University in May, 2009, were fed with *A. pisum* in alfalfa (Hunter River) in cages (60 cm wide × 150 cm long × 50 cm high) under laboratory conditions (25℃ ± 2℃, 50% ± 10% RH, and light/dark = 14 h/10 h). Adult males and females were provided with *A. pisum* in alfalfa plants and kept in cohort for mating. After 24 h, eggs were collected and transferred into clean Petri dish (7 cm diameter × 2 cm deep), which containing a small alfalfa branch with two foliages. In order to maintain humidity, the bottom end of the alfalfa stem was wrapped in a water-saturated cotton ball. Then these eggs were conducted in artificial intellectual climate chamber (RXZ-300C) under abiotic condition: 25℃ ± 1℃, 70% ± 7% RH, light/dark = 14 h/10 h and 11000 lx. *P. japonica* larvae were also conducted in artificial intellectual climate chamber when molted and were fed with *A. pisum* in alfalfa.

A colony of *A. pisum* was also originally collected from alfalfa fields and maintained in alfalfa (Hunter River) cultivated in small pots (15 cm high × 10 cm diameter) at 20℃ ± 3℃, 50% ± 10% RH and 16 h/8 h(light/dark).

Both ladybird and aphid individuals were kept in a growth chamber (3 m × 4 m × 5 m).

1.2 Methods

P. japonica larvae (10 h post-molting), adult males and females were collected in plastic jars (11 cm long × 7 cm diameter), adults were separated and sexed according to the size (male is smaller in size than female). Predation was assessed by placing a single larva/adult in an experimental arena consisting of a plastic jar containing a small alfalfa branch with two foliages. In order to maintain humidity, the bottom end of the alfalfa stem was wrapped in a water-saturated cotton ball.

The predation potential of ladybird larva increased as larva proceeds to the next stage, prey densities were increased for later instars and adult stages. Therefore, five prey densities of *A. pisum* were evaluated: 5, 10, 15, 20, 25 aphids for the first instar; 10, 15, 20, 25, 30 aphids for the second instar; 10, 20, 30, 40, 50 aphids for the third instar; 20, 30, 40, 50, 60 aphids for the fourth instar, male and female, respectively. In addition, there was a plastic jar with 30 aphids of *A. pisum*, which contained all stages of pea aphid, for estimating the number of aphids after 24 hours. *A. pisum* were gently transferred to each experimental arena using a fine hair brush putting them on the alfalfa branch described above. Ten replicates were made for each combination with controls. The experiments were conducted in artificial intellectual climate chamber (RXZ-300C) under abiotic condition: 25℃ ± 1℃, 70% ± 7% RH, light/dark = 14 h/10 h, and 11000 lx. After 24 h, all dead and living aphids were recorded.

1.3 Data analysis

The relationships between mean rate of consumption (prey consumed/prey offered × 100) by adult males, females and different larval instars related to prey density were analyzed using general regression equation (SPSS 13.0 for windows and Excel 2003). Holling curvilinear type Ⅱ equation[11] was used to fit the data for functional response. In this model, the number of prey consumed (N_a) is a function of prey density (N) as follows:

$$N_a = (a' T_t N)/(1 + a' T_h N),$$

Where a' is the attack (discovery) rate of the prey, T_t is the total time available (1 d or 24 h in this experiment), and T_h is a handling time for one prey.

Make $T_t = 1$, $B = 1/a'$, $A = T_h$, Transform $N_a = (a' T_t N)/(1 + a' T_h N)$ into $1/N_a = B(1/N) + A$, calculate value of a' and T_h by using the least square method.

2 Results

The percentage of prey consumed by each predatory stage is negatively correlated with the prey densities offered (Fig. 1) with subsequent r^2 values being highest (0.956) for second instar and lowest (0.759) for adult females. The rate of prey consumption declined with the increasing prey density for all predatory stages, suggesting that these stages exhibit a type Ⅱ functional response (Fig. 2). The type Ⅱ functional responses of all stages of *Propylaea japonica* are followed:

First instar: $N_a = 0.229N/(1+0.022579N)$;

Second instar: $N_a = 0.512N/(1+0.025037N)$;

Third instar: $N_a = 0.656N/(1+0.020336N)$;

Fourth instar: $N_a = 0.870N/(1+0.01218N)$;

Adult male: $N_a = 0.568N/(1+0.009826N)$;

Adult female: $N_a = 0.779N/(1+0.009738N)$.

The fourth instars showed the highest (0.870) rate of attack (Table 1). However, the first instars exhibited the lowest (0.338) rate of attack. Handling time decreased as predatory larva proceeded to the next stage, and the adult females had the shortest handling time (18 min). Subsequently, the model predicts a maximum consumption of 6.5, 20.4, 32.3, 71.4, 58.8, 80.0 *A. pisum* aphids by first, second, third and fourth instars, adult males and females, respectively in 24 hours (Table 1).

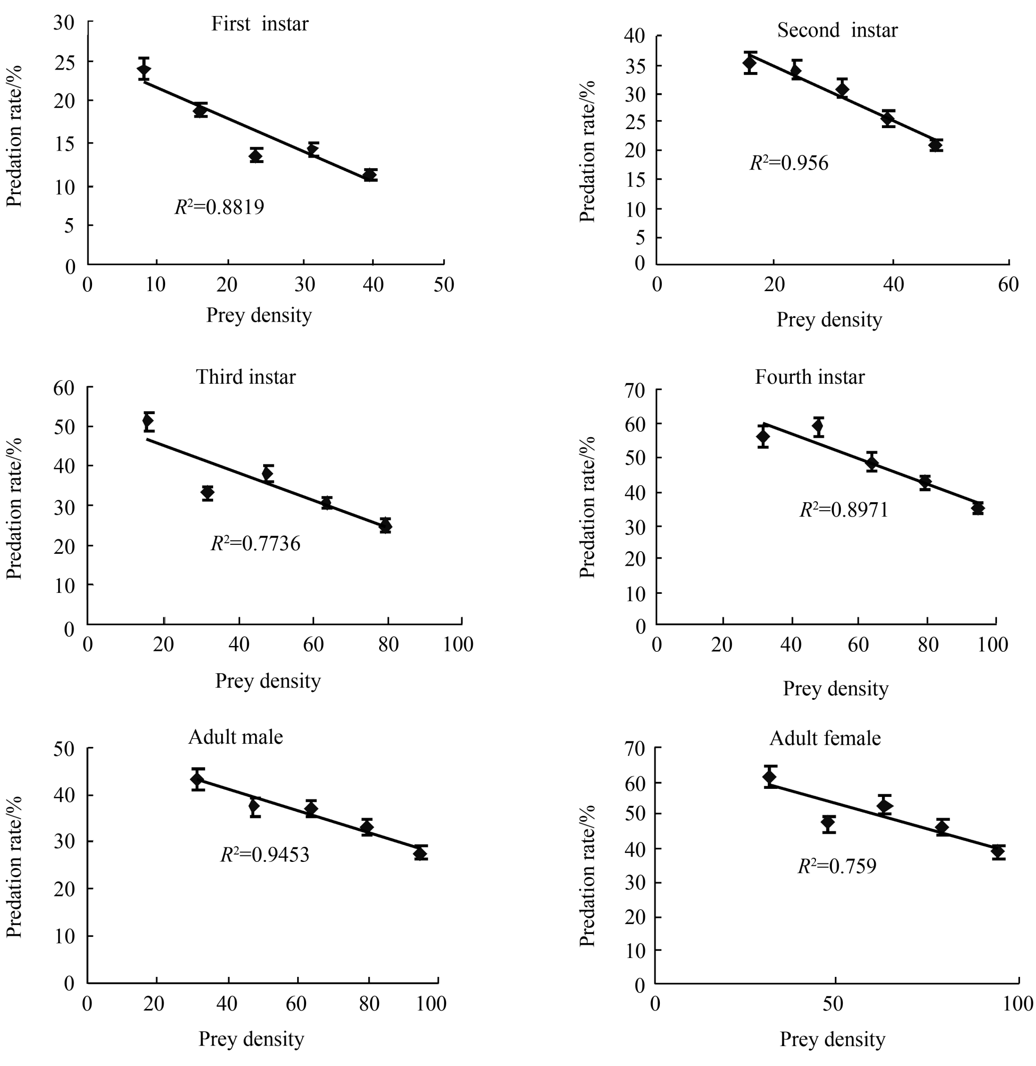

Fig. 1 Percent predation of *P. japonica* to *A. pisum* on alfalfa

Note: Points show average number of aphids eaten or killed by *P. japonica* at each level of prey availability. Error bars show standard errors

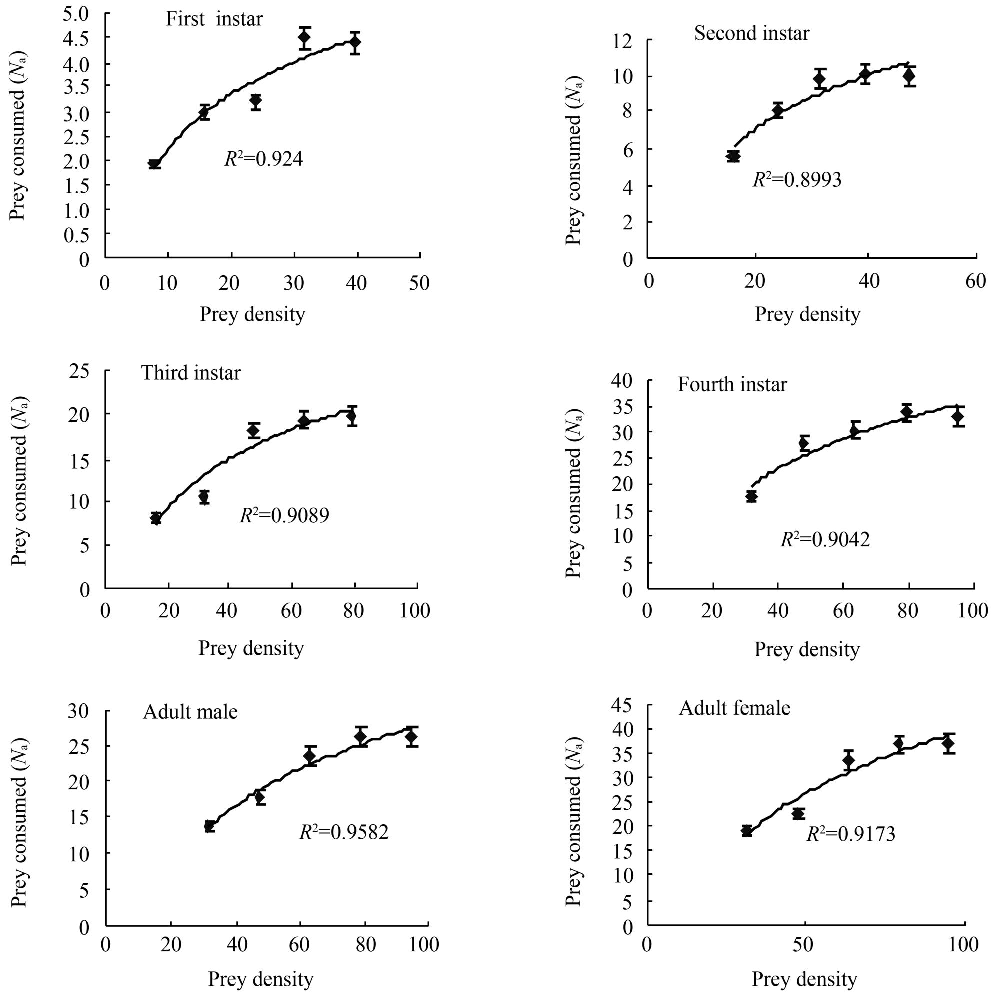

Fig. 2 Functional response of *P. japonica* to *A. pisum* on alfalfa

Note: Points show average number of aphids eaten or killed by *P. japonica* at each level of prey availability. Error bars show standard errors. The line shows the Holling's model for a type Ⅱ functional response

Table 1 Type Ⅱ functional response parameters of *Propylaea japonica* at different densities of *Acyrthosiphon pisum*

Stage/instar	n	a'±SE	T_h±SE/min	T_t/T_h	R
First instar	10	0.338±0.015	0.1533±0.0279(220.75)	6.5	0.985**
Second instar	10	0.512±0.032	0.0489±0.0112(70.42)	20.4	0.970**
Third instar	10	0.656±0.041	0.0310±0.0090(44.64)	32.3	0.959**
Fourth instar	10	0.870±0.056	0.0140±0.0040(20.16)	71.4	0.961**
Male	10	0.568±0.022	0.0173±0.0029(24.91)	58.8	0.990**
Female	10	0.779±0.045	0.0125±0.0042(18.00)	80.0	0.962**

Note: R is the correlation coefficient between $1/N$ and $1/N_a$

3 Discussion and Conclusion

The data indicates that all tested stages of *P. japonica* exhibited typical type Ⅱ functional response (Fig. 2). Similar reponse were earlier reported for other aphidophagous, such as, *Sitobion avenae*[5], *Aphis gossypii*[6], *Rhopalo siphum maidis*[7], *Macrosiphum sanborni*[8], *Myzus persicae*[9], or other coccinellids such as *Hippodamia* (*Adonia*) *variegate*[12-16], *Coccinella septempunctata*[17-18], *Adonia variegate*[19], *Hippodamia tredecimpunctata*[13], etc.

The relative rate of prey consumption by *P. japonica* was higher at low densities, which indicated that this predator would be more effective at controlling the *A. pisum* population at low densities. The failure causes of *P. japonica* in controlling the aphid at higher population densities could be: (i) Honeydew excreted by aphids affects the searching behavior of the predators[20]; (ii) More importantly, a high rate of prey consumption is not a feature of aphidophagous coccinellids[21] that leads to the failure of aphid biological control programs using only predatory coccinellids. Therefore, for consideration of *A. pisum* biocontrol, the coccinellids have to be released early before the aphids reach high density.

The release stage of predators influences its effectiveness and potential economic benefits because the search rates and handling times of predators are different in different developmental stages [12,14]. Therefore, the data provide insights to assess which stages for the release of *P. japonica* against *A. pisum*. The first instars larvae of *P. japonica* become easily the prey of other ladybird due to its small size which should be cautious to be released. The fourth instars and adult stages of *P. japonica* could be efficient to suppress *A. pisum* density because of higher attack rates and shorter handling times. The third instars larvae may be cost-effective stages because of shortening mass rearing periods.

The elevated functional response curve of adult female over that of the adult male indicates the possibility of delayed satiation, faster digestion [22], more large body size than males; hence greater food required and higher nutritional demands, particularly for egg formation.

In natural conditions, lady beetle will prey on all stages of aphids encountered, rather than just higher instars nymphs. If only using higher instars nymphs, it would not reflect the reality. It is also difficult to distinguish between higher instars nymphs and adult aphids. If only adult aphids were used, it would reduce consumption of ladybug. For reaction of natural enemy predation on aphids in the field, all stages of *A. pisum* were used in the experiment.

The study of functional response in the laboratory could be used to infer basic mechanisms underlying natural enemy-prey interaction[23]. Such studies provide valuable information for biological control programs. During biological control evaluation processes, comparisons of parameter values of two or more predators may be more meaningful and convenient than similar comparisons involving functional response curves[24].

However, the relations between functional responses observed in the laboratory and field performance of natural enemies is not identical, some studies showed a significant difference between the responses observed in laboratory and field environment[23]. It is recognized that functional responses derived from laboratory research may have little resemblance to those that could be measured in the field. For instance, other factors, such as intrinsic growth rates, host patchiness, predation and competition, host traits, and environmental complexities (abiotic and biotic factors) also have a major influence on the efficiency of predator in managing the prey population. The data provided here indicate how these predators will respond to increasing prey density under simplified experimental conditions. For conclusive estimations of their biocontrol potential, further field studies are needed.

Recent years, pea aphid population is steadily increasing and has been being a dominance population in alfalfa field at Lanzhou, Gansu province. Therefore it is needed to make proper prevention and control of pea aphid in order to avoid a large yield loss.

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