

Article

Parasitism Potential and Laboratory Rearing of *Psyllaephagus* sp., a Newly Discovered Parasitoid of *Cacopsylla chinensis*

Zifang Qin ¹, Mingyue Feng ¹, Liu Zhang ², Yang Ge ^{3,4}, Xinzheng Huang ¹ and Wangpeng Shi ^{1,*} 

¹ Department of Entomology and MOA Key Lab of Pest Monitoring and Green Management, China Agricultural University, Beijing 100193, China; zifangqin351@cau.edu.cn (Z.Q.)

² Institute of Plant Quarantine, Chinese Academy of Inspection and Quarantine, Beijing 100176, China

³ National Resource Center for Chinese Materia Medica, State Key Laboratory Breeding Base of Dao-Di Herbs, China Academy of Chinese Medical Sciences, Beijing 100700, China

⁴ Key Laboratory of Biology and Cultivation of Herb Medicine, Ministry of Agriculture and Rural Affairs, Beijing 100700, China

* Correspondence: wpsshi@cau.edu.cn

Abstract: *Psyllaephagus* sp. (Hymenoptera: Encyrtidae) is a newly recognized and important parasitoid of *Cacopsylla chinensis* (Yang and Li) (Hemiptera: Psyllidae), a pest of pear orchards. Its parasitism potential and rearing were studied in the laboratory. The studies showed that the most suitable hosts were fourth- and fifth-instar nymphs of pear psyllids (*C. chinensis*). The development duration of *Psyllaephagus* sp. females and males was 15.25 ± 0.37 and 13.57 ± 0.27 days when laying eggs in fourth-instar psyllid nymphs. The wasps did not survive longer than three days when they were fed only water, while they survived about an average of 20 days (23.20 ± 1.12 for females and 19.00 ± 0.80 for males) when fed 20% honey water. The provision of honey water could thus increase adult parasitoid longevity significantly. The lifetime fecundity of *Psyllaephagus* sp. females was 21.60 ± 0.88 . Tests of parasitoid efficiency showed that the wasp's functional response was Holling type II, with the number of hosts parasitized increasing with the host density to a maximum parasitism rate. The model suggested that a single *Psyllaephagus* sp. female could parasitize a maximum of 13.66 nymphs per day. The mutual interference of foraging *Psyllaephagus* sp. females occurred at high parasitoid densities. *Psyllaephagus* sp. has potential as a biocontrol agent for use against *C. chinensis*.

Keywords: pear psylla; Psyllidae; parasitoid wasp; biological characteristics; biological control



Citation: Qin, Z.; Feng, M.; Zhang, L.; Ge, Y.; Huang, X.; Shi, W. Parasitism Potential and Laboratory Rearing of *Psyllaephagus* sp., a Newly Discovered Parasitoid of *Cacopsylla chinensis*. *Agronomy* **2023**, *13*, 943. <https://doi.org/10.3390/agronomy13030943>

Academic Editor: Andrea Liliana Clavijo McCormick

Received: 25 February 2023

Revised: 20 March 2023

Accepted: 20 March 2023

Published: 22 March 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The pear psylla, *Cacopsylla chinensis* (Yang and Li) (Hemiptera: Psyllidae), is an important pest in pear orchards in China [1,2]. Pear psyllids damage pear trees by direct feeding on the phloem and xylem, and, more importantly, cosmetic damage from sooty mold growing on the pest's honeydew reduces the photosynthesis of pear leaves and the quality of pear fruits [3–5]. Currently, chemical insecticides are commonly used options to control *C. chinensis*. However, due to psyllids' cryptic habits and the honeydew they produce, chemical control is not always effective, and side-effects of use can include harm to natural enemies, environmental pollution, and resistance of *C. chinensis* to chemical insecticides. However, the natural enemies of pear psyllid are often abundant and may be a practical alternative for biological control.

The genus *Psyllaephagus* is a cosmopolitan group of 246 known species [6,7], of which 18 have been recorded in China [7–15]. Species of *Psyllaephagus* are believed to be all primary endoparasitoids of psyllids [16]. Several *Psyllaephagus* species have been used for biological control of psyllids. For instance, (1) *P. yaseeni* Noyes has been used against *Heteropsylla cubana* Crawford in a number of countries; it has been established in some of these countries

and at least partially controlled the psyllids effectively [17]. (2) *P. pilosus* Noyes was released at eight sites in California and psyllid *Ctenarytaina eucalypti* Maskell populations declined somewhat at most sites [18]. (3) *P. bliteus* Riek is a parasitoid of red gum lerp psyllid, *Glycaspis brimblecombei* Moore, which was imported from Australia and released in California after this psyllid invaded California [19]. (4,5) *P. parvus* Riek and *P. perplexans* Cockerell were introduced into southern California to control *Eucalyptolyma maiden* Froggatt and *Cryptoneossa triangula* Taylor, respectively [20]. However, little information is available about *Psyllaephagus* parasitoids of *Cacopsylla*.

We found an undescribed species of *Psyllaephagus* attacking *C. chinensis* in pear orchards in China. It was first discovered in Dangshan, Anhui Province, through field investigation. The wasp is widely distributed in China, being found in pear orchards in Dangshan in Anhui Province and Fangshan, Beijing, and Taian in Shandong Province, covering parts of both southern and northern China. Parasitism rates often reach 50% in pear orchards (unpublished data), suggesting the wasp has potential for augmentative or conservation biocontrol of pear psyllids in the field. Here, we present information on the developmental duration, host stage preference, fecundity, functional response, and laboratory rearing of this new *Psyllaephagus* species.

2. Materials and Methods

2.1. Insects

Colonies of *C. chinensis* were reared as described by Ge et al. [21]. That is, *Cacopsylla chinensis* colonies originated from field-collected individuals harvested from a pear orchard in Anhui Academy of Agricultural Sciences, China (31°89'48" N, 117°25'23" E). The insect colonies were maintained in growth chambers under the following conditions: 25 ± 2 °C, RH 75 ± 5%, and 16:8 h L:D photoperiod. Psyllids were maintained on two-month-old pear seedlings. The pear (*Pyrus bretschneideri* Rehder) leaves and seedlings were obtained from potted pear plants grown under controlled conditions. Discrimination of the different instars of *C. chinensis* followed the method reported by Gai et al. [22]. The *Psyllaephagus* sp. used in the experiments was collected from Fangshan, Beijing, China (39°38'25" N, 116°4'13" E), as parasitized pear psylla nymphs, and when adult wasps emerged, they were paired and placed into 1.5 mL centrifuge tubes. Its COI sequence can be obtained with the accession number OP787025 in GenBank. The wasps were fed on 20% honey water and provided with 4th- and 5th-instar nymphs of *C. chinensis* for oviposition. The insect colonies were maintained in an insectarium at a 16 L:8 D photoperiod, 25 ± 2 °C, and 75 ± 5% RH.

2.2. Wasps' Host Stage Preference

To determine the optimal host life stage for parasitoid rearing, a fresh pear leaf was inserted aslant into water agar in a Petri dish (9 cm diameter), and then the dish was covered with a sheet of stretched parafilm that had been punctured multiple times with a pin (diameter = 0.3 mm). In the choice test, 15 psyllid nymphs of each of the three instars (third, fourth, and fifth) were introduced on the pear leaf in the Petri dish and allowed to settle for one hour. Ten replications were set. A pair of newly emerged (<24 h old) and mated *Psyllaephagus* sp. adults was introduced into each Petri dish and left for 24 h. The wasps were then removed. To allow close observation of individual nymphs, all the exposed psyllids were placed individually in 1.5 mL centrifuge tubes and held until they molted to adults, died, or parasitoids emerged. We then calculated the pear psyllids' mummies as the parasitism number for each psyllid instar.

In a no-choice test, groups of 40 psyllids of each of the three nymphal instars were placed on pear leaves in the Petri dish, with instar groups in separate Petri dishes. Then, a pair of newly emerged (<24 h) and mated wasps was introduced into the Petri dish and left for 24 h. From this point on, the procedures for post-exposure observations were the same as described above for the choice test. This experiment was replicated 10 times.

Both experiments were carried out in an incubator at 16 L:8 D photoperiod, 25 ± 2 °C, and $75 \pm 5\%$ RH.

2.3. Developmental Duration and Fecundity of *Psyllaephagus* sp.

To measure the life stage duration of *Psyllaephagus* sp., one potted pear seedling (3 months old) bearing 300 4th instar psyllids was put in a cage (30 cm × 30 cm × 40 cm), and then 10 pairs of *Psyllaephagus* sp. were introduced into the cage for 24 h to initiate a single large cohort of parasitoid offspring. Thereafter, we dissected 10 or more parasitized psyllids daily under a stereoscopic microscope. All parasitoid immature stages seen in these host dissections were classified to life stages, and the data was used to calculate the duration of each immature parasitoid stage.

To measure total fecundity of *Psyllaephagus* sp. females, a potted pear seedling bearing 20 4th-instar nymphs was put in a cage (as above). Then a pair of newly emerged (<24 h old) adults of *Psyllaephagus* sp. was introduced into the cage. The pear seedling with 4th-instar psyllids was replaced daily until the female parasitoids had died. Honeydew excreted by pear psyllids can supply the diet for parasitic wasps, so we did not additionally provide honey water for the parasitic wasps. After its day of parasitoid exposure, each potted pear was held, the nymphs on it were examined daily, and the number of psyllid mummies was noted. Mummified psyllids were collected daily as they formed. They were then held for parasitoid emergence. The emerging parasitoids were counted and sexed to determine the progeny number and sex ratio. This experiment was replicated 10 times.

2.4. Influence of Supplementary Nutrition on *Psyllaephagus* sp. Adult Longevity

To determine an optimal concentration of honey water for feeding adult parasitoids, newly emerged adults of *Psyllaephagus* sp. were put in centrifuge tubes plugged with absorbent cotton and fed on absorbent cotton soaked with 0, 5%, 10%, or 20% honey water. The absorbent cotton was changed daily, and parasitoids were checked daily until all had died. Ten replicates (5 adults in each replicate) of each sex were run per treatment. During the test, wasps were held at 25 ± 2 °C, $75 \pm 5\%$ RH, and a 16:8 L:D photoperiod.

2.5. Functional Response of *Psyllaephagus* sp. Females to *C. chinensis* Nymphs

To determine the response of parasitoids to the host density, the species' functional response to hosts was measured. Newly emerged (1 d old) isolated female parasitoids reared with 20% honey water were presented with either 8, 16, 24, 32, 40, 50, or 70 newly molted 4th- or 5th-instar psyllids for 24 h, after which the females were removed. The number of mummified *Psyllaephagus* sp. nymphs from each treatment was recorded for each wasp. Since the differences in functional responses between type II and type III are mainly the result of different parasitism rates at lower host densities, more replications at lower host densities are recommended. Therefore, there were ten replicates for treatments with host densities of 8 or 16, but only five for host densities of between 24 and 70.

2.6. Optimal Host/Parasitoid Ratios

To determine the effect of various host/parasitoid ratios, we placed 1, 2, or 3 pairs of newly emerged (<24-h old) *Psyllaephagus* sp. wasps in Petri dishes with 20 newly molted 4th- or 5th-instar nymphs of *C. chinensis* for 24 h. The numbers of nymphs parasitized in each treatment were recorded to estimate the parasitism rates for each host/parasitoid ratio. The experiment was replicated ten times for each treatment.

2.7. Statistical Analysis

All data were checked for normality and homoscedasticity before comparison analysis. The number of parasitized pear psyllids in the choice and no-choice tests were compared using Kruskal–Wallis tests. The data were first subjected to a square-root transformation because 1st- and 2nd-instar nymphs were not parasitized; 0.5 was first added to the original data, and then the transformed total was used to analyze the host stage preference

results [23]. Differences in the wasps' adult longevity between different levels of supplementary nutrition were analyzed using a one-way ANOVA. The least significant difference (LSD) test was used to determine the difference among different treatments at $p < 0.05$. The development period and longevity data of the parasitic wasps were analyzed with Student's t -test. The data were analyzed statistically with the software SPSS v21.0.

A one-way ANOVA at a significance level of $p < 0.05$ for mean separation was used to detect the effect of host/parasitoid ratios on parasitism. The parasitoid search efficiency in relation to the parasitoid density (i.e., indirect mutual interference) was estimated from Equation (1) [24].

$$s = \frac{1}{P_t} \ln \left[\frac{N_t}{N_t - N_a} \right] \quad (1)$$

where P_t is the density of *Psyllaephagus* sp. females, N_t is the density of *C. chinensis* nymphs, and N_a is the number of nymphs parasitized. The searching efficiency (s) was regressed on the \log_{10} -transformed female *Psyllaephagus* sp. densities using least-squares regression [23].

A two-step method was used to analyze the results of the functional response test [25]. First, a logistic regression was applied to determine the relationship between the percentage of *C. chinensis* nymphs parasitized by *Psyllaephagus* sp. and the *Psyllaephagus* sp. density, using the logistic model (Equation (2)).

$$\frac{N_a}{N_0} = \frac{\exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)}{1 + \exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)} \quad (2)$$

where N_a is the number of *C. chinensis* nymphs parasitized; N_0 is the nymphs' density; and P_0, P_1, P_2 , and P_3 are the intercept, linear, quadratic, and cubic coefficients, respectively. The type of functional response can be determined by the signs of the linear and quadratic coefficients P_1 and P_2 , where $P_1 = 0$ indicates a type I functional response, $P_1 < 0$ indicates a type II functional response, and a type III functional response is indicated by $P_1 > 0$ and $P_2 < 0$ [26–28]. When there was no significant difference between P_1 and 0, the cubic and quadratic coefficients in Equation (2) were successively deleted until there was a significant difference between the linear coefficient P_1 and 0 [28–30]. The P_0, P_1, P_2 , and P_3 values in the logistic regression model were calculated using GLM in R [31]. In this case, a type II functional response was obtained; hence, Holling's disc equation for a type II functional response was fit to calculate the handling time per host T_h and the attack constant a (Equation (3)).

$$N_a = \frac{aTN_0}{1 + aT_h N_0} \quad (3)$$

where N_a is the number of *C. chinensis* nymphs parasitized, N_0 is the nymphal density, T is the total exposure time in the experiment (1 d), and T/T_h is the maximum number of hosts parasitized per female and day.

3. Results

3.1. Wasps' Host Stage Preference

The results from both choice (Figure 1a) and no-choice (Figure 1b) tests show that *Psyllaephagus* sp. preferred fourth- or fifth-instar psyllids to younger instars. The first- and second-instar nymphs were not parasitized by the wasps, and parasitism on the fourth or fifth instars was significantly higher than on the third instar, but no significant difference occurred between fourth- and fifth-instar parasitism in the choice test (Kruskal–Wallis test, $F = 42.894$; $df = 4.45$; $p < 0.001$). In the no-choice test, the first- and second-instar nymphs were also not parasitized by the wasps, and the parasitism on the third, fourth, and fifth instars had no significant difference (Kruskal–Wallis test, $F = 45.353$; $df = 4.45$; $p < 0.001$).

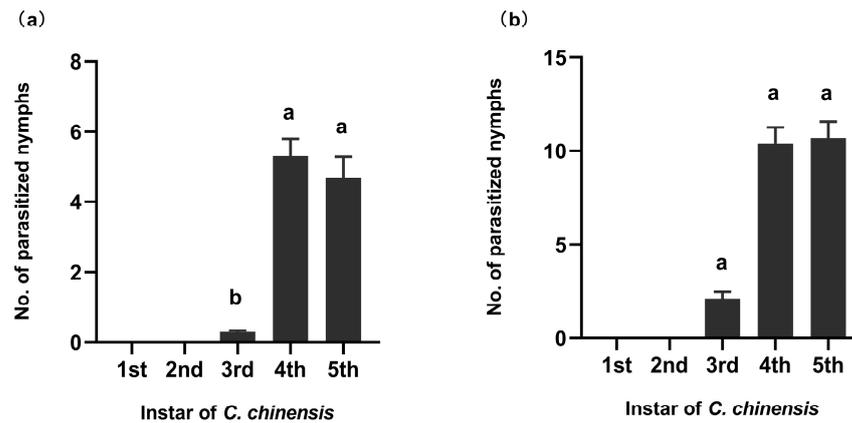


Figure 1. Preference of *Psyllaephagus* sp. to *Cacopsylla chinensis* among different nymphal stages. (a) Choice test; (b) No-choice test. Black bars (\pm standard deviation) followed by different lowercase letters indicate significant differences among instars ($p < 0.001$, Kruskal–Wallis test).

3.2. Developmental Duration and Fecundity of *Psyllaephagus* sp.

At 25 °C, the developmental durations of the egg, larval, and pupal stages of *Psyllaephagus* sp. were 1.52 ± 0.06 , 6.34 ± 0.20 , and 6.23 ± 0.15 d, respectively. The developmental duration of the immature stages combined of female and male adult wasps was 15.25 ± 0.37 and 13.57 ± 0.27 d, respectively, and the adult longevity was 22.80 ± 1.41 and 18.00 ± 0.97 d for females and males, respectively. The female parasitoids had a significantly longer developmental duration and greater longevity than males (developmental duration: two-sample *t*-test, $t = 3.80$; $df = 37$; $p = 0.001$; longevity: two-sample *t*-test, $t = 2.93$; $df = 36$; $p < 0.05$).

The lifetime fecundity of *Psyllaephagus* sp. females was 21.60 ± 0.88 , and their daily oviposition pattern is shown in Figure 2. The results showed that the daily oviposition number decreased with the increasing age of female parasitic wasps, and the numbers of female and male progeny were 7.00 ± 0.87 and 12.90 ± 0.80 , respectively, with a sex ratio (proportion female) of 0.35 ± 0.03 and an adult emergence rate of 0.92 ± 0.02 .

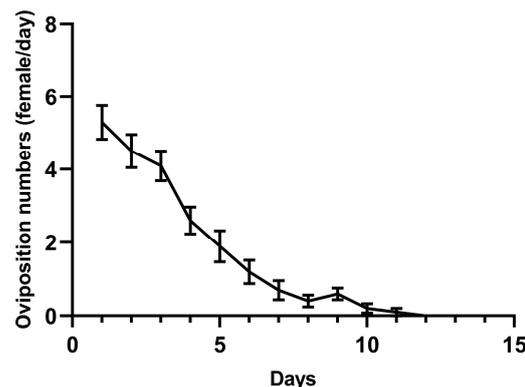


Figure 2. The daily oviposition pattern of *Psyllaephagus* sp. females.

3.3. Influence of Supplementary Nutrition on *Psyllaephagus* sp. Adult Longevity

Supplementary nutrition in the form of honey water significantly influenced the longevity of the wasp *Psyllaephagus* sp. (female: one-way ANOVA, $F = 172.88$; $df = 3196$; $p < 0.001$; male: $F =$ one-way ANOVA, 218.05 ; $df = 3196$; $p < 0.001$) (Figure 3). When fed on distilled water, the longevity of the wasp adults was not more than 2 days (Table 1). The longevity of the parasitoids fed on 20% honey water was longer than those fed 5 or 10% honey water (Table 1). Moreover, female wasps survived significantly longer than male wasps whatever level of honey water they were fed (distilled water: two-sample *t*-test, $t = 2.17$, $p < 0.05$; 5% honey water: two-sample *t*-test, $t = 2.68$, $p < 0.05$; 10% honey water:

two-sample *t*-test, $t = 2.41, p < 0.05$; 20% honey water: two-sample *t*-test, $t = 3.05, p < 0.05$, Table 1).

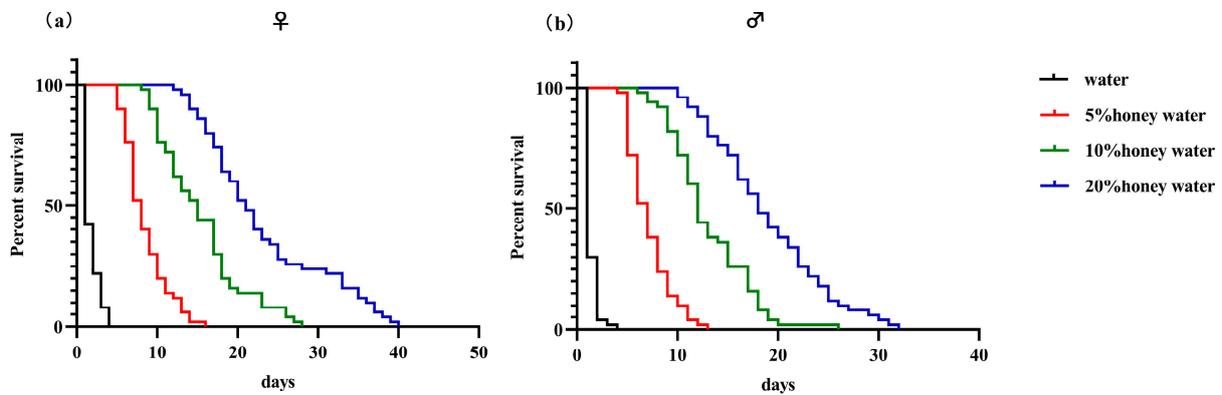


Figure 3. Survival for (a) *Psyllaephagus* sp. females and (b) males fed distilled water (black line), 5% honey water (red line), 10% honey water (green line), or 20% honey water (blue line).

Table 1. Longevity (d) of *Psyllaephagus* sp. female and male adults feeding on different food (mean ± SE).

	Distilled Water	5% Honey Water	10% Honey Water	20% Honey Water
Females	1.72 ± 0.14 Da	8.44 ± 0.38 Ca	15.32 ± 0.74 Ba	23.20 ± 1.12 Aa
Males	1.36 ± 0.09 Db	7.14 ± 0.30 Cb	13.08 ± 0.57 Bb	19.00 ± 0.80 Ab

Different uppercase letters in the same row indicate a significant difference among foods, and different lowercase letters in the same column indicate significant differences between genders ($p < 0.05$).

3.4. Functional Response of *Psyllaephagus* sp. Females to *C. chinensis* Nymphs

Since the results of the logistic regression analysis showed that there was no significant difference between the P_1 value and 0 in the initial cubic-term model, the cubic term and quadratic term in Equation (2) were gradually deleted until the linear term coefficient showed a significant difference from 0. After calculation and deletion, the P_1 values in the model were significantly less than 0 ($P_1 = -0.015, p < 0.001$). Therefore, the logistic regression showed that the response of the parasitoid to changes in the pear psyllid density was a Holling type II response, with the number of hosts parasitized increasing with the host density until the parasitoid reached its maximum reproductive capacity (Figure 4). The instantaneous attack rate (a), handling time (T_h/d), maximum daily parasitism, and a/T_h of *Psyllaephagus* sp. females in response to *C. chinensis* nymphs were $0.490 \pm 0.094, 0.073 \pm 0.015, 13.660$, and 6.712 , respectively.

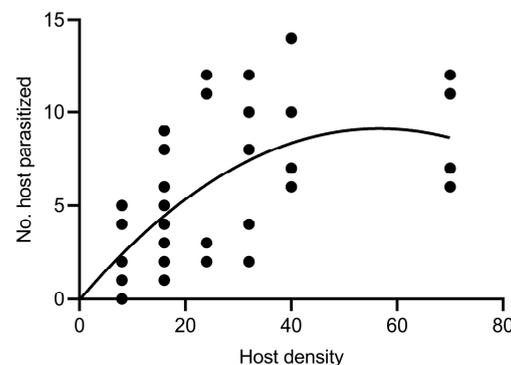


Figure 4. Functional response of *Psyllaephagus* sp. to increasing host density (Holling type II response, $Na = 0.490N_0 / (1 + 0.036N_0)$; $R^2 = 0.996$; $p < 0.001$).

3.5. Optimal Host/Parasitoid Ratios

When the host parasitism ratio varied, we found that the per capita parasitism decreased with an increase in the parasitoid number. As the number of wasps increased from one to three (with a fixed number of hosts), the average number of hosts parasitized per *Psyllaephagus* sp. female decreased significantly from 8.50 ± 0.79 to 4.60 ± 0.40 . Single adult females parasitized significantly more nymphs per capita than did females in groups of two or three (one-way ANOVA, $F = 13.18$; $df = 2, 27$; $p = 0.001$). There was a significant negative correlation between the \log_{10} searching efficiency (s) and parasitoid number ($\log s = -0.4359 \log P_t + 0.2321$) (Figure 5).

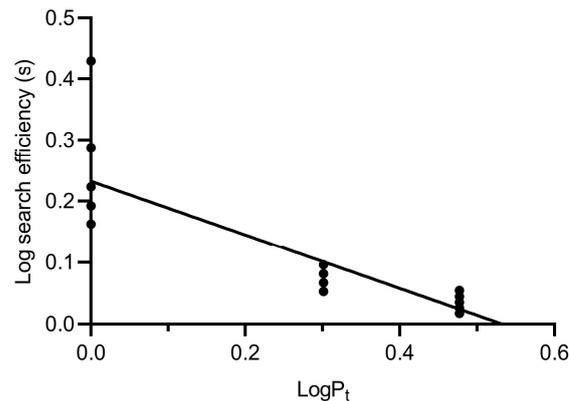


Figure 5. Density interference of *Psyllaephagus* sp. females.

4. Discussion and Conclusions

The biology and behavior of parasitoids are the most complex in the animal kingdom [32]. The information on the development, fertility, and life history of a parasitoid is important when its field establishment, efficiency, and long-term effects in a system need to be evaluated [33]. Our study provides information on the biology and parasitic behavior of a new species of psyllid parasitoid, *Psyllaephagus* sp., which is a koinobiont parasitoid, so that *C. chinensis* kept feeding and growing until mummification after parasitization. The total development period of *Psyllaephagus* sp. males and females from parasitism to the death of adult wasps was 31 and 38 days, respectively. Mummification of *C. chinensis* nymphs occurs two weeks after parasitism and only one wasp emerges from each mummy. This species' biology is similar to that of other parasitoids such as *P. bliteus* Riek, which attacks *G. brimblecombei* Moore (Hemiptera: Psylloidea) [19], and *Blepyrus clavicornis* Compere parasitizing *Pseudococcus viburni* Signoret [34]. The longevity of adult wasps differed between the sexes and among the supplementary foods, which was also observed in *Blepyrus clavicornis* Compere parasitizing *Pseudococcus viburni* Signoret. Nectar from flowers and honeydew can be important sources of carbohydrates for psyllid parasitoids in the field, and honey water as a supplementary food could provide the carbohydrates used by parasitoid wasps in laboratory. Sandanayaka et al. [35] reported that *Acerophagus maculipennis* (Mercet) had a similar longevity when it fed on honey and on honeydew from *P. viburni*. Generally, parasitic wasps have female-biased sex ratios [36,37], but in our study, the result of the sex ratio was male-biased with a very low proportion of female offspring, which is similar to *Neodryinus typhlocybae* Ashmead [38]. Sex allocation in parasitoid wasps is under maternal control; females develop from fertilized eggs and males develop from unfertilized eggs [39]. Females typically manipulate the sex of their progeny according to many factors, such as the host quality, age, and especially host size [40–44]. Environmental factors also influence the offspring sex ratio of hymenopteran parasitoids [45]. The reason for the low proportion of female offspring in our study may be the presence of unmated females, because the wasp was only one pair when mated and the mated time was short.

Host preferences have proven to be important to assess the dynamics of parasitoid interactions [46]. This study showed that there was no *Psyllaephagus* sp. emergence from

first- and second-instar nymphs, and *Psyllaephagus* sp. prefers fourth- or fifth-instar nymphs of *C. chinensis*. This may be because later instars can provide enough nutrition for the development of parasitoid wasps, thus reducing the mortality of parasitoid wasps, which is similar to the pattern shown by *Tamarixia triozae* (Burks), an ectoparasitoid of the potato psyllid *Bactericera cockerelli* (Šulc) [47]. There were three possible reasons that the wasps preferred the old stages: (1) hosts at young stages are rarely encountered by the parasitoid; (2) it is difficult to oviposit in first- or second-instar nymphs because of their small body size; (3) hosts at young stages as food resources are insufficient for parasitoid development [48]. The preference of *Psyllaephagus* sp. for older nymphs shows that releases of wasps would have to be well-timed to ensure the presence in the field of the preferred psyllid instar.

Understanding the parasitoid functional response to its host is essential to evaluate its efficiency [49]. The functional response of *Psyllaephagus* sp. was type II, similar to many other parasitoids [50–54]. In contrast, the functional responses for *T. triozae*, *Aphidius matricariae* Haliday, and *Necremnus tutae* Ribes and Bernardo were type III [47,53,54]. Some have suggested that the type III functional response might be more common than reported, due to flaws in experimental designs [55,56]. Because the parasitoids are limited to a single space for the entire experimental period and forced to revisit the parasitized hosts in laboratory experiments, type II functional response was more likely to appear. In contrast, under natural conditions, parasitoids often cannot find enough hosts available, and the parasitoids showed a type III functional response [57]. Holling [58] suggested that the use of functional response studies was better to understand predator–prey dynamics compared to parasitism-capacity indices. Now, the type of functional response is not considered a very good predictor of a novel natural enemy [59]. In laboratory conditions, many factors can influence the response of a bioagent, especially small arenas that cannot represent the field conditions; therefore, the exact searching abilities may not be assessed. In spite of this, the consequence of functional response experiments cannot be ignored; at least, its potential to reduce pest populations can be obtained by estimating the attack rate and the handling time as long as factors such as the host age/size [60], parasitoid age [58], temperature [61], and experimental conditions [62] are controlled. However, the other model parameters of natural enemies should also be investigated to access the overall biocontrol potential of the natural enemies better [63]. The effects of mutual interference on the search efficiency of parasitoid wasps were more prominent when multiple female wasps were restricted. Our results showed that the female *Psyllaephagus* sp.'s search efficiency was significantly reduced when the parasitoid density was more than one. The results of our study on the female *Psyllaephagus* sp. were consistent with the results of *T. triozae* previously reported [47]. The functional response and mutual interference of *Psyllaephagus* sp. indicate that releases of wasps would have to have the proper density to ensure a higher parasitism rate in the field.

The findings of the present study provide insights into the potential value of *Psyllaephagus* sp. for biological control of *C. chinensis* in pear orchards, and the details provided should help optimize mass rearing schemes for this species.

Author Contributions: W.S. and Z.Q. conceived and designed the study. Z.Q. and M.F. collected the data. X.H., L.Z. and Y.G. contributed the resources. Z.Q. and W.S. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Twelve-Five National Science and Technology Support Program of China (Grant numbers [2014BAD16B07] and [2014BAD23B01]).

Data Availability Statement: Datasets from the study are available from the corresponding author on request.

Acknowledgments: We thank Y.Z. Zhang of the Institute of Zoology, Chinese Academy of Sciences, for identifying the parasitic wasp collected in this study.

Conflicts of Interest: All authors declare that they have no conflicts of interest.

References

- Dong, Y.H.; Qian, J.R.; Zhou, L.Q.; Wang, H.S. Study on occurrence law and control strategy of *Psylla chinensis* in southern China. *Acta Agric. Jiangxi* **2009**, *21*, 118–120.
- Qiao, L.X. Occurrence and comprehensive control of *Psylla chinensis* in China. *Mod. Rural Sci. Technol.* **2017**, *6*, 32.
- DuPont, S.T.; Strohm, C.; Nottingham, L.; Rendon, D. Evaluation of an integrated pest management program for central Washington pear orchards. *Biol. Control* **2021**, *152*, 104390. [[CrossRef](#)]
- Gullan, P.J.; Martin, J.H. Sternorrhyncha (jumping plant-lice, whiteflies, aphids and scale insects). In *Encyclopedia of Insects*, 2nd ed.; Resh, V.H., Cardé, R.T., Eds.; Academic Press: Cambridge, MA, USA, 2009; pp. 957–967. [[CrossRef](#)]
- Li, D.L.; Wang, P.; Zhang, C.T. Review of research status and control of *Cacopsylla chinensis* in China. *Shanxi Fruits* **2003**, *4*, 30–31.
- Noyes, J.S. Universal Chalcidoidea Database. Available online: <https://www.nhm.ac.uk/chalcidooids> (accessed on 22 November 2022).
- Wu, F.; Zhen, W.; Yang, Z.; Zu, G. A new species of *Psyllaephagus* (Hymenoptera: Encyrtidae) from China.; parasitoid of *Macrohormotoma sinica* (Hemiptera: Homotomidae) on *Ficus concinna*. *Biodiv. Data J.* **2021**, *9*, e63253. [[CrossRef](#)]
- Ma, F.L. Systematic Studies on Encyrtidae (Hymenoptera: Chalcidoidea) from Northeastern China. Ph.D. Thesis, Northeast Forestry University, Liaoning, China, 2004.
- Li, H.L. The Taxonomic and Biodiversity of Encyrtidae from Shanghai City. Ph.D. Thesis, Shanghai Normal University, Shanghai, China, 2010.
- Tan, Y.G.; Zhao, J.Z. One new species and one newly recorded species of the genus *Psyllaephagus* Ashmead from China (Hymenoptera: Encyrtidae). *J. Hubei Univ.* **1999**, *21*, 174–176.
- Xu, Z.H.; Chen, W.; Yu, H.; Li, B.J. Notes on *Psyllaephagus*.; a genus new to China with descriptions of two new species (Hymenoptera: Encyrtidae). *Sci. Silvae Sin.* **2000**, *36*, 39–41.
- Xu, Z.H.; Chou, L.Y.; Hong, S.C. Notes on three encyrtid parasitoids of *Triozasyzygii* in Taiwan with description of one new species (Hymenoptera: Encyrtidae). *Chin. J. Entomol.* **2000**, *20*, 9–12.
- Zhang, Y.Z. Generic Revision of Chinese Encyrtidae (Hymenoptera: Chalcidoidea). Ph.D. Thesis, Chinese Academy of Sciences, Beijing, China, 2001.
- Tang, X.L.; Zhang, Y.Z.; Hu, H.Y. Seven new record species of *Psyllaephagus* (Hymenoptera: Encyrtidae) from China. *Entomotaxonomia* **2016**, *38*, 63–78. [[CrossRef](#)]
- Zhang, X.; Wu, P.C.; Ma, B.X.; Zhang, Y.Z. *Psyllaephagus arenarius* (Hymenoptera: Encyrtidae), a newly recorded parasitoid of *Bactericera gobica* (Hemiptera: Psyllidae) in China. *Acta Entomol. Sin.* **2017**, *60*, 842–846. [[CrossRef](#)]
- Noyes, J.S.; Fallahzadeh, M. *Psyllaephagus zdeneki* sp. nov. (Hymenoptera: Encyrtidae) from Iran.; A parasitoid of *Euphyllura pakistanica* (Hemiptera: Psyllidae). *Acta. Soc. Zool.Bohem.* **2005**, *69*, 203–208.
- Dahlsten, D.L.; Rowney, D.L.; Copper, W.A.; Tassan, R.L.; Chaney, W.E.; Robb, K.L.; Tjosvold, S.; Bianchi, M.; Lane, P. Parasitoid wasp controls blue gum psyllid. *Calif. Agr.* **1998**, *52*, 31–34. [[CrossRef](#)]
- Noyes, J.S.; Hanson, P. Encyrtidae (Hymenoptera: Chalcidoidea) of Costa Rica: The genera and species associated with jumping plant-lice (Homoptera: Psylloidea). *Bull. Nat. Hist. Museum. Entomol. Ser.* **1996**, *65*, 105–164.
- Daane, K.M.; Sime, K.R.; Dahlsten, D.L.; Andrews, J.W.; Zuparko, R.L. The biology of *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae); a parasitoid of the red gum lerp psyllid (Hemiptera: Psylloidea). *Biol. Control* **2005**, *32*, 228–235. [[CrossRef](#)]
- Jones, M.E.; Daane, K.M.; Paine, T.D. Establishment of *Psyllaephagus parvus* and *P. perplexans* as serendipitous biological control agents of Eucalyptus psyllids in southern California. *BioControl* **2011**, *56*, 735–744. [[CrossRef](#)]
- Ge, Y.; Liu, P.P.; Zhang, L.; Snyder, W.E.; Smith, O.M.; Shi, W.P. A sticky situation: Honeydew of the pear psylla disrupts feeding by its predator *Orius sauteri*. *Pest Manag. Sci.* **2020**, *76*, 75–84. [[CrossRef](#)] [[PubMed](#)]
- Gai, Y.P.; Ji, X.L.; Sun, X.G.; Liu, Y.S. Studies on the external morphological characters of *Psylla chinensis* Yang et Li nymphs. *J. Shandong Agric. Univ.* **2000**, *31*, 253–256.
- Zar, J.H. *Biostatistical Analysis*, 5th ed.; Prentice Hall: Upper Saddle River, NJ, USA, 2010.
- Visser, M.E.; Driessen, G. Indirect mutual interference in parasitoids. *Neth. J. Zool.* **1991**, *41*, 214–227. [[CrossRef](#)]
- Juliano, S.A. Nonlinear Curve Fitting: Predation and Functional Response Curves. Ph.D. Thesis, Oxford University, Oxford, UK, 2001.
- Campos, V.; Araya, J.E. Functional response of *Nabis punctipennis* Blanchard to *Acyrtosiphon pisum* Harris in the laboratory. *Chile J. Agric. Anim. Sci.* **2017**, *33*, 64–72. [[CrossRef](#)]
- Costa, J.F.; Matos, C.H.; de Oliveira, C.R.; Da Silva, T.G.; Lima Neto, I.F. Functional and numerical responses of *Stethorus tridens* Gordon (Coleoptera: Coccinellidae) preying on *Tetranychus bastosi* Tuttle, Baker & Sales (Acari: Tetranychidae) on physic nut (*Jatropha curcas*). *Biol. Control* **2017**, *111*, 1–5. [[CrossRef](#)]
- Ge, Y.; Zhang, L.; Qin, Z.F.; Wang, Y.; Liu, P.P.; Tan, S.Q.; Fu, Z.; Olivia, M.S.; Shi, W.P. Different predation capacities and mechanisms of *Harmonia axyridis* (Coleoptera: Coccinellidae) on two morphotypes of pear psylla *Cacopsylla chinensis* (Hemiptera: Psyllidae). *PLoS ONE* **2019**, *14*, e0215834. [[CrossRef](#)]
- Jalali, M.A.; Tirry, L.; De Clercq, P. Effect of temperature on the functional response of *Adalia bipunctata* to *Myzus persicae*. *BioControl* **2010**, *55*, 261–269. [[CrossRef](#)]
- Mahdian, K.; Vantornhout, I.; Tirry, L.; De Clercq, P. Effects of temperature on predation by the stinkbugs *Picromerus bidens* and *Podisus maculiventris* (Heteroptera: Pentatomidae) on noctuid caterpillars. *Bull. Entomol. Res.* **2006**, *96*, 489–496. [[CrossRef](#)]

31. R Core Team, R. *R Core Team R: A Language and Environment for Statistical Computing*; R Core Team R: Vienna, Austria, 2022.
32. Stehr, F.W. Parasitoides and predators in pest management. In *Introduction to Insect Pest Management*; Mecalff, R.L., Luckmann, W.H., Eds.; Wiley & Sons, A Wiley-Interscience Publication: New York, NY, USA, 1982.
33. Hemerik, L.; Harvey, J.A. Flexible larval development and the timing of destructive feeding by a solitary endoparasitoid: An optimal foraging problem in evolutionary perspective. *Ecol. Entomol.* **1999**, *24*, 308–315. [[CrossRef](#)]
34. Pacheco da Silva, V.C.; Garcia, M.S.; Botton, M. Biology of *Blepyrus clavicornis* (Compere) (Hymenoptera: Encyrtidae), a parasitoid of *Pseudococcus viburni* (Signoret) (Hemiptera: Pseudococcidae). *Rev. Brasil. Entomol.* **2017**, *61*, 257–261. [[CrossRef](#)]
35. Sandanayaka, W.R.M.; Charles, J.G.; Allan, D.J. Aspects of the reproductive biology of *Pseudaphycus maculipennis* (Hym: Encyrtidae), a parasitoid of obscure mealybug, *Pseudococcus viburni* (Hem: Pseudococcidae). *Biol. Control* **2009**, *48*, 30–35. [[CrossRef](#)]
36. Boulton, R.A.; Collins, L.A.; Shuker, D.M. Beyond sex allocation: The role of mating systems in sexual selection in parasitoid wasps. *Biol. Rev.* **2015**, *90*, 599–627. [[CrossRef](#)] [[PubMed](#)]
37. Broadley, H.J.; Gould, J.R.; Sullivan, L.T.; Wang, X.Y.; Hoelmer, K.A.; Hickin, M.L.; Elkinton, J.S. Life history and rearing of *Anastatus orientalis* (Hymenoptera: Eupelmidae), an egg parasitoid of the spotted lanternfly (Hemiptera: Fulgoridae). *Environ. Entomol.* **2021**, *50*, 28–35. [[CrossRef](#)] [[PubMed](#)]
38. Mazzon, L.; Visentini, A.; Girolami, V. Sex ratio of European populations of *Neodryinus typhlocybae* (Ashmead) (Hymenoptera: Dryinidae) parasitoid of *Metcalfa pruinosa* (Say) (Homoptera: Flatidae). *Frustrula Entomol.* **2000**, *23*, 30–36.
39. King, B.H. Offspring sex ratios in parasitoid wasps. *Q. Rev. Biol.* **1987**, *62*, 367–369. [[CrossRef](#)]
40. Bugila, A.A.A.; Franco, J.C.; Silva, E.B.; Branco, M. Suitability of five mealybug species (Hemiptera, Pseudococcidae) as hosts for the solitary parasitoid *Anagyrus* sp. nr. *pseudococci* (Girault) (Hymenoptera: Encyrtidae), *Biocontrol. Sci. Techn.* **2015**, *25*, 108–120. [[CrossRef](#)]
41. King, B.H. Offspring sex ratio and number in response to proportion of host sizes and ages in the parasitoid wasp *Spalangia cameroni* (Hymenoptera: Pteromalidae). *Environ. Entomol.* **2002**, *31*, 505–508. [[CrossRef](#)]
42. Li, J.; Gong, X.M.; Chen, Y.Z.; Pan, S.Y.; Dai, Y.N.; Hu, H.Y.; Liu, P.C. Effect of maternal age on primary and secondary sex ratios in the ectoparasitoid wasp *Pachycrepoideus vindemmiae*. *Entomol. Exp. Appl.* **2022**, *170*, 468–476. [[CrossRef](#)]
43. Liu, P.C.; Men, J.; Zhao, B.; Wei, J.R. Fitness-related offspring sex allocation of *Anastatus disparis*, a gypsy moth egg parasitoid, on different-sized host species. *Entomol. Exp. Appl.* **2017**, *163*, 281–286. [[CrossRef](#)]
44. Liu, P.C.; Wei, H.X.; Cao, D.D.; Wei, J.R. Relationships amongst sex ratio of progeny in *Anastatus disparis* (Hymenoptera: Eupelmidae), sperm depletion and decreased fecundity. *Appl. Entomol. Zool.* **2020**, *55*, 25–30. [[CrossRef](#)]
45. Cochard, P.; Galstian, T.; Cloutier, C. The proportion of blue light affects parasitoid wasp behavior in LED-extended photoperiod in greenhouses: Increased parasitism and offspring sex ratio bias. *Biol. Control* **2019**, *133*, 9–17. [[CrossRef](#)]
46. Murray, T.J.; Withers, T.M.; Mansfield, S. Choice versus no-choice test interpretation and the role of biology and behavior in parasitoid host specificity tests. *Biol. Control* **2010**, *52*, 153–159. [[CrossRef](#)]
47. Yang, X.B.; Campos-Figueroa, M.; Silva, A.; Henne, D.C. Functional response, prey stage preference, and mutual interference of the *Tamarixia triozae* (Hymenoptera: Eulophidae) on tomato and bell pepper. *J. Econ. Entomol.* **2015**, *108*, 414–424. [[CrossRef](#)]
48. Islam, K.S.; Copland, M.J.W. Host preference and progeny sex ratio in a solitary koinobiont mealybug endoparasitoid, *Anagyrus pseudococci* (Girault), in response to its host stage. *Biocontrol Sci. Techn.* **1997**, *7*, 449–456. [[CrossRef](#)]
49. Tillman, P.G. Functional response of *Microplitis croceipes* and *Cardiochiles nigriceps* (Hymenoptera: Braconidae) to variation in density of tobacco budworm (Lepidoptera: Noctuidae). *Environ. Entomol.* **1996**, *25*, 524–528. [[CrossRef](#)]
50. Savino, V.; Coviella, C.E.; Luna, M.G. Reproductive biology and functional response of *Dineulophus phthorimaeae*, a natural enemy of the tomato moth *Tuta absoluta*. *J. Insect Sci.* **2012**, *12*, 153. [[CrossRef](#)]
51. Luo, S.P.; Li, H.M.; Lu, Y.H.; Zhang, F.; Tim, H.; Ulrich, K.; Wu, K.M. Functional response and mutual interference of *Peristenus spretus* (Hymenoptera: Braconidae), a parasitoid of *Apolygus lucorum* (Heteroptera: Miridae). *Biocontr. Sci. Technol.* **2014**, *24*, 247–256. [[CrossRef](#)]
52. Ebrahimifar, J.; Jamshidnia, A.; Allahyari, H. Functional Response of *Eretmocerus delhiensis* on *Trialeurodes vaporariorum* by Parasitism and Host Feeding. *J. Insect Sci.* **2017**, *17*, 56. [[CrossRef](#)]
53. Tazerouni, Z.; Talebi, A.A.; Fathipour, Y.; Soufbar, M. Agespecific functional response of *Aphidius matricariae* and *Praon volucre* (Hym.: Braconidae) on *Aphis gossypii* (Hem.: Aphididae). *J. Entomol. Soc. Iran* **2017**, *36*, 239–248.
54. Bodino, N.; Ferracini, C.; Tavella, L. Functional response and age specific foraging behaviour of *Necremnus tutae* and *N. Cosmopterix*, native natural enemies of the invasive pest *Tuta absoluta* in Mediterranean area. *J. Pest Sci.* **2019**, *92*, 1467–1478. [[CrossRef](#)]
55. Hassell, M.P.; Lawton, J.H.; Beddington, J.R. Sigmoid functional responses by invertebrate predators and parasitoids. *J. Anim. Ecol.* **1977**, *46*, 249–262. [[CrossRef](#)]
56. Van Lenteren, J.; Bakker, K. Functional responses in invertebrates. *Neth. J. Zool.* **1975**, *26*, 567–572. [[CrossRef](#)]
57. Sagarra, L.; Vincent, C.; Peters, N.; Stewart, R.J. Effect of host density, temperature, and photoperiod on the fitness of *Anagyrus kamali*, a parasitoid of the hibiscus mealybug *Maconellicoccus hirsutus*. *Entomol. Exp. Appl.* **2000**, *96*, 141–147. [[CrossRef](#)]
58. Holling, C.S. Some characteristics of simple types of predation and parasitism. *Can. Entomol.* **1959**, *91*, 385–398. [[CrossRef](#)]
59. Tazerouni, Z.; Talebi, A.A.; Rezaei, M. Functional response of parasitoids: Its impact on biological control. In *Parasitoids: Biology, Behavior and Ecology*; Donnelly, E., Ed.; Nova Science Publishers: Hauppauge, NY, USA, 2019; pp. 35–58.
60. Streams, F.A. Effect of prey size on attack components of the functional response by *Notonecta undulata*. *Oecologia* **1994**, *98*, 57–63. [[CrossRef](#)]

61. Ziaei Madbouni, M.A.; Samih, M.; Namvar, P.; Biondi, A. Temperature-dependent functional response of *Nesidiocoris tenuis* (Hemiptera: Miridae) to different densities of pupae of cotton whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Eur. J. Entomol.* **2017**, *114*, 325–331. [[CrossRef](#)]
62. Hemerik, L.; Yano, E. Scaling up from individual behaviour of *Orius sauteri* foraging on Thrips palmi to its daily functional response. *Popul. Ecol.* **2011**, *53*, 563–572. [[CrossRef](#)]
63. Okuyama, T. On selection of functional response models: Holling's models and more. *BioControl* **2013**, *58*, 293–298. [[CrossRef](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.