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Development of Microplitis similis (Hymenoptera: Braconidae) on Two Candidate Host Species, Spodoptera litura and Spodoptera exigua (Lepidoptera: Noctuidae)

Authors: Li, Shun-Ji, Huang, Ju-Ping, Chang, Yang-Yang, Quan, Si-Yuan, Yi, Wen-Ting, et al.

Development of *Microplitis similis* (Hymenoptera: Braconidae) on two candidate host species, *Spodoptera litura* and *Spodoptera exigua* (Lepidoptera: Noctuidae)

Shun-Ji Li[1,2], Ju-Ping Huang[1], Yang-Yang Chang[1], Si-Yuan Quan[1], Wen-Ting Yi[1], Zi-Shu Chen[1,2], Shuang-Qing Liu[1], Xiao-Wen Cheng[3], and Guo-Hua Huang[1,2,]*

Abstract

Microplitis similis Lyle (Hymenoptera: Braconidae) is a solitary endoparasitic braconid that generally parasitizes larvae of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) and many other noctuid species. To understand host preference, fitness, and the effects of *M. similis* on the hosts, we compared percentage parasitism, development periods, and the effects on host growth in candidate noctuid species. We found high levels of parasitism of *S. exigua* and *Spodoptera litura* (F.) larvae but not of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) larvae. The parasitoid wasp larvae took similar amounts of time for development on *S. exigua* and *S. litura* larvae, i.e., 13.87 ± 0.15 and 13.69 ± 0.42 d, respectively. Compared with the control larvae, the growth and development of the hosts were severely affected. The hosts were able to molt to 4th instars after being parasitized as early 3rd instars, but were unable to develop to the 5th instar. The body weight was similar between parasitized and non-parasitized larvae within the first 4 d (3 d in *S. litura*) but later began to show a significant difference from the 5th day on (4th day in *S. litura*). The host larvae eventually weighed up to 50 to 80% less than the non-parasitized larvae. Furthermore, the host larvae lived for an extended period in the same instar after egression of the parasitoid, but the body mass did not increase.

Key Words: braconid; noctuid host; growth; development; age-specific survival rate; life table

Resumen

Microplitis similis Lyle (Hymenoptera: Braconidae) es un bracónido endoparásito solitario que generalmente parasita larvas de *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) y de muchas otras especies de noctuidos. Para entender la preferencia de hospedero, la aptitud y los efectos de *M. similis* en los hospederos, comparamos el porcentaje de parasitismo, el período de desarrollo y los efectos sobre el crecimiento del hospedero en los candidatos de especies de nóctuidos. Encontramos altos niveles de parasitismo en larvas de *S. exigua* y *Spodoptera litura* (F.) pero no en *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). Las larvas de la avispa parasitoide tardó una cantidad similar de tiempo para desarrollarse en larvas de *S. exigua* y *S. litura*, 13.87 ± 0.15 y 13.69 ± 0.42 días, respectivamente. En comparación con las larvas del control, el crecimiento y el desarrollo de los hospederos fueron gravemente afectados. Los hospederos pudieron mudar al cuarto estadio después de ser parasitadas al principio de la tercera estadio, pero no pudieron desarrollarse hasta el quinto instar. El peso corporal fue similar entre las larvas parasitadas y larvas no parasitadas durnate de los primeros 4 días (3 días en *S. litura*), pero más tarde comenzaron a mostrar una diferencia significativa en el peso corporal en el quinto día (cuarto día en *S. litura*). Las larvas de los hospederos a lo largo pesaron hasta un 50-80% menos que las larvas no parasitadas. Por otra parte, las larvas de los hospederos vivieron por un período prolongado en el mismo estadio después de la egresión del parasitoide, pero la masa del cuerpo no aumentó.

Palabras Clave: bracónido; hospederos noctuidos; crecimiento; desarrollo; tasa de sobrevivencia específica por edad; tabla de vida

Spodoptera exigua (Hübner) (Lepidoptera: Noctuidae) and *Spodoptera litura* (F.) are economically important pest species. Both of them have a wide distribution, broadly overlapping generations, and broad host ranges, and cause significant crop losses. Traditionally, management of *S. exigua* and *S. litura* relies mainly on chemical pesticides, which tend to be highly efficient in reducing pest populations in the field. However, the abuse of chemical pesticides and factitious interventions to increase crop yield have led to many negative effects (Luttrell et al. 1994; Chau 1995). As the concept of Integrated Pest Management (IPM) has become generally accepted, natural enemies of the insect pests, such as parasitoid wasps, have been used as alternatives (Xu et al. 2001; Beckage & Gelman 2004).

The biological characteristics of certain parasitoid wasps enable them to strongly suppress insect pest species that are their hosts. The development of parasitoid wasps has been shown to have dramatic impacts on host larval growth, development, and even metamorphosis

(Webb & Dahlman 1985; Beckage & Gelman 2004). As soon as the egg is laid in the host body, the development of the egg induces a series of major physiological changes in the host (Brodeur & Boivin 2004; Zhang et al. 2012). Most of these changes are likely to be caused by the venom — which contains various functional proteins — injected into the host during oviposition (Goecks et al. 2013). Also, during the development time of the immature parasitoids, the synthesis of proteins favorable to the parasitoids themselves has effects on host larval growth (Kaeslin et al. 2005). Therefore, the host larvae cannot gain weight, and often feed and molt less than non-parasitized hosts (Liu & Li 2006).

By means of field research and surveys conducted for many years, a number of parasitoid wasp species have been discovered, and the Natural Enemy of Pests database has been enriched greatly in China. *Spodoptera exigua* was shown to be vulnerable to 33 parasitoid wasp species (He et al. 2002a) and *S. litura* to 40 species (He et al. 2002b), but more studies on parasitoid wasp species are still in needed to fill knowledge gaps.

Microplitis similis Lyle (Hymenoptera: Braconidae) — with *Agrotis ypsilon* (Rottemberg) (Lepidoptera: Noctuidae) as its original host (Lyle 1921) — was one of the major larval endoparasitoids in the Indo-Australian Region (Wilkinson 1930; Shepard & Barrion 1998). We studied the biological features and geographical distribution of *M. similis*. Recently, we discovered that this species was first recorded in Changsha City, Hunan Province, China. In addition, the interactions between *M. similis* and its hosts remained unrevealed, and its effects on its hosts have not been studied systematically. These questions are theoretically important because their answers underlie the potential of biological control of insect pests.

In this study, we examined the effects of *M. similis* on the hosts *S. exigua* and *S. litura*. Gross percentage parasitism of *S. exigua* and *S. litura* was determined, and *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) was used as negative control to show the inability of *M. similis* to parasitize it. By dissection of the parasitized host larvae, the instar duration and age-specific survival of the *M. similis* were determined and associated with host larval growth trajectories. The combination of the data for parasitoid wasp and host larvae will offer valuable information for biological control of *S. exigua* and *S. litura* in the field.

Materials and Methods

HOST INSECT AND PARASITOID COLONIES

Spodoptera litura, *S. exigua*, and *H. armigera* larvae were from colonies developed in our laboratory (Li et al. 2013). These colonies were established from wild-collected individuals in a cotton field near Hunan Agricultural University, Changsha, Hunan, China. *Microplitis similis* was obtained by collecting parasitized larvae in this cotton field.

The larvae of *S. litura* and *H. armigera* were reared on pinto bean–based diets (Burton 1970), whereas *S. exigua* larvae were reared on artificial diets following Song's method (Song et al. 2009). All larvae were reared on artificial diets until pre-pupation. Adults were fed 10% honey solution.

The *M. similis* adults were paired in a 1:1 ratio (male: female), and then fed with 40% honey solution within a test tube (2 × 10 cm). Each pair was kept in the test tube and provided with 10 healthy 2nd or 3rd instars of *S. litura* as hosts for reproduction. After the female oviposited on all the larvae in the tube for 2 h, she was transferred to another test tube with another quota of larval hosts under the same conditions. Parasitized larvae were then kept separately in test tubes for observation of cocoon production. All hosts and the parasitoid wasps were kept in an environmental chamber at 27 ± 3 °C, 70 ± 10% RH, and a 14:10 h L: D photoperiod.

COMPARISON OF PERCENTAGE PARASITISM RATES AMONG 3 MAJOR NOCTUID HOST SPECIES

To determine the different parasitism rates among noctuid hosts, *S. litura*, *S. exigua*, and *H. armigera* were provided as hosts, as these species were prevalent in the cotton field where the *M. similis* parasitoid wasps were originally collected. Thirty newly-molted 3rd instars of each host species and 1 parasitoid wasp (2-day-old mated female) were kept together in a 250 mL flask with fresh leaves for 24 h. Then, the female wasp was removed and the host larvae were transferred into separate test tubes with a piece of artificial diet. These host larvae were kept in the environmental chamber (as described above) and observed daily until either a parasitoid emerged or the host developed to pre-pupation. The gender of the *M. similis* offspring was confirmed by examination of the genitalia under a microscope. Ten females (each with 30 host larvae) were used as replicates for each host species.

DEVELOPMENT OF IMMATURE STAGES OF *M. SIMILIS*

A group of newly-molted 3rd instars of *S. exigua* (and also *S. litura*) was provided to a *M. similis* female for oviposition. In total, 20 female parasitoid wasps were each provided with 10–15 hosts in a test tube. After 24 h, all the parasitized larvae were transferred separately to test tubes as described above. A batch of 10 host larvae was dissected on each consecutive day to assess the development of the eggs. Each immature stage was recorded as egg, larva, or pupa (Qiu et al. 2013).

EFFECTS ON HOSTS OF *M. SIMILIS* DEVELOPED IN HOST *S. EXIGUA* AND *S. LITURA* LARVAE

The remaining parasitized host larvae mentioned above that were not dissected were examined for the head capsule width and body mass each day until death or pre-pupation. These data were compared with corresponding data from non-parasitized larvae, which were reared separately from the early 3rd instar. The measurement of head-capsule widths (mm) was aided by a microscope, whereas body mass was measured on an electronic balance.

DATA ANALYSES

Data were checked for normality and homoscedasticity (Zhou et al. 2010). All data were evaluated with SPSS software (SPSS Inc®, version 16.0). For percentage parasitism, a Student's t-test was performed to analyze the significance among data of the 2 candidate hosts *S. exigua* and *S. litura* and the negative control host *H. armigera*. For daily body weight after parasitism of the 2 candidate hosts, a 1-way analysis of variance (ANOVA) was used to compare the overall differences among treatments (Li et al. 2013). For head capsule width data, a 2-way ANOVA was conducted to show the impact of both species and status (parasitized and non-parasitized) on the change of head capsule width of *S. exigua* and *S. litura* (McLoud 2011). Daily change of head capsule width was presented with histogram (means and standard errors) and a 1-way ANOVA analysis showing significance between parasitized and non-parasitized groups.

Life table parameters, which include the intrinsic rate of increase (r), net production rate (R_0), generation time (T), and finite rate of increase (λ) (Chi 1988; Chi & Su 2006) were used to examine the development of *M. similis* in the 2 hosts by the computer program TWOSEX-MSChart (Chi 2005).

Results

PARASITISM LEVELS AMONG 3 MAJOR NOCTUID HOST SPECIES

The percentage parasitism was 68.05 ± 0.43% on *S. exigua* and 61.72 ± 0.31% on *S. litura*. Because *H. armigera* larvae did not show any evidence of parasitism, Student's *t*-test was only performed with *S. exigua* and *S. litura*, and it showed that the percentage parasitism in both host species did not differ significantly (*P* = 0.267). Based on this result, we concluded that the *M. similis* females prefer both *S. exigua* and *S. litura* as host species.

AGE-SPECIFIC SURVIVAL OF *M. SIMILIS* PARASITIZING *S. EXIGUA* AND *S. LITURA*

In general, the duration of the total immature stage of both *S. exigua* and *S. litura* averaged almost 14 d (Table 1). Age-specific survival rates of immature *M. similis* developed on *S. exigua* and *S. litura* did not show significant differences (egg: $F_{1,39}$ = 2.80, *P* = 0.10; larva: $F_{1,34}$ = 8.08, *P* = 0.008; pupa: $F_{1,32}$ = 0.31, *P* = 0.58; immature: $F_{1,31}$ = 0.17, *P* = 0.68). The overlapping regions (also referred to as stage overlapping) between adjacent curves indicated the variations in rates of development among individuals (Fig. 1).

The age–stage specific survival rates indicate the probability that newly hatched eggs of *M. similis* of either *S. exigua* or *S. litura* will survive to a specific age-stage of the (Fig. 1). When *M. similis* developed in *S. exigua*, the mortality of its immature stages largely occurred in the larval and pupal stages (Fig. 1A). Male wasps emerged earlier and lived longer than the females. The development of *M. similis* in *S. litura* was similar to that in *S. exigua* except for an obvious later emergence of the females (Fig. 1B). Also, the survival rate of pupae was higher in *S. litura* than in *S. exigua*. Most male wasps lived longer than the females.

GROWTH AND DEVELOPMENT OF PARASITIZED *S. EXIGUA* AND *S. LITURA* LARVAE

The body weight of both parasitized and non-parasitized *S. exigua* larvae increased within the first 3 d (Fig. 2). However, from day 4 to day 7, the body weight of parasitized larvae increased at a slower rate than that of the non-parasitized larvae, and this resulted in significant difference between the weights of parasitized and non-parasitized larvae. After the larvae of *M. similis* egressed from the hosts (8 d after parasitism), the body weight of the hosts dropped somewhat and then remained unchanged until death. Some hosts died of parasitism soon after the egression of the parasites, but some hosts lived for additional 5 to 7 d (Fig. 2A).

The changes of body weight in parasitized *S. litura* were similar to those in *S. exigua*. The effect of parasitism was noticed from day 3 after the hosts had been exposed to the parasitoid wasp. Significant differences between the parasitized and the non-parasitized larvae

were detected from day 3 until day 8, when the non-parasitized larvae pupated. After emergence of the adult wasps, the hosts' body weight started to drop (Fig. 2B).

The changes in head-capsule width also suggested the retarded growth of the parasitized hosts. From the early 3rd instar when larvae were parasitized, the head capsule (in parasitized and non-parasitized larvae) expanded during and after the first 2 d; however, from day 3 until *M. similis* larvae pupated, the head capsule of parasitized hosts changed little whereas that of non-parasitized larvae expanded with the passage of time (Fig. 3). This indicated that the 3rd-instar time of parasitized larvae was extended. The non-parasitized *S. litura* larvae, on the other hand, developed to the prepupal stage within 7 to 8 d (non-parasitized *S. exigua* larvae pupated on days 6 to 7), and therefore significant differences in head capsule widths between parasitized and non-parasitized larvae occurred at days 3 and 4 in *S. exigua* and days 5, 6, and 7 in *S. litura* (Fig. 3). The changes of head-capsule width in parasitized *S. exigua* and *S. litura* were significantly affected by species ($F_{1,68}$ = 249.64, *P* < 0.05) and status (parasitized and non-parasitized; $F_{1,68}$ = 1653.84, *P* < 0.05), and by interaction between these factors ($F_{1,68}$ = 37.95, *P* < 0.05).

Overall, when egg deposition began in the early 3rd instar, the development of host larvae slowed down in the first 3 d, so that the growth rates of gross body weight and of head-capsule width were all significantly reduced.

LIFE TABLE PARAMETERS OF *M. SIMILIS* DEVELOPED IN *S. EXIGUA* AND *S. LITURA*

In *S. exigua*, the intrinsic rate of increase (*r*) of *M. similis* was 0.22, the net reproduction rate (R_0) was 40.55, the generation time (*T*) was 16.65, and the finite rate of increase (λ) was 1.23. In *S. litura*, *r* of *M. similis* was 0.21, R_0 was 41.10, *T* was 17.73, and λ was 1.24.

Discussion

In the past 2 yr we worked in cotton fields to understand the relationship between *M. similis* and its natural hosts. We found that from Jul to Oct, when the populations of *S. exigua* and *S. litura* were significantly high, the percentage parasitism in these 2 species was correspondingly high, as a result. The parasitoids select among their natural hosts in the field, and search the hosts by recognizing the chemical and physical parameters in the environment (Vinson 1976; Hilker & McNeil 2008). In this study, the newly-molted 3rd instars of *S. exigua*, *S. litura*, and *H. armigera* were maintained with fresh cotton leaves as food resource. As the feeding habitat provided orientation for parasitoids, the 3 species may all produce parasitoids after being attacked, but not all of them were able to support the growth of parasitoid offspring (Vinson 1975).

The ovipositional abilities of parasitoids in the genus *Microplitis* were investigated in the past (Jones & Lewis 1971; Belz et al. 2013). Most of the studies revealed that host age is an important factor that is considered by the parasitoids (Jowyk & Smilowitz 1978; Vinson & Iwantsch 1980) because the parasitoids must be provided with a suitable environment (the hosts and their feeding habitat) for their offspring (Qiu et al. 2013; Chu et al. 2014). Unlike gregarious koinobiont parasitoids, which prefer later instars in order to produce more offspring (Elzinga et al. 2003), solitary parasitoids choose slowly-growing larvae as their hosts (Mironidis & Savopoulou-Soultani 2009; van Nouhuys et al. 2012) and they regulate and adjust host development rate in favor of their own development (Khafagi & Hegazi 2004).

Table 1. Durations of the developmental stages of immature stages of *Microplitis similis* reared on 3rd instars of *Spodoptera exigua* and *S. litura*.

Host species	Developmental stage (d)[a]			
	Egg	Larva	Pupa	Total immature stage[b]
S. exigua	1.50 ± 0.11	6.39 ± 0.11	5.94 ± 0.18	13.87 ± 0.15
S. litura	1.25 ± 0.10	6.82 ± 0.10	5.68 ± 0.42	13.69 ± 0.42

[a] Data are shown as the means ± SE.
[b] Immature stage includes the egg, larva, and pupa until the adult's emergence.
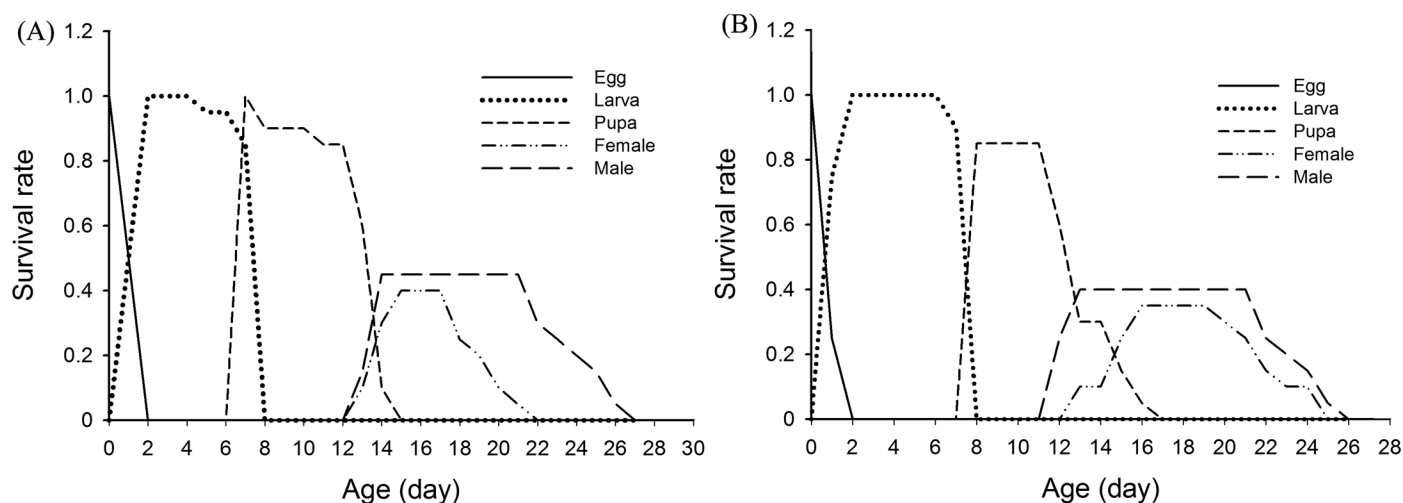
Fig 1. Age–stage specific survival rates (S_x) of *Micropletis similis* that developed in *Spodoptera exigua* (A) and in *S. litura* (B).

Endoparasitoids induce many physiological and behavioral changes in the hosts (Webb & Dahlman 1985; Hegazi et al. 2005). Overall, body weight and duration of instars served as fundamental and important indices for studying the growth and development of the hosts. Regulation of hosts' growth by parasitoids can be seen in body weight–changes of the hosts. The changes of body mass and head-capsule width in *S. exigua* and *S. litura* during *M. similis* development suggested larval growth was retarded significantly resulting from parasitism. Although the growth rate of parasitized larvae was less than that of non-parasitized larvae, the body mass of parasitized larvae continued to increase after oviposition by *M. similis*. This phenomenon was observed previously (Sato et al. 1986). It has been shown that protein synthesis continued during the growth of immature stages of parasitoids in the host body. However, the developing parasitoid larvae did not use newly composed host proteins but used dissociated proteins in the host body (Ferkovich & Dillard 1986).

Parasitoids also regulate the hosts' growth by slowing down their development rates (Iwantsch & Smilowitz 1975; Chu et al. 2014). Therefore, in addition to body mass, head capsule width was used as another index to indicate host larval growth because it relates to the larval instar in this study (Caltagirone et al. 1983; Godin et al. 2002).

The parasitized larvae were able to develop to the 4th but not 5th instar, whereas the non-parasitized larvae developed to pupation and eventually became adults. This phenomenon was also observed in previous studies (Strand 1990; Chu et al. 2014) .

Overall, our studies on effects of parasitoid behavior and development on different insect hosts will contribute to biological control of insect pests theoretically and practically. The introduction of parasitoids and predators in pest control relies greatly on rearing of hosts and parasitoids or predators (Landis et al. 2000). As *M. similis* is a solitary parasitoid wasp, as for a predator, the numbers of its offspring can be defined as the numbers of its prey. The relationship between the predator and the prey is important to the study of their coevolution (Chi & Yang 2003). The life table parameters indicated that the *M. similis* population would keep expanding over following generations, suggested by the finite rate of increase (both of the values were beyond 1). The generation time of *M. similis* development in *S. exigua* (T = 16.65) was shorter than in *S. litura* (T = 17.73). However, we cannot simply conclude which host likely would be more suitable for the growth and development of *M. similis* because of the following reasons. Following the intrinsic rate of increase (r), we know that the population of *M. similis* developing in *S. exigua* (r = 0.22) is assumed to gain more
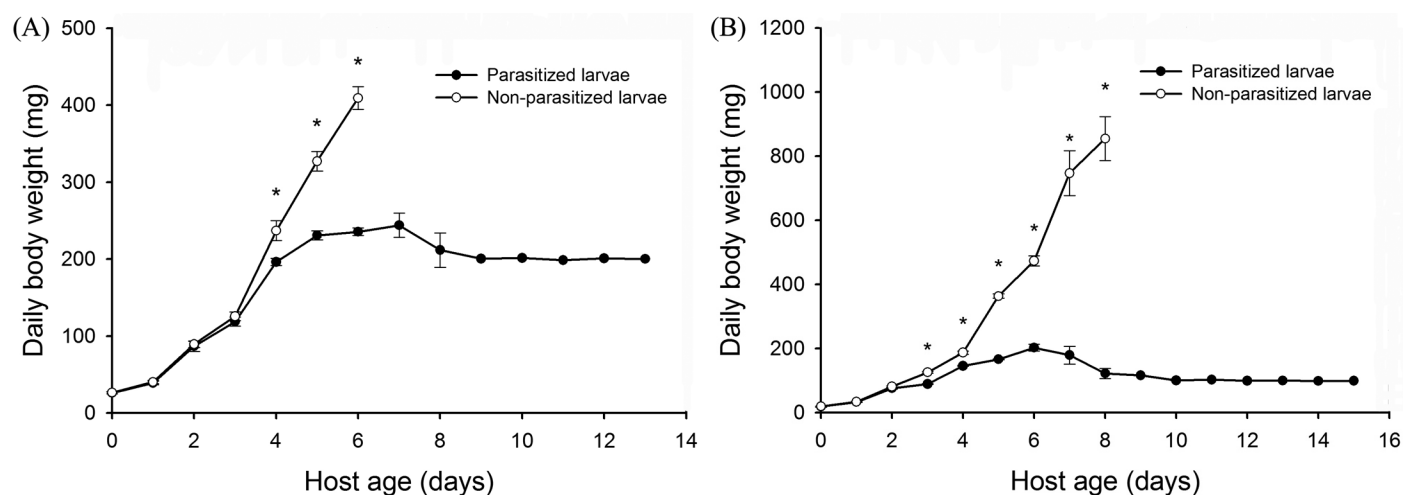

Fig 2. Daily body weight of parasitized and non-parasitized *Spodoptera exigua* (A) and *S. litura* (B). Each datum is shown as a mean ± SE.
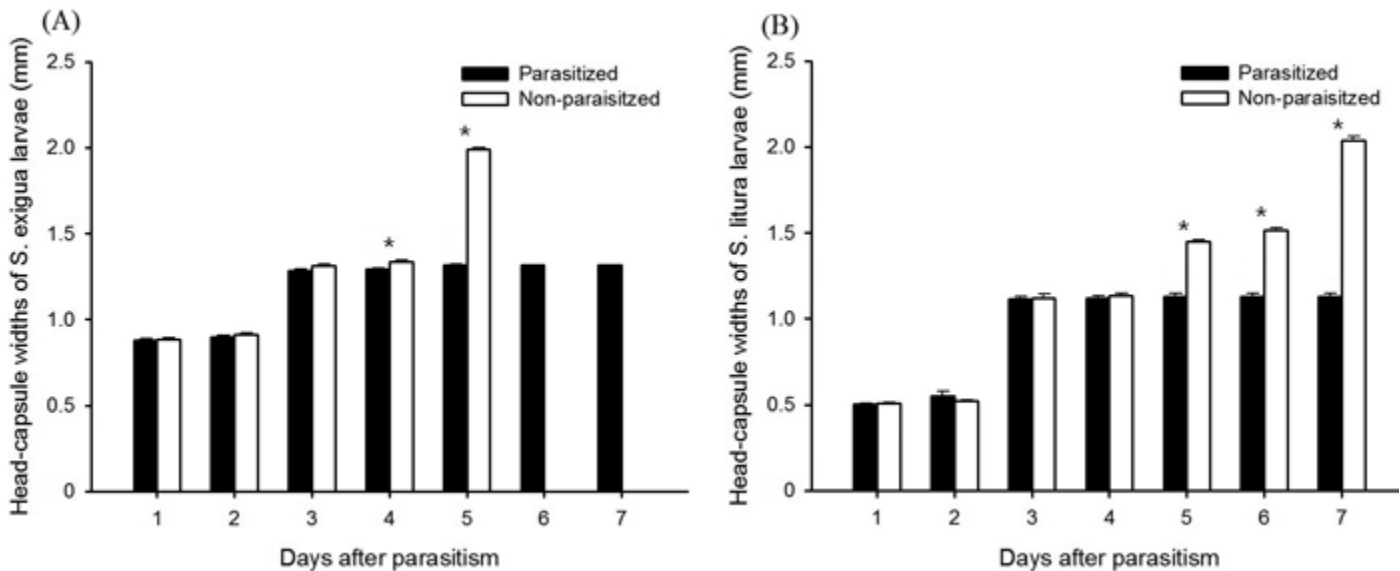
Fig 3. Daily head-capsule width of parasitized and non-parasitized *Spodoptera exigua* (A) and *S. litura* (B). Each datum is shown as a mean ± SE. The asterisk means the referred columns differ significantly. Non-parasitized *S. exigua* larvae pupated on days 6 and 7, and thus for these 2 days no head-capsule width data for them are shown.

individuals per day than in *S. litura* ($r = 0.21$). However, the net reproduction rate (R_0) suggests the population of *M. similis* developing in *S. litura* ($R_0 = 41.10$) is likely to increase 41.10 fold, which is a little more than in *S. exigua* ($R_0 = 40.55$). We accept that intrinsic rate of increase is an instantaneous parameter, so the r value would be used to represent the rising trend of the population.

On the other hand, we showed that when parasitism took place in 3rd instars of the hosts, the population of *M. similis* peaked in 15 d. However, the population of parasitized *S. exigua* and *S. litura* declined about 8 d after the disappearance of the parasitoid wasp. In order that *M. similis* can reproduce continuously, the use of chemical agents that directly kill the host must be considered carefully. In other words, chemical control of *S. exigua* and *S. litura* larvae must not be excessive during high population density and overlapping generations (Basri et al. 1995).

Acknowledgments

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