Biocontrol performance and mass production potential of the larval endoparasitoid Campoletis chlorideae against Spodoptera frugiperda

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Abstract

*Spodoptera frugiperda* is an invasive pest that causes severe economic losses in outbreak regions. The use of natural enemies of *S. frugiperda*, particularly native parasitoids, has been suggested as a promising control strategy due to high rates of pesticide resistance. *Campoletis chlorideae* is a solitary larval endoparasitoid with a broad host range that includes *S. frugiperda*; however, its parasitism rate, developmental stages, and population dynamics during *S. frugiperda* infection remain unclear. Here, we performed a systematical analysis to evaluate the biological control performance of *C. chlorideae* on the fall armyworm through age-stage, two-sex life tables. Due to their reproductive ability and short-life cycle, fall armyworms have the potential to be developed as a mass production host of *C. chlorideae*, we investigated the mass production potential by group-rearing fall armyworms using a low cannibalism rate artificial diet. Our results revealed an adequate biological control performance of *C. chlorideae* on *S. frugiperda* with a lifetime fecundity of 301.5 eggs/female, net reproduction rate (*R_*₀, adult females reproduced by a female) of 62.5, longevity of 28 days, and intrinsic rate of increase (r) of 1.2148. However, mass production factors require further optimization to improve efficiency and reduce the cost, as there was a lower net reproduction rate (6.02) due to the parasitization-induced cannibalism. This study provides instruction and guidance for the application and release of *C. chlorideae* for control of the fall armyworm.

Introduction

The fall armyworm, *S. frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is a notorious pest native to tropical regions of the Americas (Kenis et al. 2022; P. Wang et al. 2022) that has recently spread to Africa, Asia, and Oceania. Since 2019, this pest has rapidly expanded into 1,538 counties in 26 Chinese provinces (municipalities and districts) within a relatively short period of time (Zhang et al. 2021). This has harmed more than 150 thousand hm² of corn and continues to pose a serious threat to agriculture and food production (Qiulin et al. 2019). Previous reports of corn yield loss from *S. frugiperda* range from 40–72%, but can be as high as 100% (De Groote et al. 2020). The International Centre for Agriculture and Biological Sciences has reported that the yield losses caused by the fall armyworm across 12 African countries will likely reach to 8.3 million to 20.6 million tonnes per year, costing $2.48 billion to $6.19 billion, without any control measures (Murúa et al. 2006). As a highly polyphagous agricultural pest, *S. frugiperda* can feed on up to 186 plant species across 42 families, with maize, rice, and sorghum are the preferable hosts (Montezano et al. 2018; Wu et al. 2021). The reproduction ability and growth rate of *S. frugiperda* is remarkable - females can produce more than 1000 eggs and their life cycle is approximately 30 days (Firake & Behere 2020). Due to the significant risk this pest poses to crops, it has been designated as the top 10 most dangerous plant pests in the world by the CAB International (CABI) in 2017 ( https://www.cabi.org/isc/fallarmyworm ).

Control of fall armyworms has primarily relied on chemical pesticides, however their overuse has resulted in development of resistance, toxic residues, and resurgence of secondary pests(Desneux et al. 2007; Palma-Onetto et al. 2021; Richardson et al. 2020). Thus, alternative control strategies are a pressing
necessity. Natural enemies are a category of important biological pest control agents that have been broadly used in both prevention and control (Kenis et al. 2022; Zang et al. 2021). *Campoletis chlorideae* (Ichneumonidae: Hymenoptera) is an endoparasitic wasp that can parasitize more than 30 species of young larvae of the family of Lepidoptera including *Helicoverpa armigera* (Hübner), *H. assulta* (Guenée), *Spodoptera litura* (Fabricius), and *Mythimna separata* (Walker) (Suguo et al. 2012). *C. chlorideae* also plays an important role in limiting the population size of lepidopteran pests. Recently, *C. chlorideae* has been identified as an effect native parasitoid of *S. frugiperda* larvae in China (Hongtao et al. 2021) and India (Navik et al. 2021). Previous studies have shown that *C. chlorideae* prefer to parasitize 2-3rd instar larvae of *S. frugiperda* by laying eggs in their host then growing within the host hemolymph, eating host tissues and depriving them of nutrition. The life cycle of *C. chlorideae* in *Helicoverpa armigera* larvae is around 12–16 days, and though one host larvae can be parasitized by several *C. chlorideae* eggs, sometimes only one parasitoid survives to the cocoon stage before emerging from the epidermis of the 3rd instar host. Laboratory studies have also found that the parasitism and eclosion rates of *C. chlorideae* from the 2nd and 3rd instar larvae of fall armyworms can reach 82.6% and 85.1%, respectively (Liangheng et al. 2021). Thus, *C. chlorideae* is a promising natural enemy resource that may be developed as a biological control agent used to control *S. frugiperda* in the field. However, the developmental stages and biocontrol performance of *C. chlorideae* against *S. frugiperda* are unclear.

In addition, the use of parasitoids in the field requires reliability, feasibility, and economic mass production (Zang et al. 2021). To improve adaption, hosts must be mass reared, and proper mass production techniques are essential to reduce production cost and improve production efficiency (Y. Wang et al. 2020). *S. frugiperda* has a high reproductive capacity and short life cycle, which suggest it may be an ideal host for mass produced *C. chlorideae*. Similar to many other Lepidoptera, *S. frugiperda* larvae exhibit cannibalism when reared in groups (Hou et al. 2022). To combat this, an artificial diet that limits the rate of cannibalism in this setting was recently developed by a collaborator, though the potential of group-reared *S. frugiperda* in *C. chlorideae* mass production is worth further examination.

Here, we performed a field investigation to determine the emergence rates and sex ratio of *C. chlorideae* from cocoons collected in several regions of the Guizhou province during 2021 and 2022. Developmental stages of *C. chlorideae* within *S. frugiperda* were recorded by stereo-microscope to reveal morphological characteristics. An age-stage, two-sex life table analysis was then performed in individually-reared *S. frugiperda* to estimate *C. chlorideae*’s biological control capacity. In addition, we assessed the mass production potential using an age-stage, two-sex life table when *S. frugiperda* were reared in group conditions. These results revealed that *C. chlorideae* is a high potential larvae parasitoid with advantageous biocontrol performance and acceptable mass production potential when *S. frugiperda* are group-reared and fed an optimized artificial diet.

**Materials And Methods**

**Rearing the host insect** *S. frugiperda*
A laboratory colony of the host insect *S. frugiperda* was started with larvae collected from maize fields in Qiannan Bouyei and Miao Autonomous Prefecture, Guizhou Province, China and cultivated in a climate-controlled room (25°C ± 1°C, 70% ± 5% RH, and 14 L: 10 D). Larvae were maintained in polystyrene Petri dishes and fed an artificial diet. To avoid cannibalism (He et al. 2021), all fourth instar larvae were reared separately in 28-compartment boxes (hereafter called rearing boxes) until pupation. Fall armyworm pupae were collected and placed into transparent plastic boxes covered with white PP (polypropylene) non-woven fabric as an egg-laying substrate for the emerged adults. A 10% honey solution (v/v) was provided as a food source and replaced daily. After egg laying, substrates with egg masses were collected daily.

**Field investigation and rearing of C. chlorideae**

A total of five field surveys were conducted on August 9 and October 17, 2021 and July 21, August 1, and August 22, 2022. The first two surveys were conducted in maize fields at Huaxi District, Guiyang, Guizhou Province, and the final three were conducted in maize fields in Pingtang country, Qiannan Bouyei and Miao Autonomous Prefecture, Guizhou Province. The emergence rate and sex ratio of the collected cocoons were quantified in the same condition for rearing *S. frugiperda*. During parasitization a pair of newly emerged parasitoids were placed into transparent plastic boxes (13.5 × 8.2 × 6.8 cm) with gauze (200-mesh). A cotton ball soaked in a 10% honey-water solution (v/v) was attached to the inner wall of the box and refreshed every 24 h. Seventy 2nd instar *S. frugiperda* with artificial diet were transferred into the box for parasitization. After a 24 h exposure, the wasps were removed and the hosts were separated into multiple rearing boxes until the wasps emerged.

**Morphology and molecular identification of C. chlorideae**

For the morphological analysis, 20 3-day-old females that had mated but not laid eggs were put into 20 separate glass tubes containing 30 larvae 2nd instar armyworms allowed parasitization for 30 min. The *in vivo* growth stages of the *C. chlorideae* parasitoid developing within the fall armyworm were recorded by daily dissection 15–20 of the individually-reared host larvae using a stereo-microscope. The pupal growth stages were observed similarly, by dissecting and recording the parasitoid cocoons each day. The morphology of three day old adult females and males was also examined with a stereo-microscope.

For COI sequence analysis, the genome of adult parasitoids were extracted using genomic DNA extraction kit according to manufacturer’s instructions (Tiangen, Beijing, China). The genome DNA was used as a template for PCR amplification with a COI primer (Vrijenhoek 1994) with the following PCR conditions: 94°C 3 min; 94°C 30 s, 50°C 30 s, 72°C 30 s, 35 cycles; 72°C 5 min. The PCR products are purified by gel extraction kit (Tiangen, Beijing, China) before sequencing by Chengdu Sangon Biotech. The sequencing results were analyzed using DNAMAN8.0 and BLAST in NCBI. A phylogenetic tree based on the partial COI sequences was constructed using MEGA X with Neighbor-Joining method for 1000 bootstrap (Kumar et al. 2018).

**Life table study and parasitization rate of C. chlorideae on S. frugiperda 2nd instar larvae**
In order to determine the pre-adult stage parameters of two-sexes life table of *C. chlorideae* on the *S. frugiperda*, 70 2nd instar armyworm larvae were introduced into a box used for parasitism and provided with artificial diet. A pair of 3-day-old *C. chlorideae* were released into the box, allowed to oviposit for 24 h, then removed. Twenty parasitized *S. frugiperda* were randomly selected from the box and transferred into rearing boxes. Every 24 h, the immature *C. chlorideae* were checked and the developmental time was recorded until all *C. chlorideae* had either emerged or died. This was repeated with 10 pairs, and ultimately a total of 209 parasitized larvae were used for experimental observation to record the developmental stage and survival rate. The pre-adult parameters of two-sexes life table of *C. chlorideae* on the group-reared *S. frugiperda* are investigated similarly, except for the pre-parasitized and parasitized *S. frugiperda* are reared in a group condition and a total of 204 parasitized larvae were investigated.

The adult stage parameters, including fecundity, longevity, and predation rate of adult *C. chlorideae* on 2nd instar *S. frugiperda* larvae are determined as following: a pair of newly emerged (< 6 h) female and male wasps were first mated in a glass tube (12 × 3 cm) with 70 2nd instar *S. frugiperda* larvae for parasitism and rearing in the container as previous description. If the male wasp died prior to the female, it was replaced with another male. For each replicate, the exposed larvae from the previous day were separated into multiple rearing boxes and monitoring until the emergence of wasps. To measure lifetime fecundity, the number of *C. chlorideae* eggs on *S. frugiperda* larvae were counted and recorded under stereoscope without dissection before each transfer. Each treatment was performed in 17 replicates. The fecundity, longevity, and predation rate of adult *C. chlorideae* on group-reared 2nd instar *S. frugiperda* larvae were analyzed similarly, except for *S. frugiperda* are group-reared after parasitism until the emergence of wasps. The lifetime fecundity data in group-rearing condition are investigated with the number of pupae rather than the number of *C. chlorideae* eggs and this treatment was performed with 27 replicates.

**Data analysis**

Life table data (i.e., the developmental duration, survival rate, longevity and daily fecundity) of *C. chlorideae* parasitizing 2nd instar larvae of *S. frugiperda* were analyzed using the age-stage, two-sex life table method (Chi & Liu 1985; Chi & Su 2006; Chi et al. 2020; Tuan et al. 2014) according to the TWOSEX-MSChart (Chi 2022c). The age-stage-specific predation rate (*c*<sub>xj</sub>, where *x* is age and *j* is stage) was calculated based on the method of Chi and Yang (2003) by using the CONSUME-MSChart computer program (Chi 2022a). The population growth and predation capacity of *C. chlorideae* was projected using Timing-MSChart (Chi 2022b). The variances and standard errors (SE) of these population parameters were estimated by the bootstrap resampling method with 100,000 resampling (B = 100,000) (Y.-B. Huang & Chi 2011). The 0.025th and 0.975th percentiles of the finite rate of increase (*λ*) from the sorted 100,000 bootstrap samples were used to represent the variability of population growth and predation potential according to H.-W. Huang et al. (2018). All figures were created using Sigma Plot 14.0.

**Results**
C. chlorideae identification, morphology and field investigations

The ontogenetic development of *C. chlorideae* can be divided into four stages: the egg stage (embryonic period, Fig. 1A-B), larvae stage (postembryonic development, Fig. 1C-E), pupae stage (Fig. 1F-H), and adult stage (Fig. 1O-P). The egg is around 50–100 µm × 300–400 µm lasted for around 2 days. After that, the egg/embryo development was completed with a light brown skull, smooth and transparent body, an elongated tail, and a complete digestive tract, which will last for 2–3 days (Fig. 1C-D). At the late larval stage (~ 3 days), its morphology changed from caudal to membranoptin, and the intestine changed from pale yellow to pale green (Fig. 1E-G). Approximately 7 days post-paratization the mature larvae emerged from the host and appeared pale pink throughout the dimorphic body. Mature female larvae (Fig. 1H) had three pairs of ova-like depressions on the surface of the ventral segments 10, 11, and 12, whereas mature male larvae had an unpaired ova-like depression near the posterior edge of segment 12. The entire larval stage lasted 6 ~ 8 days with the body length around 1–4 mm.

The cocoon formed immediately following emergence of the mature larvae from the dead host appearing white or off-white with small black dots in the cocoon silk and measured about 2 mm × 6 mm. (Fig. 1I). The dissected pupae were long, ovate, and milky white with colorless compound eyes that gradually turned red then black, their body color also changed from light to dark over time (Figs. 1J-N). Three days into the cocoon stage, the female pupal ovipositor developed at the end of the abdomen (Fig. 1K). The pre-pupal and pupal stages lasted 5 ~ 7d with body length about 4 ~ 6 mm. Male *C. chlorideae* typically underwent eclosion earlier than females. The body length of male and female adults were around 5 ~ 6mm, both with a pair of long antennae and a yellow, narrow abdomen. However, adult males were usually smaller than females, and the females displayed a long, pronounced ovipositor (Fig. 1O) which was absent in male adults.

The COI gene sequence was analyzed by BLAST via the NCBI database. The query cover showed 100% COI homology, and the sequence identity was 99.85% with *C. chlorideae* voucher SCAU 3048973 (GenBank accession number: MW250777). The phylogenetic tree revealed that our COI sequence clustered with the previously identified COI sequences of *C. chlorideae* on a single branch (Fig. 2A), but was distinguishable from other species of the same genus, which identified the species in this study as *C. chlorideae* on the molecular level, as expected.

The number of collected of *C. chlorideae* cocoons in field investigations were 20, 19, 34, 41, and 119, from earliest to latest collection date. The emergence rates of *C. chlorideae* from cocoons collected from Guiyang were ~ 84% and 88%, whereas the eclosion rates of cocoons collected from Pingtan were 16%, 52%, and 15%, respectively (Fig. 2B). In contrast, the proportion of female *C. chlorideae* was around 30% in the emerged adults collected from Guiyang and 60–72% from Pingtang (Fig. 2C).

**Longevity and lifetime fecundity of C. chlorideae parasitizing S. frugiperda larvae**

The developmental periods for each stage of *C. chlorideae* from the individually-reared and group-reared on 2nd instar larvae *S. frugiperda* are summarized in Table 1. In individually-rearing, the total pre-adult
Developmental time was 17.11 ± 0.16 days with an egg–larva and pupa duration of 9.98 ± 0.12 and 7.28 ± 0.08 days, respectively. The proportion of males in the treatment (Nf/N = 0.2057 and Nm/N = 0.3733) was significantly higher than the proportion of females (p < 0.05). The female adult longevity (11.02 days) was also slightly longer than males (9.04 days), resulting in a slightly longer overall life span of females (28.3 days) compared with males (26.05 days). Females started to lay eggs as soon as they emerged, thus they showed no obvious adult preoviposition period (APOP, Adult pre-oviposition period). Moreover, the total preoviposition period (TPOP, Total pre-oviposition period) (17.28 days) was similar to the total pre-adult developmental time. Duration of the oviposition period was 9.95 days, with a mean daily oviposition rate of 30.3 eggs per female and a lifetime fecundity (F) of 301.5 eggs per female.
Table 1
Developmental times (means ± SE), adult longevity, total longevity, adult preoviposition period (APOP), total preoviposition period (TPOP), oviposition days, and fecundity of *Campoletis chlorideae* parasitizing the 2nd instar larvae of *Spodoptera frugiperda* (2nd Sf) in individually-rearing and group-rearing.

<table>
<thead>
<tr>
<th>Parameters</th>
<th><em>C. chlorideae</em> on 2nd Sf in IR(^1)</th>
<th><em>C. chlorideae</em> on 2nd Sf in GR(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of individuals</td>
<td>Means ± SE</td>
</tr>
<tr>
<td>Egg-larva duration (days)</td>
<td>171</td>
<td>9.98 ± 0.12</td>
</tr>
<tr>
<td>Pupa duration (days)</td>
<td>121</td>
<td>7.28 ± 0.08</td>
</tr>
<tr>
<td>Total pre-adult duration (days)</td>
<td>121</td>
<td>17.11 ± 0.16</td>
</tr>
<tr>
<td>Female adult longevity (days)</td>
<td>43</td>
<td>11.02 ± 0.49</td>
</tr>
<tr>
<td>Female total longevity (days)</td>
<td>43</td>
<td>28.3 ± 0.52</td>
</tr>
<tr>
<td>Male adult longevity (days)</td>
<td>78</td>
<td>9.04 ± 0.47</td>
</tr>
<tr>
<td>Male total longevity (days)</td>
<td>78</td>
<td>26.05 ± 0.53</td>
</tr>
<tr>
<td>Adult preoviposition period (APOP) (days)</td>
<td>43</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Total preoviposition period (TPOP) (days)</td>
<td>43</td>
<td>17.28 ± 0.26</td>
</tr>
<tr>
<td>Oviposition days ((O_d))</td>
<td>43</td>
<td>9.95 ± 0.38</td>
</tr>
<tr>
<td>Total fecundity ((F)) (eggs/female)</td>
<td>43</td>
<td>301.5 ± 16.4</td>
</tr>
<tr>
<td>Proportion of female individuals ((N_f/N))</td>
<td>209</td>
<td>0.2057 ± 0.03</td>
</tr>
<tr>
<td>Proportion of male individuals ((N_m/N))</td>
<td>209</td>
<td>0.3733 ± 0.03</td>
</tr>
<tr>
<td>Proportion of preadult mortality ((N_n/N))</td>
<td>209</td>
<td>0.4210 ± 0.03</td>
</tr>
</tbody>
</table>

Standard errors were estimated by using 100,000 bootstrap resampling. \(^1\) Individually-reared fall armyworm, \(^2\) Group-reared fall armyworm.
In contrast, the developmental times of the egg–larval, pupal, and pre-adult stages of *C. chlorideae* from the group-reared *S. frugiperda* condition. The developmental times for eggs–larae, pupae, and pre-adults were 8.82, 7.02, and 15.72 days, respectively. The proportion of males in the group-reared cohort (Nf/N = 0.0687 and Nm/N = 0.2452) was much higher than in the individually-reared condition. The average adult longevity of *C. chlorideae* females (8.79 days) was shorter than males (9.54 days), but the average overall life span between females and males was similar (25.21 and 25.06 days) and slightly longer than in the individually-reared condition. There was also no obvious APOP in *C. chlorideae* female adults (0.07 days) in this condition; they demonstrated similar TPOP (16.5 days) and total pre-adult development times (15.72 days). The oviposition period of *C. chlorideae* females was 7.21 days, and the lifetime total fecundity (F) (replaced by the number of pupae) was 87.71 eggs with a mean daily oviposition rate of 12.17 eggs per female. These were significantly lower than in the individually-reared condition.

**Age-Stage and age-Specific survival rate and fecundity of *C. chlorideae* parasitizing *S. frugiperda* larvae**

The survival rates of *C. chlorideae* in the egg-larva stage maintained high levels in the first eight days and decreased sharply thereafter (Fig. 3A). Additionally, the survival rates of both pupae and adults increased initially before decreasing. The highest survival rate of pupae was 70.81% which occurred on the 12th day. In the adult stage, the highest survival rate of males (32.54%) was greater than that of females (19.62%), and their peak values were observed on the 19th day and 20 ~ 22th days, respectively. The similar decreasing trend in survival rates of both sexes occurred on the 25th day and lasted until the end of the observation period.

With respect to the group-reared host, the values of $S_{xj}$ at the egg-larva stage dropped sharply at 7 days old, which was similar to what was observed in the individually-reared condition. The peak of the $S_{xj}$ at the pupal stage was 62.25% at 9 days, which was 8% lower and 3 days shorter than in the individually-reared condition. Female adults began to emerge at 15 days old with the highest emergence rate occurring at 19 days. Male adults began to emerge at 14 days and peaked at 20. Similar to the individually-reared condition, the peak age-stage survival rate of adult female *C. chlorideae* (6.86%) was lower than adult males (24.51%) at the age of 19 days (Fig. 3B).

The value of age-specific survival rate ($l_x$) of *C. chlorideae* decreased steadily in individually reared larvae (Fig. 3C). All the values of age-specific fecundity $f_{x3}$ (3 represent egg-larvae, pupae, and adult stage), age-specific fecundity of population ($m_x$), and age-specific net reproductive rate ($l_xm_x$) displayed a trend wherein they first increased followed by a steady decrease. In female adults, each index reached its highest value on the 5th day after emergence, and these peaks were 42.41, 14.14, and 7.91, as shown in Fig. 3C.

In the group-reared condition, the value of $l_x$ decreased fastest at 9–15 days (Fig. 3D). The value of $f_{x3}$ nearly reached its maximum value (19.33) initially, but it then decreased rapidly with minor fluctuations. The values of $m_x$ and $l_xm_x$ displayed a similar trend as in the individually-reared condition, in which they reached their highest values at 19 days post-parasitism (3.03 and 0.95, respectively) and increased prior
to decreasing. The cumulative fecundity ($l_xm_x$) of female parasitoids reached 93.02% in the larvae of *S. frugiperda* at 23 days.

**Life expectancy and reproductive value of** *C. chlorideae* **parasitizing** *S. frugiperda* **larva**

The life expectancy values ($e_{xj}$) of a newly laid egg of *C. chlorideae* (age of 0 day) under individually-reared conditions was 20.36 days, after which the life expectancy decreased with age (Fig. 4A). The highest $e_{xj}$ for the *C. chlorideae* pupae was 14.61 days at 8 days post-parasitism, while the peak life expectancy of adult females was 13.29 days at 15 days post-parasitism. This $e_{xj}$ was longer than in male adults which peaked at 11.64 days at 14 days post-parasitism.

In contrast, the $e_{xj}$ was 14.61 days for newly laid eggs under the group-rearing condition (Fig. 4B), after which the life expectancy of eggs steadily decreased with age (Fig. 4B). The highest $e_{xj}$ for the pupae, adult females, and adult males was 10.11, 10.21, and 11.06 days at the ages of 15, 15, and 14 days post-parasitism, respectively.

The female-specific reproductive value of *C. chlorideae* ($v_{xj}$) was 169.07 d$^{-1}$ at 16 days post-parasitism when the female adults emerged immediately in the individually-reared condition. This value decreased gradually with age growing until 35 days post-parasitism (Fig. 4C). The reproductive value at the age of zero ($v_{0t}$) was equal to the finite rate of increase ($\lambda$) with the identical number of 1.21.

On the other hand, the peak age-stage reproductive value of *C. chlorideae* ($v_{xj}$) occurred at 15 days (87.28 d$^{-1}$) after parasitization in the group-reared larvae, and then gradually decreased with age (Fig. 4D). The reproductive value at the age of zero ($v_{0t}$) or finite rate of increase ($\lambda$) for this group-reared condition was 1.10.

**Population parameters of** *C. chlorideae* **parasitizing** *S. frugiperda* **larva**

Table 2 presents the population demographic parameters of *C. chlorideae* parasitizing the 2nd instar larvae of *S. frugiperda* in individually-rearing and group-rearing conditions. In individually, the net reproduction rate ($R_0$) was 62.03. This could be attributed to the low pre-adult survival rate (57.90%) and low adult female proportion (20.57%). Although the large proportion of N-type offspring (42.10%) was not useful for the reproduction of *C. chlorideae*, it was important for the biological control of the host pest *S. frugiperda*. Conversely, the mean generation time ($T$, the time required to achieve R0 when the population reaches a steady growth rate ($\lambda$ and $r$)) of the *C. chlorideae* population in the *S. frugiperda* larvae was 21.21, which was shorter than the $R_0$ of *S. frugiperda* when feeding on maize (40.92 days) (Xie et al. 2021).
Table 2
Population parameters (means ± SE) of *Campoletis chlorideae* parasitizing the 2nd instar larvae of *Spodoptera frugiperda* in individually-rearing and group-rearing conditions.

<table>
<thead>
<tr>
<th>Population Parameters</th>
<th><em>C. chlorideae</em> on 2nd <em>Sf</em> in IR(^1)</th>
<th><em>C. chlorideae</em> on 2nd <em>Sf</em> in GR(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrinsic rate of increase ((r)) (day(^{-1}))</td>
<td>0.1946 ± 0.0076</td>
<td>0.0899 ± 0.0148</td>
</tr>
<tr>
<td>Finite rate of increase ((\lambda)) (day(^{-1}))</td>
<td>1.2148 ± 0.0092</td>
<td>1.0941 ± 0.0162</td>
</tr>
<tr>
<td>Net reproductive rate ((R_0)) (eggs/individual))</td>
<td>62.03 ± 9.07</td>
<td>6.02 ± 1.61</td>
</tr>
<tr>
<td>Preadult survival rate ((S_a)) (%)</td>
<td>0.5790 ± 0.03</td>
<td>0.3138 ± 0.03</td>
</tr>
<tr>
<td>Mean generation time ((T)) (day)</td>
<td>21.21 ± 0.27</td>
<td>19.57 ± 0.50</td>
</tr>
</tbody>
</table>

Values are mean ± SE, and standard errors were estimated by using 100,000 bootstrap resampling. \(^1\)Individually-reared fall armyworm, \(^2\)Group-reared fall armyworm.

In group conditions, the intrinsic rate of increase (\(r\)) and finite rate of increase (\(\lambda\)) were > 0 and > 1, respectively, but much lower than determined for the individually-reared condition. The net reproduction rate (\(R_0\)) of *C. chlorideae* was 6.02 with low pre-adult survival rate (31.38%) and adult female proportion (6.87%), and large proportion of N-type offspring (68.82%). The mean generation time (\(T\)) of *C. chlorideae* populations group-reared hosts was 19.57, which was slightly shorter than in the individually-reared hosts.

3.6 Predation rates, population and predation projection of *C. chlorideae* parasitizing individually-reared *S. frugiperda* larva

Since only *C. chlorideae* female adults parasitize and kill *S. frugiperda* larvae for population reproduction, both \(k_x\) and \(q_x\) were 0 before the adult stage (Fig. 5A). These values then peaked at 19 days old at 11.99 and 6.71 larvae per parasitoid, respectively, before decreasing.
Table 3
Predation rate parameters (means ± SE) of *Campoletis chlorideae* parasitizing the 2nd instar larvae of *Spodoptera frugiperda* in individually-rearing conditions.

<table>
<thead>
<tr>
<th>Predation rate Parameters</th>
<th><em>C. chlorideae on 2nd Sf</em>¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net predation rate (<em>C₀</em>) (prey/individual)</td>
<td>52.90 ± 7.64</td>
</tr>
<tr>
<td>Transformation rate (<em>Qₚ</em>) (*C₀' <em>R₀</em>)</td>
<td>0.85 ± 0.02</td>
</tr>
<tr>
<td>Finite predation rate (<em>ω</em>) (d⁻¹)</td>
<td>0.2422 ± 0.0282</td>
</tr>
<tr>
<td>Stable predation rate (<em>ψ</em>) (d⁻¹)</td>
<td>0.1993 ± 0.0217</td>
</tr>
</tbody>
</table>

Values are mean ± SE, and standard errors were estimated by using 100,000 bootstrap resampling.

The conversion rate (*Pₓ*) decreased from the onset of offspring production (15 d) until age 20 d, and then remained flat relative to the *Rₓ* and *Cₓ* rates (Fig. 5B). The cumulative predation number of each female *C. chlorideae* was 257.12. The net predation rate (*C₀*) showed that, on average, *C. chlorideae* consumed 52.90 fall armyworm larvae during its lifespan (Table 3). The transformation rate (*Qₚ*) was 0.85 pest/predator (Table 3). The possibility of over-parasitism with a sufficient number of host larvae per day may explain the transformation rate of < 1. Due to the low survival rate and predation rate after age 26 d, the age-specific cumulative net reproductive rates (*Rₓ*) and cumulative consumption rates (*Cₓ*) increased only slightly during the remaining 10 days (Fig. 5B). As shown in Table 3, finite predation rate (*ω*) and predation rate (*ψ*) were 0.2422 and 0.1993 per day in individually-reared conditions, respectively.

The total number of *C. chlorideae*, beginning with an initial population of 10 eggs with unlimited prey assumed, are shown in Fig. 5C. The variability, an innate characteristic of population growth, was projected using life tables from the 0.025th and 0.975th percentiles of the net reproductive rate (Fig. 5C). The projected total predation rate and its variability show a slow increase in initial population size and predation rates but a rapid increase in population size and predation rates over time (Fig. 5D).

**Discussion**

Age-Stage, Two-Sex life tables are a powerful tool used to study the impact of external environmental factors on the growth, development, reproduction, and survival of insect populations, including estimation of predation rates and population dynamics of parasitoids. In this study, Age-Stage, Two-Sex Life Table analysis are carried out in both individually-reared and group-reared *S. frugiperda*. The individually reared fall armyworm can mimic the field condition, where only one larvae usually appear in the leaf heat of corn, whereas the group-reared fall armyworm can stimulate the mass production, where reliability, feasibility, and economic conditions are required. *C. chlorideae* had a superior biocontrol performance to *S. frugiperda* with high fecundity rate (301.5 ± 16.4 eggs/female), relative long longevity (~ 28 days), and
a high Finite rate of increase ($\lambda = 1.2148$) in individually-reared *S. frugiperda*, but the mass production condition needs further optimization.

The life cycle of *C. chlorideae* in individually-reared fall armyworms was around 28.3 days in 25 °C with an egg–larva and pupa duration of 9.98 ± 0.12 and 7.28 ± 0.08 days for females and 11.02 days and 9.04 days for males, respectively. This egg-larvae period is comparable with *C. chlorideae* growth in *H. armigera* at 27 °C (Dhillon & Sharma 2008), however the adult longevities in *S. frugiperda* were shorter than in *H. armigera* at 18 and 14.9 days for female and male adults, respectively. Temperatures between 18–27 °C have been previously shown to negatively affect the duration times of egg-larvae, pupae, and adult stages. The life cycle of *C. chlorideae* in individually-reared fall armyworms was similar to the life cycle of fall armyworms (~ 30 days) at a similar temperature. This indicates that new *C. chlorideae* emergence would line up with the next generation of fall armyworms if some larvae escaped *C. chlorideae* in the field. Our *in vivo* morphological analysis of *C. chlorideae* in *S. frugiperda* larvae on various days following parasitization was an effective tool to estimate the parasitoid stages in the field and predict *C. chlorideae* emergence. Combining the morphological and age-stage specific duration time informs the release of *C. chlorideae* at an ideal time for *S. frugiperda* control and provides guidance for the mass production of *C. chlorideae* as to reduce its shelf life before major fall armyworm outbreaks.

Our results found that the total number of *C. chlorideae* offspring in individually-reared fall armyworms were as high as 301.5 with an emergence rate of 57.9% and a composition of 62.03 and 112.55 adult females and males, respectively. This high offspring output resulted in a cumulative predation number of 257.12 for female *C. chlorideae* and a Finite rate of increase ($\lambda$) (day-1) of 1.2148. The relatively small predation number compared with the offspring numbers could be attributed to the fact that *C. chlorideae* may have laid more than one egg in some larvae and because some parasitized larvae can overcome the parasitoids. The predicted population size after 60 days was determined to be 386,764 composed of 365,970 egg-larva, 11,415 pupae, and 3,462 female and 5,916 male adults from ten viable eggs, a remarkable theoretical population growth rate. The eclosion rate (57.9%) of *C. chlorideae* in individually-reared *S. frugiperda*, however, was lower than the ecolotion rate of *C. chlorideae* in corn-reared *S. frugiperda* (~ 80%). This could be a result of the components in the artificial diet, which may have affected the development of *C. chloridea* in the *S. frugiperda* larvae. Foods and/or supplements in artificial diets have been shown to affect herbivore performance and defense of host insects (Johnson 2008), hence they may have impacted the emergence rate of parasitoids growing on various foods (Behmer 2008; Sarfraz et al. 2009). Similar studies have reported that maize genotypes can also influence the parasitism rate of *C. sonorensis* on corn-reared *S. frugiperda* (Barreto-Barriga et al. 2021).

In *S. frugiperda* group-reared conditions, the *C. chlorideae* eclosion rate and sex ratio was much lower than in the individually-reared conditions, with each female parasitoid producing about 87.71 eggs. Moreover, the number of emerged female and male adults was 6.02 and 21.48 (female-male ratio of 1:3.569), respectively. The proportion of N-type parasitic wasps (parasitoids dead before pupae stage) was as high as 68.61%, which is also much higher than in the individually-reared larvae (42.10%). This indicates that even being fed the low cannibalism rate artificial diet, many parasitized larvae were still
consumed by others. These consumed larvae were likely parasitized larvae as they grow at a slower rate than non-parasitized larvae. These results suggest that the low cannibalism rate artificial diet cannot completely inhibit cannibalism by parasitized larvae. However, the higher adult male emergence rate indicates that the larvae parasitized by male parasitoids escaped cannibalism more successfully. Nutrition and larvae density are not hypothesized to be the primary cause of this cannibalism, as more than enough food was provided and the larvae density was equal. Additionally, high cannibalism rates have also been reported in larvae kept in very low density (2 per box) (He et al. 2021). Thus, the potential mechanisms underlying this cannibalism as well as optimal artificial diets to reduce it warrant further investigation, despite this, the population parameter ($r = 1.0941$) indicates the population size can still steadily increase.

Biological control agents are attracting increasing attention as an eco-friendly, continual control methods that are beneficial to biodiversity. The habitat of $C. chlorideae$ is broad and overlaps with the invasion regions of the fall armyworm which occurs in both south and north China. This suggests that $C. chlorideae$ has the potential to be an excellent biological fall armyworm control agent. The use of $C. chlorideae$ would also avoid the introduction of alien organisms thereby reducing the potential ecological risks caused by these foreign organisms. The age-stage two-sex life tables coupled with our morphological analysis provided an accurate description and prediction of the developmental time of various forms of parasitoids, survival rates, predation rate, and reproduction potential. Results from the individually-reared host $S. frugiperda$ larvae after parasitism indicate that $C. chlorideae$ is a promising biocontrol tool for control of fall armyworm infestations in the field. Further, our results suggest that the mass production potential of $C. chlorideae$ is acceptable though not economic when using group-reared fall armyworms as the reproduction host as many parasitized larvae are cannibalized. Future investigations should focus on development of superior mass production techniques, including optimized artificial diets and/or alternative insect hosts.

Declarations

Ethical Approval

Not applicable.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors’ contributions

ZR performed the two sex life tables experiments, data collection, analyses and prepared figures and wrote the original draft. XL and CL performed the field investigations and prepared the host insects. QZ, AM and NL performed the validation, data analysis and writing - review & editing. WZ conceived the
project and supervised the study, wrote the original draft and performed data analysis. LZ conceived the project and supervised the study, and performed writing - review & editing. All authors read and approved the final version for publication.

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Availability of data and materials

Not applicable.

References


**Figures**
Figure 1

Morphological changes of *Campoletis chlorideae*. (A-B) 1-2 day-old eggs of *Campoletis chlorideae*; (C-E) 3-7 day-old larvae of *Campoletis chlorideae*; (H) the mature larvae-female emerged from the host body. (I) cocoon of *Campoletis chlorideae*; (J-N) pupae stages of *Campoletis chlorideae*; (O) adult female *Campoletis chlorideae*; (P) adult male *Campoletis chlorideae*. 
Figure 2

Phylogenetic tree and field investigations. (A) Phylogenetic analyses of the identified partial COI of *C. chlorideae* and 10 homologies from other species, including 9 homologies of *Campoletis*, *C. chlorideae* (MW250777 and MW241326), *C. latator* (MZ627404), *C. thomsoni* (MZ626699), *C. flavicincta* (KR900099), *C. horstmanni* (KF604424), *Campoletis rostrata* (KT959701), *C. sonorensis* (HQ929710), *C. zonata* (MZ628534) and 1 homology from *Diadegma semiclausum* (KY846410). (B) Field investigations of *C. chlorideae* Emergence rate. (C) Sex ratio of *Campoletis chlorideae* collected from the field.
Figure 3

Age-stage survival rate and age-specific survival rate of *Campoletis chlorideae* parasitizing the individually-reared and group-reared *Spodoptera frugiperda*. Age-stage survival rate ($s_{xy}$) of *Campoletis chlorideae* parasitizing the 2nd instar larvae of *Spodoptera frugiperda* in individually-rearing (A) and group-rearing condition (B). Age-specific survival rate ($l_x$), age-specific fecundity ($f_{x3}$), age-specific fecundity of population ($m_x$) and age-specific net reproductive rate of population ($l_x m_x$) of *Campoletis chlorideae* parasitizing the 2nd instar larvae of *Spodoptera frugiperda* in individually-rearing (C) and group-rearing condition (D).
Figure 4

Life expectancy and reproductive values of *Campoletis chlorideae* parasitizing the individually-reared and group-reared *Spodoptera frugiperda*. Life expectancy ($e_{xj}$) of * Campoletis chlorideae* parasitizing the 2nd instar larvae of *Spodoptera frugiperda* in individually-rearing (A) and group-rearing (B). Reproductive values ($v_{xj}$) of *Campoletis chlorideae* parasitizing the 2nd instar larvae of *Spodoptera frugiperda* in individually-rearing (C) and group-rearing (D) conditions.
Figure 5

Population Parameters of *C. chlorideae* parasitizing individually-reared *S. frugiperda* larva. (A) Age-specific survival rate ($l_x$), age-specific predation rate ($k_x$) and age-specific net predation rate ($q_x$) of *Campoletis chlorideae* parasitizing the 2nd instar larvae of *Spodoptera frugiperda* in individually-rearing conditions. (B) Age-specific cumulative consumption rates ($C_x$), cumulative net reproductive rates ($R_x$), and conversion rates ($P_x$) of *Campoletis chlorideae* parasitizing the 2nd instar larvae of *Spodoptera frugiperda* in individually-rearing conditions. Simulated population growth of *Campoletis chlorideae* parasitizing the 2nd instar larvae of individually-reared *Spodoptera frugiperda* over a period of 60 d. (C) Uncertainty of population projection based on the original, 0.025th percentile, and 0.975th percentile of life tables starting with an initial population of 10 eggs. (D) Predation potential and uncertainty of population projection based on the original, 0.025th percentile, and 0.975th percentile of life table starting with an initial population of 10 eggs.