Biological Control and Crop Protection

Influence of host preference, mating, and release density on the parasitism of *Telenomus remus* (Nixon) (Hymenoptera, Platygastridae)

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ABSTRACT

We evaluated the influence of host preference, mating, and release density on *Telenomus remus* (Nixon, 1937) (Hymenoptera: Platygastridae) parasitizing eggs of *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae). First, we tested host preference of *T. remus* (free choice test) offered a choice between eggs of *Corcyra cephalonica* (Stainton, 1865) (Lepidoptera: Pyralidae) and *S. frugiperda*. Parasitism capacity and host preference (*S. frugiperda*) of *T. remus* reared on either of the two hosts did not differ. Secondly, we evaluated the influence of mating behavior of *T. remus* females on its parasitism. Only the offspring sex ratio differed between treatments, indicating that the species reproduces by parthenogenesis of the arrhenotoky type. Finally, we evaluated the influence of release density on *T. remus* parasitism. This was tested by releasing different numbers of the parasitoid per *S. frugiperda* egg using *T. remus* reared for different numbers of generations on *C. cephalonica* eggs. The regression analysis between percentage of parasitism and density of released *T. remus* females showed a quadratic effect for all tested parasitoid generations (F_{35}, F_{40}, and F_{45}) with maximum parasitism from 65.07% to 71.69%. Our results allow the conclusion that (a) *T. remus* prefers *S. frugiperda* eggs, regardless of the host on which this parasitoid was reared, showing no preimaginal conditioning; (b) Mating does not affect the number of eggs parasitized by *T. remus* or the development of its offspring; and (c) The optimal *T. remus* release density when reared on *C. cephalonica* is between 0.133 and 0.150 females/*S. frugiperda*.

Introduction

Telenomus remus parasitizes eggs of various species of the order Lepidoptera, many of which are global crop pests (Cave, 2000). Despite possessing traits favorable for their use as biological control, this parasitoid is currently only reared on a small scale due to the difficulties of rearing it on its natural host *Spodoptera frugiperda* (Pomari-Fernandes et al., 2015). Alternatively, *T. remus* can be reared on a factitious hosts that may not be the parasitoid's preference but is still adequate for its successful development (Parra, 1997). In this context, *Corcyra cephalonica*, which can be reared in the laboratory more easily and at a lower cost than *S. frugiperda* (Kumar et al., 1986), has been suggested as a possible factitious host of *T. remus* (Kumar et al., 1986; Pomari et al., 2012). However,

continuous rearing of a parasitoid on a factitious host may affect its parasitism or host preference, and may directly influence its efficiency against the target pest. This is probably due to preimaginal conditioning occurring during larval development (Cobert, 1985), a biological process which needs further study for *T. remus* reared on *C. cephalonica* eggs.

Knowledge of the biology and ecology of insects and their natural enemies is a prerequisite for the success of biological control in Integrated Pest Management (IPM) (Cave, 2000). Adult mating can impact parasitism, and should be taken into account when testing the use of an egg parasitoid in massive field releases (Pratissoli et al., 2009). Males of *T. remus* have one larval instar less than females (Cave, 2000), and therefore emerge earlier than females from the same host egg mass. The newly emerged males guard egg masses to ensure their mating with females as soon as they emerge (Cave, 2000). Because parasitism capacity may differ between mated and unmated *T. remus* females, the influence of mating at the time of emergence should be assessed prior to adopting the recently

developed technology of aerial release of individual pupae close to emergence.

Additionally, superparasitism can decrease the number of parasitized eggs, and may occur when an excessive number of parasitoids per host egg is released (Cave, 2000). Research is needed to test the influence of release density on parasitism with the long-term goal of determining the optimal number of parasitoids to be released into the field (Sá and Parra, 1993). To this end, this study evaluates the influence of host preference, mating, and release density on *T. remus* parasitism on eggs of *S. frugiperda*. The results yield crucial information for the success of rearing *T. remus* and its release in the field.

Material and methods

The studies (bioassay 1 and 2) were carried out under controlled laboratory conditions (25 °C ± 2 °C, relative humidity 80% ± 10%, photoperiod 14/10 h [light/dark]) and in a semifield (greenhouse) (bioassay 3) at Embrapa Soybean, Londrina, State of Paraná, Brazil. This work involved three independent bioassays. In the first bioassay we assessed host preference of the parasitoid *T. remus* offered a choice between eggs of *C. cephalonica* and *S. frugiperda*. In the second bioassay we evaluated the influence of mating on *T. remus* parasitism of *S. frugiperda* eggs. In the third bioassay we determined the optimal number of parasitoids to be released for the successful control of *S. frugiperda* in maize. All hosts and parasitoids used in the experiments were obtained from the rearing laboratory at Embrapa Soybean.

Bioassay 1: host preference of Telenomus remus

All *T. remus* colonies evaluated in the host preference test originated from insects reared on *C. cephalonica* eggs at the F_{40} generation and on *S. frugiperda* eggs at the F_{350} generation (Parra, 1997) in order to compare the two insect populations. The experiment was carried out in a completely randomized 2 × 2 factorial design (2 parasitoid colonies by 2 host eggs) and 15 replicates. Each replicate consisted of an arena according to Thuler et al. (2007): a polyethylene bottle (4 and 2 cm in diameter) for parasitoid release was placed in the middle of the arena around which four tubes (volume 1.5 mL) containing the host specimens were arranged at equal distances.

Approximately 150 eggs of a single host (*C. cephalonica* or *S. frugiperda*) were glued to labeled, white cardboard cards (2.5 × 5 cm) with white glue (Tenaz®). Two cards per host were individually introduced into the tubes located on opposite sides of the arena. Next, four newly emerged (24 h) *T. remus* females fed on honey were released into the arena from the central bottle. After 24 h, the cards were removed and placed individually in 1.5 mL Duran tubes until the emergence of adults. At this step, the number of parasitized eggs was recorded.

The results (Table 1) were analyzed for normality (Shapiro and Wilk, 1965) and homogeneity of variance of treatments (Burr and Foster, 1972) and, whenever necessary, transformed to perform ANOVA. The number of parasitized eggs was transformed by log ($x + 1$). The treatment means were compared using Tukey's test at a probability level of 5% (SAS Institute, 2009).

Bioassay 2: influence of parasitoid mating on its parasitism

Mated and unmated *T. remus* females reared on *C. cephalonica* eggs (F_{40} generation) were offered eggs of *S. frugiperda* for a 24 h period. Each female was placed in a Duran type tube (1.5 mL) containing a droplet of honey as food, and offered approximately 100 *S. frugiperda* eggs (up to 24 h old) glued to white cardboard cards (2.5 × 5 cm) (six replicates per treatment). Afterwards, cards

Table 1

Bioassay 1: number of eggs (*Spodoptera frugiperda* and *Corcyra cephalonica*) parasitized by *Telenomus remus* from different colonies (reared on *S. frugiperda* and *C. cephalonica* eggs). Temperature 25 ± 2 °C, relative humidity of 80% ± 10%, and the photoperiod of 14/10 h (light/dark).

Host	Parasitoid colony		
	T. remus reared on *C. cephalonica*	*T. remus* reared on *S. frugiperda*	Mean
C. cephalonica	2.29 ± 1.03	0.00 ± 0.00	**1.19 ± 0.57 b**
S. frugiperda	94.36 ± 12.71	81.08 ± 13.53	87.96 ± 9.18 a
Mean	48.32 ± 10.85 A	40.53 ± 10.47 A	
CV (%)	39.15		
$F_{parasitoid}$	1.57		
F_{host}	259.46		
$F_{parasitoid*host}$	1.73		
$p_{parasitoid}$	0.2161		
p_{host}	<0.0001		
$p_{parasitoid*host}$	0.1950		
$DF_{parasitoid}$	1		
DF_{host}	1		
$DF_{parasitoid*host}$	1		
DF_{total}	53		

Means (±SE) followed by identical upper-case letters within a row (different parasitoid colonies), and lower-case letters within a column (different egg hosts) did not statistically differ according to Tukey's Studentized range test at 5% probability. Original data followed by statistics performed on data transformed in log ($x + 1$).

were removed and placed individually in Duran-type tubes until the emergence of adults. The number of parasitized eggs, parasitoid emergence (%), sex ratio, and longevity of parental females was recorded. The results (Table 2) were analyzed for normality (Shapiro and Wilk, 1965) and homogeneity of variance of treatments (Burr and Foster, 1972) and, whenever necessary, transformed to perform ANOVA. The number of parasitized eggs was transformed by log ($x + 1$) and parasitoid emergence (%) transformed by arcsin $\sqrt{X/100}$. Then, treatment means were compared by Tukey's test at a probability level of 5% (SAS Institute, 2009).

Bioassay 3: influence of T. remus release density on its parasitism

The influence of the release density of *T. remus* on its parasitism was determined by releasing different numbers of *T. remus* in relation to a given number of eggs of *S. frugiperda*, using *T. remus* reared for a different number of generations (F_{35}, F_{40}, and F_{45}) on *C. cephalonica* eggs. We chose *T. remus* reared on *C. cephalonica* eggs because this parasitoid is planned to be released in the field on a large scale for biological control programs. An independent trial was carried out for each parasitoid generation under greenhouse conditions, using a fully randomized experimental design with ten treatments (0, 0.017, 0.033, 0.050, 0.067, 0.083, 0.100, 0.117, 0.133, and 0.150 *T. remus* females per *S. frugiperda* egg) and five replicates.

Eggs of the pest species were obtained from laboratory rearing and exposed to treatments inside iron-framed cages (40 cm × 40 cm × 120 cm) covered with voile fabric in each replicate. A pot of 40-cm diameter with two hybrid IPR 114 maize plants was placed inside each of these cages, attaching a white cardboard card with 150 eggs to the whorl of each plant. Different numbers (5, 10, 15, 20, 25, 30, 35, 40 and 45) of previously mated females (Pomari et al., 2013) were released, representing the proportions of 0.017, 0.033, 0.050, 0.067, 0.083, 0.100, 0.117, 0.133, and 0.150 *T. remus* females per *S. frugiperda* egg. No parasitoids were released in the control treatment. Parasitism was allowed for 24 h, and temperature and humidity were recorded using a digital data logger (Instrutherm HT-500) (Table 3). Next, eggs were collected and maintained in Petri dishes at 25 °C until they darkened and parasitoids emerged for subsequent evaluation. We recorded total percent of parasitism per replicate. This procedure was repeated

Table 2

Bioassay 2: biological parameters of mated and unmated *Telenomus remus* females (from *Corcyra cephalonica* eggs) that parasitized *Spodoptera frugiperda* eggs. [Temperature of $25 \pm 2\,^{\circ}C$, relative humidity of $80\% \pm 10\%$, and the photoperiod of 14/10 h (light/dark).].

Treatment	Number of parasitized eggs[1]	Parasitoid emergence (%)[2]	Sex ratio	Longevity of parental females (days)
Mated females	53.33 ± 5.68^{ns}	99.13 ± 0.26^{ns}	0.61 ± 0.04 a	5.63 ± 0.34^{ns}
Unmated females	46.00 ± 5.27	98.78 ± 0.81	0.00 ± 0.00 b	6.08 ± 0.18
CV (%)	11.84	4.46	19.88	10.52
p	0.3506	0.9264	<0.0001	0.2835
F	0.98	0.01	253.13	1.32
DF_{error}	8	8	8	8

[1] Means ($\pm$SE) followed by identical upper-case letters within a row (different parasitoid colonies), and lower-case letters within a column (different egg hosts) did not statistically differ according to Tukey's Studentized range test at 5% probability Original data followed by statistics performed on data transformed in log $(x + 1)$.

[2] Original data followed by statistics performed on data transformed by arcsin $\sqrt{X/100}$.

ns ANOVA not significant.

Table 3

Weather data recorded by a data Logger during bioassay 3 (influence of the *T. remus* release density on its parasitism).

Generation – stadium	Temperature			Relative humidity		
Phenological	Minimum	Maximum	Average	Minimum	Maximum	Average
F35 – V2/V3	19.8	27.1	23.45	47.3	95.5	71.4
F35 – V4/V5	23.2	36.8	30	54.3	85.1	69.7
F35 – V8/V9	25.6	38.4	32	49.1	90.2	69.6
F40 – V2/V3	23.3	29.3	26.3	53.6	84.2	68.9
F40 – V4/V5	21.4	36.4	28.9	40.1	90.1	65.1
F40 – V8/V9	19.3	26.7	23	77.1	95.8	86.4
F45 – V2/V3	21.8	38.8	30.3	52.1	96.1	74.1
F45 – V4/V5	24.9	37.3	31.1	55.2	80.6	67.9
F45 – V8/V9	21.8	38.8	30.3	52.1	96.1	74.1

at various developmental stages of the plants (V2/V3, V4/V5, and V8/V9 according to Magalhães and Durães, 2006) because different leaf surfaces might provide different sizes of area for a parasitoid to find eggs and therefore may impact its parasitism. The average parasitism observed for each parasitoid density during different plant stages was used in the analyses (Fig. 1). The number of *T. remus* females per egg of *S. frugiperda* and percentage of parasitism were used in a regression analysis (SAS Institute, 2009). Parasitism data was analyzed for normality (Shapiro and Wilk, 1965) and homogeneity of variance of treatments (Burr and Foster, 1972), and whenever necessary transformed to perform ANOVA. Parasitism values of *T. remus* generations F_{40} and F_{45} were transformed by $(x + 1)^{0.5}$. The treatment means were then compared by Tukey's test at a probability level of 5% (SAS Institute, 2009).

Results

Bioassay 1: host preference of Telenomus remus

There was no interaction between *T. remus* colonies (*T. remus* reared on *C. cephalonica* or *S. frugiperda* eggs) and parasitized hosts (*C. cephalonica* or *S. frugiperda*) ($p_{parasitoid*host} = 0.1950$, $F_{parasitoid*host} = 1.73$) (Table 1). *Telenomus remus* reared on *C. cephalonica* eggs and *S. frugiperda* eggs exhibited similar parasitism capacity regardless of the host species they were reared on. The number of parasitized eggs was similar for both parasitoid colonies (48.32 and 40.53 eggs for parasitoids reared on *C. cephalonica* and *S. frugiperda*, respectively, $p_{parasitoid} = 0.2161$, $F_{parasitoid} = 1.57$) (Table 1). In contrast, higher numbers of *S. frugiperda* eggs (87.96) were parasitized compared with *C. cephalonica* eggs (1.19) ($p_{host} < 0.0001$, $F_{host} = 259.46$) (Table 1), indicating a clear preference for *S. frugiperda* eggs regardless of the host the parasitoid was reared on.

Bioassay 2: influence of parasitoid mating on its parasitism

No differences were found between mated and unmated females with respect to the number of parasitized eggs, parasitoid emergence (%) and longevity of parental females (days) (Table 2). In contrast, offspring sex ratio differed between treatments (0.61 for mated females and 0.00 for unmated females, Table 2), indicating that *T. remus* reproduces parthenogenetically with characteristics of the arrhenotoky type.

Bioassay 3: influence of the release density of T. remus on its parasitism

For *S. frugiperda* eggs attached to maize leaves, we found a quadratic effect for the regression analysis between the percentage of parasitism and density of released *T. remus* females (number of parasitoid per pest egg) in all parasitoid generations tested (Fig. 1). Maximum parasitism was 71.69%, 71.69%, and 65.07% for F_{35}, F_{40}, and F_{45}, respectively. The maximum parasitism rate was reached at densities between 0.133 and 0.150 of *T. remus* females per *S. frugiperda* egg (Fig. 1). Throughout the study, climatic data were recorded with a data logger at greenhouse conditions. Differences in temperature and relative humidity between all evaluated growth stages are shown in Table 3.

Discussion

Parasitoid rearing on factitious hosts is essential for the success of its mass release in augmentative biological control programs (Bueno et al., 2008) and requires the establishment of procedures to monitor the quality of the produced insect. According to Cobert (1985), continuous rearing of parasitoids on factitious hosts can cause the loss of their ability to recognize and choose a host and therefore reduce their efficiency against the targeted pest species. In our study, this negative effect was not observed for *T. remus* reared on *C. cephalonica* eggs. Parasitism of eggs of the target field pest (*S. frugiperda*) was similar in parasitoid females reared on both *C. cephalonica* and *S. frugiperda*. However, the influence of the factitious host on parasitoid quality might also depend on the number of generations the parasitoid is reared on the same host. Pratissoli et al. (2004) reported that parasitism capacity of species reared on factious hosts was inversely proportional to the number
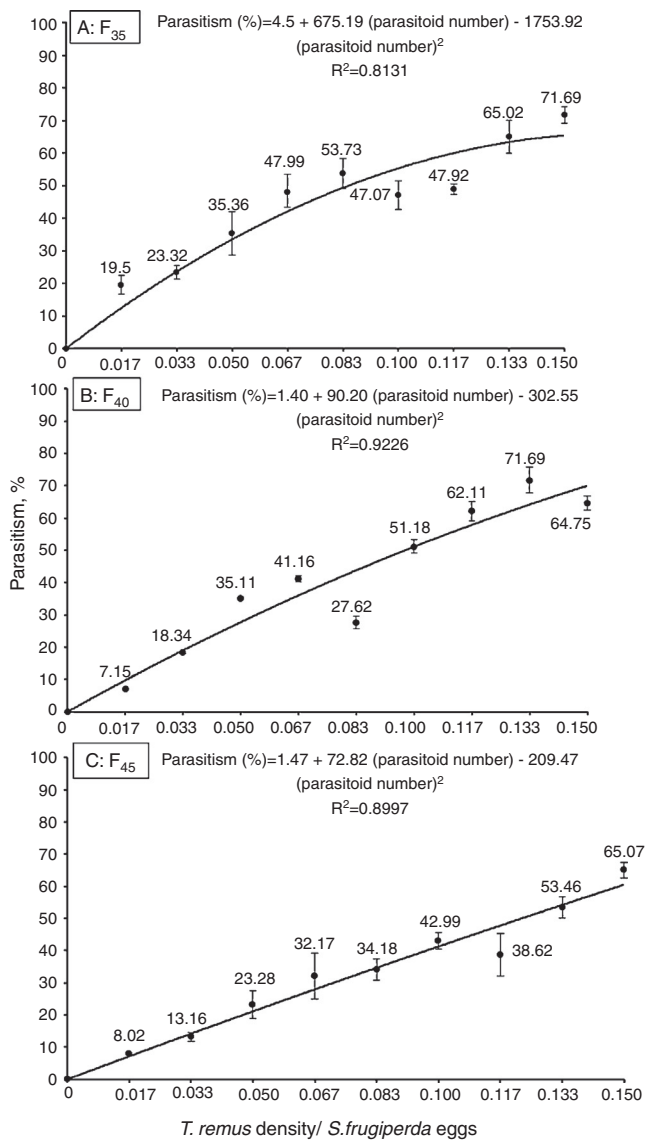
Fig. 1. Bioassay 3: parasitism (%) in *Spodoptera frugiperda* eggs after the release of different densities of *Telenomus remus* per host egg using parasitoids from different colonies. *T. remus* reared on *Corcyra cephalonica* for 35 generations (A), 40 generations (B) and 45 generations (C). Average values from trials repeated at maize at V2/V3, V4/V5 and V8/V9 development stage.

of generations for which they were kept on the same host species in the laboratory. Therefore, quality control must assess parasitism capacity of all generations throughout parasitoid rearing. We observed similar parasitism for *T. remus* on *S. frugiperda* regardless of the origin of the parasitoid (reared on either *C. cephalonica* or *S. frugiperda* eggs) even after rearing it on *C. cephalonica* eggs for 45 generations (F_{45} generation). These results highlight the potential of *C. cephalonica* as a factitious host for *T. remus* rearing.

In addition, *S. frugiperda* eggs continued to be preferred by *T. remus* reared on *C. cephalonica* eggs in the free-choice test supporting the hypothesis that it constitutes an adequate factitious host. This result also suggests that the host acceptance behavior of *T. remus* females cannot be attributed to experience acquired during larval development (preimaginal conditioning) as previously described by Cobert (1985) and Kaiser et al. (1989) and more recently by Pomari-Fernandes et al. (2015). Our finding also excludes learning or α-conditioning, where females associate new stimuli (acquired) with innate stimuli and thus can adapt their behavior to the environment in which they lived as adults or young

individuals (Kaiser et al., 1989; Nurindah et al., 1999). The absence of preimaginal conditioning or learning was previously reported for *T. remus* reared on *S. frugiperda* eggs and, when tested for different hosts (*S. frugiperda* versus *S. cosmioides*, *S. frugiperda* vs. *S. albula*, and *S. cosmioides* vs. *S. albula*) this species preferred *S. cosmioides* as a host (Goulart et al., 2011). This supports the use of *T. remus* in biological control because of the possibility of rearing it on a single host for several generations without reducing its effectiveness against different target pests in the field.

This host preference of *T. remus* is probably related to the nutritional quality of the host. Adding more complexity to this phenomenon, Molina et al. (2005) claim that not only the nutritional quality of the future host but also of the previous host on which the parasitoid was reared might influence host preference. Egg surface, color and other traits of the host can also influence host preference in a more complex decision-making process (Cônsoli et al., 1999). However, the preference of *T. remus* for *S. frugiperda* eggs when reared on *C. cephalonica* may be more closely related to the superior nutritional value of *S. frugiperda* compared to *C. cephalonica*.

The importance of adult mating must also be considered when rearing *T. remus*, because it can compromise the maintenance of the parasitoid in the field (Pratissoli et al., 2009; Farrokhi et al., 2010). A recent strategy to release egg parasitoids in the field has been to use isolated pupae that are sprayed on plants, for example of *Trichogramma* spp. in Brazil. However, males of *T. remus* which emerge earlier than females due to their shorter lifecycle await females to emerge from the same parasitized egg mass (Cave, 2000), allowing for high mating rates directly after emergence. Therefore, the spraying of *T. remus* individual pupae in the field could negatively impact the reproductive behavior of the parasitoid, highlighting the importance of evaluating mating deprivation.

The absence of mating directly influenced offspring sex ratio, leading to the production of males only. However, other biological parameters were not affected. The observed reproduction mode of *T. remus* via parthenogenesis of the arrhenotoky type (i.e. fertilized females give rise to diploid females, whereas unfertilized females give rise to haploid males), has been described as the most common reproductive type for insects of the order Hymenoptera (Pratissoli et al., 2014).

In contrast to sex ratio, the numbers of parasitized eggs per mated and unmated female were similar, as well as parasitoid emergence (%) and longevity of parental females. It should be noted that in our study the longevity of the parental females was similar to that observed for *T. remus* of generation F_{19} reared on *C. cephalonica* eggs as reported by Pomari-Fernandes et al. (2015). These results differ from Pratissoli et al. (2014) who observed an influence of mating on female longevity of the egg parasitoid *Trichogramma pretiosum* (Riley, 1879) (Hymenoptera: Trichogrammatidae). In addition, Stouthamer (1993) reports that field releases of parasitoids (genus *Trichogramma*) reproducing by parthenogenesis of the thelytoky type were more efficient compared with parasitoids with those reproducing by parthenogenesis of the arrhenotoky type, illustrating the importance of studying the reproductive mode of each parasitoid species.

Our results suggests that although parasitism of *T. remus*, and therefore its control efficiency, was not affected by mating, parasitoid permanence in the field may be impacted when unmated females are released. Some studies reported the failure to establish *T. remus* in the field after its release (Van Waddill and Whitcomb, 1982; Figueiredo et al., 1999). Since additional release might be always necessary when pest population increase, the releasing of mated females might not be important considering that the parasitism of the available eggs in the field would be the same (Carneiro et al., 2009).

Additionally, the success of *T. remus* as biological control depends on the appropriate parasitoid density per *S. frugiperda*

egg which can be evaluated by releasing different numbers of parasitoids in relation to a given number of pest eggs. We studied parasitoid release in maize, using different densities of *T. remus* females of the F_{35}, F_{40}, and F_{45} generation. Parasitism of females of all tested generations was positively related to the density of females per *S. frugiperda* egg, reaching maximum parasitism between 0.133 and 0.150 *T. remus*/*S. frugiperda* egg (40–45 *T. remus* females per 300 eggs of *S. frugiperda*). In a similar study, the optimal density was almost 50% less than in our study (25 *T. remus* females/300 *S. frugiperda* eggs of *T. remus* reared on *S. frugiperda* reported by Pomari et al., 2013). Given that parasitism can strongly depend on the parasitoid species and/or strains (Sá and Parra, 1993), these discrepancies may be due to different parasitoid colonies used in both trials. The rearing of *T. remus* on *C. cephalonica* eggs for many generations seems to generate parasitoids with lower parasitism capacity compared with those reared on *S. frugiperda* eggs, requiring a higher density of *T. remus* females to effectively control the pest. Although a greater number of parasitoids per pest egg are needed when the parasitoid is reared on a factitious host, several other factors, such as the ease of rearing and low cost of the parasitoid, should be considered. Overall, our results allow the conclusion that (a) *T. remus* prefers to parasitize *S. frugiperda* to *C. cephalonica* eggs, despite the absence of preimaginal conditioning; (b) mating does not affect parasitism capacity and development of *T. remus*; (c) the optimal release density of *T. remus* reared on *C. cephalonica* is between 0.133 and 0.150 females/*S. frugiperda* eggs, which is higher than the optimal release density of *T. remus* reared on its natural host *S. frugiperda*. These results importantly contribute to existing knowledge on the successful rearing of and field release strategies for *T. remus*.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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