Bulletin of Entomological Research (2017) **107**, 791–798
doi:10.1017/S000748531700030X

Quality control of *Telenomus remus* (Hymenoptera: Platygastridae) reared on the factitious host *Corcyra cephalonica* (Lepidoptera: Pyralidae) for successive generations

A.P. Queiroz[1], A. de Freitas Bueno[2]*, A. Pomari-Fernandes[3], M.L.M. Grande[4], O.C. Bortolotto[4] and D.M. da Silva[1]

[1]Instituto Agronômico do Paraná – IAPAR, 86051-990, Londrina, Paraná, Brazil: [2]Embrapa Soja, Laboratório de Parasitoides, 86001-970, Londrina, Paraná, Brazil: [3]Universidade Federal da Fronteira Sul – UFFS, 85301-970, Laranjeiras do Sul, Paraná, Brazil: [4]Universidade Estadual de Londrina, Centro de Ciências Agrárias, Departamento de Agronomia, 86051-990, Londrina, Paraná, Brazil

Abstract

Egg parasitoid rearing on factitious hosts is an important step to reduce costs and increase availability of the biocontrol agent but it requires quality control to achieve success in field conditions. To this end, this study evaluated the quality of *Telenomus remus* (Hymenoptera: Platygastridae) reared on *Corcyra cephalonica* (Lepidoptera: Pyralidae) for until 45 generations. In the first bioassay, we evaluated the body size of the laboratory-produced parasitoids. In the second bioassay, flight activity was examined, measuring the percentage of 'flyers', 'walkers' and 'deformed' parasitoids. The third bioassay assessed parasitism on *Spodoptera frugiperda* (Lepidoptera: Noctuidae) eggs. Our data indicate that the laboratory-reared parasitoid neither lost its ability to fly nor to parasitize *S. frugiperda* eggs. In conclusion, quality did not decrease significantly during 45 generations, and therefore rearing of *T. remus* on *C. cephalonica* as factitious host promises to be successful.

Keywords: biological control, factitious host, egg parasitoid, mass rearing

(Accepted 13 March 2017; First published online 6 April 2017)

Introduction

The rearing of egg parasitoids on factitious hosts is crucial for the success of many biological control programs because it reduces production costs and consequently increases the utility of the biocontrol agent for large-scale releases (Parra, 1997). However, the release success of a parasitoid produced on a factitious host depends on detailed information on its bioecological characteristics and its interaction with the targeted host in the field (Bourchier & Smith, 1996). A host switch might trigger changes in foraging behavior and parasitism capacity of the egg parasitoid (Jones *et al.*, 2015). Therefore, for a successful control of target pests in the field a well-designed quality control procedure is required in order to ensure that the parasitism capacity of the laboratory-reared parasitoid is similar to that of the same parasitoid species found in nature or produced on its natural host (Clarke & McKenzie, 1992).

Among various egg parasitoid species with high potential to be used in augmentative biological control programs, *Telenomus remus* (Nixon) (Hymenoptera: Platygastridae) stands out for being effective against various pest species of the genus *Spodoptera* Guenée (Lepidoptera: Noctuidae) (Pomari *et al.*, 2012), mainly due to its high reproductive capacity (Cave, 2000; Bueno *et al.*, 2008). However, due to the

*Author for correspondence
Phone: +55 (43) 3371-6208
Fax: +55 (43) 3371-6100
E-mail: adeney.bueno@embrapa.br

difficulties and costs of rearing *T. remus* on its natural host (Pomari-Fernandes *et al.*, 2015), this parasitoid to date has only been used against *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) for experimental purposes or for releases in small areas (Ferrer, 2001).

S. frugiperda rearing is time- and resource-consuming (Perkins, 1979), mainly because of larval cannibalism, which requires the rearing of larvae in individual vials to reduce preimaginal mortality (Chapman *et al.*, 2000). In this context, Kumar *et al.* (1986) and, more recently, Pomari-Fernandes *et al.* (2015) reported the successful development of *T. remus* in *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) eggs, suggesting it to be a promising factitious host, which is easily reared in the laboratory on a larger scale at lower costs compared with *S. frugiperda* (Bueno *et al.*, 2008). However, to the best of our knowledge, quality control of *T. remus* reared on *C. cephalonica* for successive generations has never been examined.

According to van Lenteren (1992), quality control aims to determine whether a parasitoid remains effective in controlling a target pest in the field after being reared for several generations in the laboratory. The development of each *T. remus* generation takes between 13 and 15 days at 25°C (Bueno *et al.*, 2008; Pomari-Fernandes *et al.*, 2015). It is unclear for how long a parasitoid colony can be kept in the laboratory while maintaining acceptable quality for releasing purposes, but longer time periods are desirable for a successful augmentative biological control programs (van Lenteren, 2003).

Quality assessment in the field can be excessively time- and labor-consuming, and as a result may not be sufficiently effective (Dias *et al.*, 2008). Therefore, laboratory procedures for a quick evaluation of laboratory-produced egg parasitoids are essential for the quality control of biocontrol agents (van Lenteren, 1992). The standardized quality control procedures established by the International Organization of Biological Control (Global IOBC Working Group: 'Quality Control of Mass Reared Arthropods') identify the number of emerged adults, sex ratio, fertility, longevity, adult size, flight activity, and performance in the field as the most important biological parameters to be evaluated (van Lenteren, 2003). Among these, parasitoid longevity, parasitism capacity, and flight activity are the main parameters when testing parasitoid quality for the use in augmentative biological control programs (Prezotti & Parra, 2002). We therefore aimed to evaluate the quality of *T. remus* reared on *C. cephalonica* eggs for successive generations (P_{35}, P_{40}, and P_{45}) by recording parasitoid size, flight activity, and parasitism capacity on its natural host (*S. frugiperda* eggs). In order to represent a profitable time period for the commercial exploitation of this parasitoid species, we reared 35–45 successive generations of *T. remus* during 455–585 days.

Material and methods

Parasitoid and host colonies

C. cephalonica and *S. frugiperda* eggs as well as *T. remus* females used in the experiments originated from insect colonies kept at Embrapa Soybean, Londrina, State of Paraná, Brazil. *S. frugiperda* was originally collected from maize plants in Rio Verde, State of Goiás. This strain was kept in the laboratory for approximately 9 years during which new field insects were introduced on a yearly basis to maintain insect quality. Perkins (1979) reported successful rearing of *S. frugiperda* in the laboratory for more than 18 years without any indication of degeneration.

Rearing in our study was carried out under controlled environmental conditions inside Biochemical Oxygen Demand (BOD) climate chambers (ELETROLab®, model EL 212, São Paulo, SP, Brazil) set at 80 ± 10% humidity, a temperature of 25 ± 2°C, and a 14/10 h photoperiod (L/D). Insects were fed on an artificial diet described by Greene *et al.* (1976) and Parra (2001). *C. cephalonica* was collected from UNESP/Jaboticabal and had been kept in the laboratory for approximately 26 generations (3 years) prior to the experiment. It was reared on a diet composed of whole-wheat flour (97%) and yeast (3%), as described by Bernardi *et al.* (2000), using an adapted rearing method for *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) (Parra, 1997).

T. remus was originally collected in Ecuador and grown at the parasitoid rearing facilities of ESALQ/USP (Luiz de Queiroz College of Agriculture/University of São Paulo), from where some specimens were transferred to Embrapa Soybean 9 years ago. In the laboratory, *T. remus* was reared using *S. frugiperda* egg masses (approximately 150 eggs each), which were glued onto a cardboard sheet (2 × 8 cm^2) and introduced into tubes together with eggs previously parasitized by *T. remus*. Small drops of honey were placed inside these tubes to feed the adults as soon as they emerged. The tubes were then closed, and the eggs allowed to be parasitized for 24 h. Adults that emerged from these eggs were used for trials or colony maintenance.

Bioassays

Three independent experiments were carried out to study parasitoid size, flight activity, and parasitism capacity on *S. frugiperda* eggs of *T. remus* emerged from *C. cephalonica* eggs. All trials were carried out in controlled environmental conditions inside BODs as previously described for the parasitoid and host colonies.

Morphological characters of T. remus *reared on* C. cephalonica *eggs for successive generations (bioassay 1)*

The experiment was carried out in a 3 × 2 factorial completely randomized design; three parasitoid generations (P_{35}, P_{40}, and P_{45}) × 2 parasitoid genders (female and male). Ten replicate adults of both genders were measured individually. Thus, ten males and ten females were measured for each parasitoid generation, totaling 60 adults in bioassay 1. Parasitoids reared on *S. frugiperda* eggs and exposed to parasitism on *C. cephalonica* eggs formed the P_0 generation. Eggs of *S. frugiperda* are almost spherical in shape (length 454.9 mm and width 390.2 mm), with a volume of approximately 0.036 mm^3. The chorion is about 2.50 mm thick, but thicker (up to 11.95 mm) where the exochorion forms a bridge or a ridge (Cônsoli *et al.*, 1999). The P_1 generation was the first generation of parasitoids reared on *C. cephalonica* eggs. In contrast to *S. frugiperda* eggs, *C. cephalonica* eggs are ellipsoid-shaped (length 573.5 mm and width 346.1 mm), but with the same volume of 0.036 mm^3. Their chorion thickness ranges from 4.18 to 5.32 mm (Cônsoli *et al.*, 1999). Length and width of the right anterior wing, length of the right hind tibia, and body length (head to tip of the abdomen) were measured in each replicate (adult insect). To measure the morphological characters, each specimen was photographed using a stereoscopic microscope (Leica Application Suite, Version 1.6.0). Images were

https://doi.org/10.1017/S000748531700030X Published online by Cambridge University Press

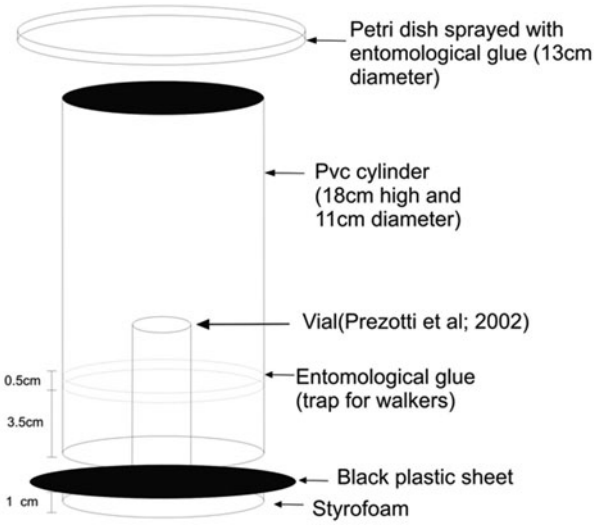

Fig. 1. Laboratory flight test unit developed by Dutton & Bigler (1995) and adapted in ESALQ-USP by Prezotti *et al.* (2002).

used for morphometric analysis with the software Image J (Version 1.47).

Flight activity of T. remus *reared on* C. cephalonica *eggs for successive generations (bioassay 2)*

The experimental design was completely randomized, consisting of four treatments [three generations (P_{35}, P_{40}, and P_{45}) of *T. remus* reared on *C. cephalonica* eggs, and *T. remus* from eggs of the natural host *S. frugiperda* (P_0)] and ten replicates. Each replicate consisted of 100–150 *T. remus* pupae (100–150 eggs parasitized by *T. remus*). Shortly before emergence, *T. remus* pupae were positioned on a plastic plate of 2.5 cm diameter and 1 cm height, which was placed at the bottom of each replicate. Emergence was allowed for 48 h to ensure complete emergence of all parasitoids from the pupae. This protocol and setup of the test unit was originally proposed by Dutton & Bigler (1995) and adapted in ESALQ-USP (Prezotti *et al.*, 2002) as described in the following: The test unit (fig. 1) consisted of a PVC cylinder (18 cm high and 11 cm in diameter). The top of the cylinder was closed using a clear Petri dish (diameter 13 cm) sprayed with entomological glue (composed of polybutene and synthetic silica) to trap flying *T. remus* ('flyers'). In order to attract the insects toward the light source at the top of the cage, the interior was painted with black ink and the bottom was sealed with a flexible black plastic sheet. Entomological glue was spread on the walls of the cage (3.5 cm from the bottom) to serve as a trap for 'walkers' (parasitoids that were unable to fly, but could walk and had no visible deformation). Originally, this test unit was used to measure parasitoid flight initiation (Dutton & Bigler, 1995). However, in addition to trapping deformed individuals, parasitoids were also caught inside the cage that did not have enough time to unfold their wings. Therefore, this test unit was modified by Prezotti *et al.* (2002) by inserting a vial (12 mm Ø × 75 mm height) inside the cage into which the pupae were placed. This modification allowed sufficient time for the emerged non-deformed parasitoids to unfold their wings during their walk inside the vial toward the entomological glue (3.5 cm from the bottom) (Prezotti *et al.*, 2002).

The number of parasitoids in the adhesive ring ('walkers'), in the Petri dish ('flyers'), and the 'deformed' individuals were recorded and used to calculate the percentages of these three groups of the total number of emerged adults. The parasitoids considered 'non-flyers' were observed under a stereoscope to determine the percentage of individuals with wing deformities ('deformed') (Prezotti *et al.*, 2002).

Parasitism capacity on S. frugiperda *eggs of* T. remus *reared on* C. cephalonica *eggs for consecutive generations (bioassay 3)*

The experiment was conducted in a completely randomized design with three treatments (*T. remus* reared on eggs of *C. cephalonica* for consecutive generations – P_{35}, P_{40}, and P_{45} parasitizing *S. frugiperda* eggs) and six replicates (each replicate consisting of five individualized females).

Mated *T. remus* individuals (newly emerged: ≤48 h old) were placed into separate glass tubes (12 mm Ø × 75 mm tall), which were then covered with the PVC film. Droplets (about 100 µl each) of pure honey were placed on the walls of the glass tubes to feed the females. Thirty glass tubes (six replicates of five females each) were prepared for each treatment. Approximately 100 eggs of *S. frugiperda* (≤24 h old) were glued onto cards made of white Bristol board paper (2.5 × 5 cm). Each paper was previously labeled with the respective treatments. Then, these cards were exposed to parasitism for 24 h. The cards were replaced daily until the death of the female parasitoid. Eggs removed from the glass tubes were maintained inside the same environmental chamber under controlled conditions until the emergence of parasitoids. Evaluated parameters were: parental *T. remus* female longevity (days), lifetime number of parasitized eggs/female, egg-to-adult duration (days), viability of parasitism (%) (percentage of parasitized eggs from which parasitoids emerged), progeny sex ratio, and the number of parasitized eggs per day (daily parasitism).

Statistical analysis

For the analysis of bioassays 1 and 3, prior to ANOVA, experimental results were subjected to exploratory analyses to assess the assumptions of normality of residuals (Shapiro & Wilk, 1965) and homogeneity of variance of the treatments (Burr & Foster, 1972) and, if necessary, transformed for ANOVA. Treatment means were then compared by Tukey's test at the 5% probability level. For the analysis of bioassay 2, percentages of 'flyers', 'walkers', and 'deformed' individuals were compared using Chi-square statistics (SAS Institute, 2009).

Results

Morphological characters of T. remus *reared on* C. cephalonica *eggs for successive generations*

Factorial analysis did not detect a significant interaction between parasitoid generation and parasitoid gender regarding the morphological characters wing length ($P_{\text{generation} \times \text{gender}} = 0.6366$; $F_{\text{generation} \times \text{gender}} = 0.46$), wing width ($P_{\text{generation} \times \text{gender}} = 0.6302$; $F_{\text{generation} \times \text{gender}} = 0.47$), body length ($P_{\text{generation} \times \text{gender}} = 0.7541$; $F_{\text{generation} \times \text{gender}} = 0.28$) and right hind tibia length ($P_{\text{generation} \times \text{gender}} = 0.3548$; $F_{\text{generation} \times \text{gender}} = 1.06$) (table 1).

https://doi.org/10.1017/S000748531700030X Published online by Cambridge University Press

Table 1. Morphological characters (mm) of *Telenomus remus* reared on *Corcyra cephalonica* eggs for successive generations (P_{35}, P_{40}, and P_{45}) (bioassay 1) under controlled environmental conditions (25 ± 2°C, 80 ± 10% RH, and photoperiod of 14/10 h L/D) ($N = 60$).

Parameter		Morphological characters (mm)[1]			
		Wing length	Wing width	Body length	Right hind length
Parasitoid generation	P_{35}	0.47 ± 0.01 b	0.14 ± 0.00^{ns}	0.49 ± 0.01^{ns}	0.13 ± 0.01^{ns}
	P_{40}	0.48 ± 0.01 ab	0.15 ± 0.00	0.52 ± 0.01	0.13 ± 0.01
	P_{45}	0.51 ± 0.01 a	0.15 ± 0.00	0.53 ± 0.01	0.13 ± 0.00
Gender	Male	0.48 ± 0.01^{ns}	0.15 ± 0.00^{ns}	0.49 ± 0.01 b	0.13 ± 0.00^{ns}
	Female	0.49 ± 0.01	0.14 ± 0.00	0.53 ± 0.01 a	0.13 ± 0.00
Statistics	CV (%)	8.65	10.06	10.79	14.55
	$P_{generation}$	0.0049	0.3391	0.1086	0.7331
	P_{gender}	0.8518	0.4886	0.0017	0.3113
	$P_{generation*gender}$	0.6366	0.6302	0.7541	0.3548
	$F_{generation}$	5.88	1.10	2.31	0.31
	F_{gender}	0.04	0.49	10.98	1.04
	$F_{generation*gender}$	0.46	0.47	0.28	1.06

[1]Means ± SE followed by the same letter in the column of each parameter are not significantly different from each other (Tukey's test, $P > 0.05$); [ns]ANOVA non-significant.

Table 2. Percentages of 'flyers', 'walkers', and 'deformed' of *Telenomus remus* when reared on different hosts for various generations (bioassay 2) under controlled environmental conditions (25 ± 2°C, 80 ± 10% RH, and photoperiod of 14/10 h L/D) ($N = 20$).

Host	Generation[1]	Flyers (%)[2]	Walkers (%)[3]	Deformed (%)[4]
C. cephalonica (CC)	P_{35}	83.21 ± 2.53 ab	13.54 ± 2.15 b	3.25 ± 0.45 a
C. cephalonica (CC)	P_{40}	70.57 ± 3.05 b	26.02 ± 3.01 a	3.42 ± 0.37 a
C. cephalonica (CC)	P_{45}	89.74 ± 1.25 a	8.46 ± 1.51 c	1.80 ± 0.37 a
S. frugiperda (SF)	P_0	91.61 ± 1.25 a	7.58 ± 1.09 d	0.80 ± 0.26 a
χ^2; *P*; df (CC P_{35} × CC P_{40})		28.77; 0.0696; 19	101.63; < 0.0001; 19	5.79; 0.7603; 19
χ^2; *P*; df (CC P_{35} × CC P_{45})		12.30; 0.8726; 19	68.29; < 0.0001; 19	2.24; 0.9871; 19
χ^2; *P*; df (CC P_{35} × SF P_0)		12.21; 0.8765; 19	66.40; < 0.0001; 19	8.16; 0.5180; 19
χ^2; *P*; df (CC P_{40} × CC P_{45})		148.65; <0.0001; 19	148.65; <0.0001; 19	4.90; 0.8431; 19
χ^2; *P*; df (CC P_{40} × SF P_0)		156.03; <0.0001; 19	156.03; <0.0001; 19	5.94; 0.7456; 19
χ^2; *P*; df (CC P_{45} × SF P_0)		4.75; 0.9996; 19	39.45; 0.0039; 19	7.46; 0.5889; 19

Means ± SE followed by the same letter in the column are not significantly different from each other (χ^2 test, $P > 0.05$).
[1]Generation of parasitoids used in the treatment [*T. remus* was reared on eggs of *S. frugiperda* for approximately 350 generations (P_0) and on eggs of *C. cephalonica* for 35 (P_{35}), 40 (P_{40}), and 45 (P_{45}) generations].
[2]Percentage of parasitoids able to fly.
[3]Percentage of parasitoids that did not fly but had no visible deformation.
[4]Percentage of parasitoids with visible deformation.

Wing length differed between generations ($P_{generation} = 0.0049$; $F_{generation} = 5.88$), with the longest wings observed in P_{45} (0.51 mm), followed by P_{40} (0.48 mm) and by P_{35} (0.47 mm). Wing length was similar between parasitoid genders ($P_{gender} = 0.8518$; $F_{gender} = 0.04$) (table 1).

Wing width did not differ between parasitoid generations ($P_{generation} = 0.3391$; $F_{generation} = 1.10$) or genders ($P_{gender} = 0.4886$; $F_{gender} = 0.49$) (table 1). Differently, body length did not differ between generations ($P_{generation} = 0.1086$; $F_{generation} = 2.31$) but was higher for females than for males ($P_{gender} = 0.0017$; $F_{gender} = 10.98$) (table 1). Right hind tibia length did not differ between generations ($P_{generation} = 0.7331$; $F_{generation} = 0.31$) or genders ($P_{gender} = 0.3113$; $F_{gender} = 1.04$) (table 1).

Flight ability of T. remus *reared on* C. cephalonica *eggs for successive generations*

The percentage of 'flyers' that emerged from *C. cephalonica* eggs in generation P_{35} was similar to the percentages in P_{40} (table 2, $\chi^2 = 28.77$, $P = 0.0696$, df = 19) and P_{45} (table 2, $\chi^2 = 12.30$, $P = 0.8726$, df = 19), and to the percentage of 'flyers' that emerged from *S. frugiperda* eggs (P_0) (table 2, $\chi^2 = 12.21$, $P = 0.8765$, df = 19). In contrast, the percentage of 'flyers' in P_{40} was lower than in P_{45} (table 2, $\chi^2 = 148.65$, $P < 0.0001$, df = 19) and P_0 (table 2, $\chi^2 = 156.03$, $P < 0.0001$, df = 19). Percentages of 'flyers' in P_{45} and P_0 were similar (table 2, $\chi^2 = 4.75$, $P = 0.9996$, df = 19).

The percentage of 'walkers' in generation P_{35} was lower than in P_{40} (table 2, $\chi^2 = 101.63$, $P < 0.0001$, df = 19) but higher than in P_{45} (table 2, $\chi^2 = 68.29$ $P < 0.0001$, df = 19) and P_0 (table 2, $\chi^2 = 66.40$, $P < 0.0001$, df = 19). The percentage of 'walkers' in generation P_{40} was higher in both P_{45} (table 2, $\chi^2 = 148.65$, $P < 0.0001$, df = 19) and P_0 (table 2, $\chi^2 = 156.03$, $p < 0.0001$, df = 19) and it was higher in generation P_{45} than in P_0 (table 2, $\chi^2 = 39.45$, $P = 0.0039$, df = 19).

The fraction of 'deformed' individuals was similar between treatments (table 2): their percentage in generation P_{35} was similar to that in P_{40} (table 2, $\chi^2 = 5.79$, $P = 0.7603$, df = 19) and P_{45} (table 2, $\chi^2 = 2.24$ $P = 0.9871$, df = 19) as well as in P_0 (table 2, $\chi^2 = 8.16$, $P = 0.5180$, df = 19). Likewise, the percentage of 'deformed' individuals that

https://doi.org/10.1017/S000748531700030X Published online by Cambridge University Press

Table 3. Biological characteristics of *Telenomus remus* reared on *Corcyra cephalonica* eggs for successive generations parasitizing *Spodoptera frugiperda* eggs (bioassay 3) under controlled environmental conditions (25 ± 2°C, 80 ± 10% RH, and photoperiod of 14/10 h L/D) ($N = 15$).

Parasitoid generation	Parental *T. remus* female longevity[1]	Lifetime number of parasitized eggs per female[1]	Egg-adult period (days)[1]	Viability of parasitism (%)[1]	Sex ratio of progeny[1]
P_{35}	5.6 ± 0.3[ns]	59.6 ± 7.7[ns]	–	99.13 ± 0.26[ns]	0.61 ± 0.04[ns]
P_{40}	6.1 ± 0.4	91.1 ± 3.3	15.06 ± 0.02 a	99.21 ± 0.15	0.62 ± 0.04
P_{45}	6.1 ± 0.4	77.6 ± 6.9	13.03 ± 0.01 b	98.51 ± 0.40	0.56 ± 0.05
CV (%)	12.95	14.75	0.28	0.64	16.39
P	0.5141	0.0767	<0.0001	0.2005	0.6395
F	0.70	5.22	6768.89	1.84	0.46

[1]Means ± SE followed by the same letter in the column are not significantly different from each other (Tukey's test, $P > 0.05$); – Data not evaluated; [ns]ANOVA non-significant.

emerged in generation P_{40} was similar to P_{45} (table 2, $\chi^2 = 4.90$, $P = 0.8431$, df = 19) and to P_0 (table 2, $\chi^2 = 5.94$, $P = 0.7456$, df = 19). Similarly, the percentage of 'deformed' individuals that emerged in P_{45} was similar to P_0 (table 2, $\chi^2 = 7.46$, $P = 0.5889$, df = 19).

Parasitism capacity on S. frugiperda *eggs of* T. remus *reared on* C. cephalonica *eggs for consecutive generations*

Parental *T. remus* female longevity ($P = 0.5141$; $F = 0.70$), lifetime number of parasitized eggs/parasitoid females ($P = 0.0767$; $F = 5.22$), viability of parasitism (% of parasitoids emerged from parasitized eggs) ($P = 0.2005$; $F = 1.84$), and progeny sex ratio ($P = 0.6395$; $F = 0.46$) did not differ between parasitoid generations (table 3). In contrast, egg-to-adult duration (days) was around 2 days shorter for P_{45} compared with P_{40} ($P < 0.0001$; $F = 6768.89$) (table 3).

More than 80% of the lifetime parasitism on *S. frugiperda* eggs of *T. remus* reared on eggs of *C. cephalonica* from generations P_{35} (fig. 2a), P_{40} (fig. 2b), and P_{45} (fig. 2c) was reached on the 2nd, 1st, and 1st day of parasitism, respectively. The number of parasitized eggs per day varied with parasitoid generations, but was higher in the first 24 h for all generations. In all treatments, the number of eggs parasitized per day decreased with time (fig. 2a–c).

Discussion

Our research aimed to evaluate the quality of *T. remus* after being reared on eggs of the factitious host *C. cephalonica* for successive generations (P_{35}, P_{40}, and P_{45}) by measuring parasitoid size and flight activity. This quality control was performed to test the fitness of these laboratory-produced parasitoids in field releases to control *Spodoptera* spp. The recorded results indicate that the quality of *T. remus* did not decrease significantly after rearing on *C. cephalonica* eggs for 45 generations. Therefore, we conclude that rearing of *T. remus* on the factitious host *C. cephalonica* promises to be successful.

The evaluated morphological characters (wing length, wing width, body length, and right hind length) have been suggested as adequate indicators of parasitoid fitness in insect-rearing facilities (Sequeira & Mackauer, 1992*a*). Because parasitoid size can be directly related to its dispersal capacity in the field (Gardner & Lenteren, 1986), these parameters must be considered when evaluating the quality of *T. remus* reared on *C. cephalonica* eggs for its successful use in applied biological control programs (Vaz *et al.*, 2004).

Although wing length, wing width, body length, and right hind length of *T. remus* were previously examined for parasitoids reared successively on *C. cephalonica* eggs, this was only done for 19 generations (Pomari-Fernandes *et al.*, 2016). The authors reported smaller values for morphological characters of parasitoids reared on *C. cephalonica* eggs compared with parasitoids reared on *S. frugiperda* eggs. Although smaller, they showed the same flight activity (percentage of 'flyers', 'walkers', and 'deformed') as bigger *T. remus* individuals reared on *S. frugiperda* eggs, indicating that a reduction of parasitoid size on the factitious host does not reduce its dispersal capacity, and is therefore adequate for biological control purposes. However, Pomari-Fernandes *et al.* (2016) studied neither those morphological characters nor the flight activity of more advanced generations of *T. remus* reared in the factitious host. It is crucial to understand whether successive *T. remus* rearing on *C. cephalonica* eggs for longer periods impairs parasitoid fitness over time.

The morphological characters of *T. remus* evaluated in our study (wing length, wing width, body length, and right hind length) were not reduced from generation P_{35} to generation P_{45}. Moreover, our results (from generation P_{35} to P_{45}) are similar to previous results reported by Pomari-Fernandes *et al.* (2016) for generations P_1 to P_{19}. More importantly, *T. remus* reared on *C. cephalonica* for 45 generations (P_{45}) had the same percentage of 'flyers' than the parasitoid reared on the natural host, *S. frugiperda*. Our results suggest that the dispersal capacity of smaller *T. remus* reared on *C. cephalonica* eggs was not impaired, similar to that of individuals from earlier generations (Pomari-Fernandes *et al.*, 2016).

The similarity in percentages of flying individuals ('flyers') from the factitious host *C. cephalonica* (generation P_{45}) and from the natural host *S. frugiperda* indicates that the size reduction of the recorded morphological characters was not sufficient to compromise flight ability. Even though the number of 'flyers' in generations P_{35} and P_{40} was smaller, their percentage was still close to 80% and that of individuals with deformities was only around 3%. The average percentage (≈80% or higher) of 'flyers' was similar to that found by other authors using the same protocol for other parasitoid species. Rodrigues *et al.* (2009) found averages from 85.9 to 97.7% of flying individuals and Prezotti *et al.* (2002) reported mean percentages between 74.7 and 90.6% for *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae). The similarity between those and our results suggests that *T. remus* rearing on *C. cephalonica* eggs provides an acceptable number of 'flyers' even when reared for successive generations (until generation P_{45}). Despite these findings, it is important to continuously examine flight ability in quality control procedures during parasitoid rearing.

In addition to flying, walking is another significant indicator of the performance of natural enemies in field conditions,

https://doi.org/10.1017/S000748531700030X Published online by Cambridge University Press

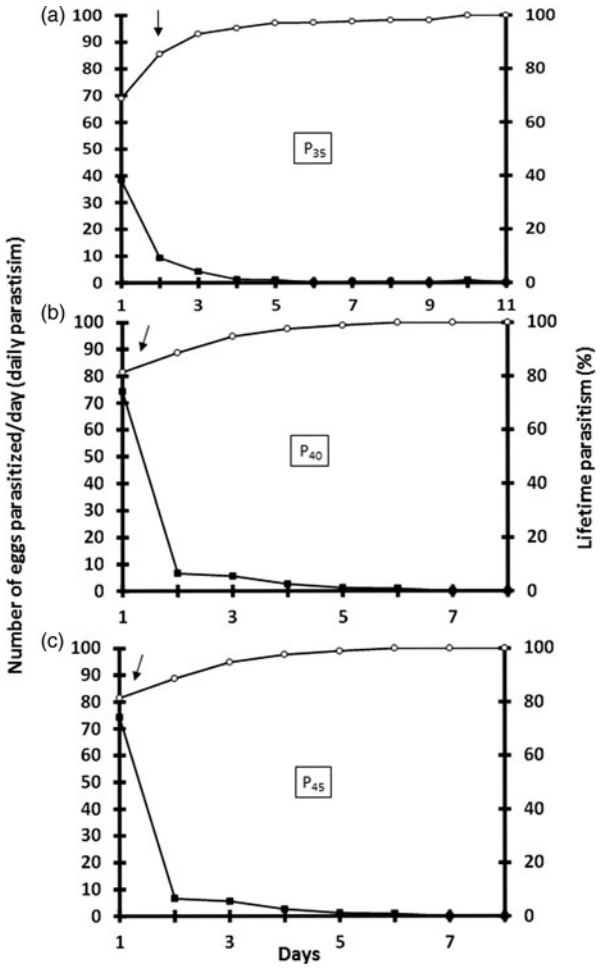

Fig. 2. Number of *Spodoptera frugiperda* eggs parasitized per day, and lifetime parasitism (%) of *Telenomus remus* reared on *Corcyra cephalonica* eggs for a variety of generations under controlled environmental conditions (25 ± 2°C, 80 ± 10% RH, and a photoperiod of 14/10 h L/D) ($N = 15$). (a) 35, (b) 40, and (c) 45 generations.

as both relate to foraging and dispersal. Therefore, the number of 'walkers' is also important for the evaluation of parasitoid quality. The average percentage of 'walkers' (from 7.58 to 26.01%) was higher than that obtained in studies using *T. pretiosum* (Prezotti *et al.*, 2002; Rodrigues *et al.*, 2009), which might be related to interspecific behavioral differences. The percentage of individuals with deformities such as stunted or folded wings was less than 4%.

Host quality is related to its size, reflecting the biomass available for consumption by the parasitoid (Chau & Mackauer, 2001; Jones *et al.*, 2015). For example, the parasitoid *Monoctonus paulensis* (Ashmead) (Hymenoptera: Braconidae) shows preference for larger aphids in order to increase its fecundity (number, size, and egg quality) (Chau & Mackauer, 2001). Another important factor is the duration of parasitoid development (egg–adult), which the species can extend as a compensatory action to recover from low-quality food and in order to reach a larger adult size (Sequeira & Mackauer, 1992*b*). Overall, *T. remus* developed more slowly on *C. cephalonica* eggs than on *S. frugiperda* eggs (Bueno *et al.*, 2014). Some differences in parasitoid development observed between *C. cephalonica* and *S. frugiperda* as hosts might be associated with the different quality of their eggs (Smith, 1996). Differences between eggs of different host species were previously identified as an important factor for survival and development of parasitoid species, such as *Trichogramma* spp. (Cônsoli *et al.*, 1999) and *T. remus* (Bueno *et al.*, 2014). Egg surface, egg size, chorion structures and other egg properties differ between host species, such as color during embryonic development, and volume. In addition, *S. frugiperda* lays its eggs in superposed masses while *C. cephalonica* lays individual eggs. All of these differences can affect not only parasitoid handling time and exploitation, but also the host's suitability for parasitoid development, which influences egg-to-adult development time (Cônsoli *et al.*, 1999).

The lifetime number of parasitized eggs may be of even greater importance than egg-to-adult duration because it determines the efficiency of biological control in the field. Lifetime numbers of parasitized eggs in our trials were smaller than those reported for parasitoids reared on eggs of *S. frugiperda* (Pomari *et al.*, 2012). However, even though it might be necessary to release a higher number of *T. remus* when the parasitoid is reared on a factitious host, laboratory-produced parasitoids of our study reared on *C. cephalonica* eggs did not lose their capacity to control *S. frugiperda* eggs. Similarly, *T. remus* reared for 75 generations on *C. cephalonica* eggs was able to parasitize *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) over all tested generations with a viability higher than 98% and a sex ratio above 0.50 (Kumar *et al.*, 1986).

The parasitization period (the period during which females are active) may vary due to differences in hosts (Reznik et al., 2001) or parasitoid species/strain (Pratissoli & Parra, 2000; Pizzol *et al.*, 2010), and can influence the success of biological control programs using egg parasitoids (Wajnberg & Hassan, 1994; Smith, 1996), or at least define the strategies for parasitoid release. Thus, whether parasitism is more intense in the first days of life or evenly distributed throughout adulthood is an important characteristic to be considered when choosing the best parasitoid release strategy (Bueno *et al.*, 2010). Parental *T. remus* lived for 5.6–6.1 days, but always reached 80% of its parasitism on the first or second day of parasitism. This might be a consequence of a pro-ovigenic development of *T. remus*. Some parasitoid species have the capacity to store a full complement of mature eggs in the ovaries or oviducts and complete oogenesis either before or shortly after adult emergence (pro-ovigenic parasitoids) (Mills & Kuhlmann, 2000). Therefore, adults emerge ready to lay eggs, as it seemed to be the case for *T. remus* in our trials. The sooner the parasitoid reaches 80% of its lifetime parasitism, the better, because while exposed to field conditions parasitoids might be susceptible to biotic and abiotic factors that can impair their action (Bueno *et al.*, 2012). Examples of such factors are the spraying of fungicides or herbicides used in crop management or an abrupt change in temperature that could kill the parasitoids (Carmo *et al.*, 2010; Denis *et al.*, 2011) but not the pests.

In conclusion, our data indicate that the quality of *T. remus* was not greatly impacted by successive rearing on *C. cephalonica* eggs. Most importantly, this laboratory-reared parasitoid did not lose its ability to parasitize *S. frugiperda* eggs. Therefore, rearing of *T. remus* on *C. cephalonica* eggs may be a successful strategy. The results presented here are from laboratory studies, requiring additional studies under field conditions to test the postulated hypotheses and to fully develop a successful biological control program using this egg parasitoid.

https://doi.org/10.1017/S000748531700030X Published online by Cambridge University Press

Acknowledgements

The authors would like to thank Embrapa Soja, the 'Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)', and the 'Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)', for funds that supported this research. Thanks are also extended to Maria Cristina Neves de Oliveira for the help with the statistics, Adair Vicente Carneiro for help with fig. 1 and Dagmar Frisch for the help with the English editing.

References

Bernardi, E.B., Haddad, M.L. & Parra, J.R.P. (2000) Comparison of artificial diets for rearing *Corcyra cephalonica* (Stainton, 1865) (Lep., Pyralidae) for *Trichogramma* mass production. *Revista Brasileira de Biologia* **60**, 45–52.

Bourchier, R.S. & Smith, S.M. (1996) Influence of environmental conditions and parasitoid quality on field performance of *Trichogramma minutum*. *Entomologia Experimentalis et Applicata* **80**, 461–468.

Bueno, R.C.O.F., Carneiro, T.R., Pratissoli, D., Bueno, A.F. & Fernandes, O.A. (2008) Biology and thermal requirements of *Telenomus remus* reared on fall armyworm *Spodoptera frugiperda* eggs. *Ciência Rural* **38**, 1–6.

Bueno, R.C.O.F., Carneiro, T.R., Bueno, A.F., Pratissoli, D., Fernandes, O.A. & Vieira, S.S. (2010) Parasitism capacity of *Telenomus remus* Nixon (Hymenoptera: Scelionidae) on *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) eggs. *Brazilian Archives of Biology and Technology* **53**, 133–139.

Bueno, R.C.O.F., Parra, J.R.P. & Bueno, A.F. (2012) *Trichogramma pretiosum* parasitism of *Pseudoplusia includens* and *Anticarsia gemmatalis* eggs at different temperatures. *Biological Control* **60**, 154–162.

Bueno, R.C.O.F., Bueno, A.F., Xavier, M.F.C. & Carvalho, M.M. (2014) *Telenomus remus* (Hymenoptera: Platygastridae) parasitism on eggs of *Anticarsia gemmatalis* (Lepidoptera: Eribidae) compared with its natural host *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America* **107**, 799–808.

Burr, I.W. & Foster, L.A. (1972) *A Test for Equality of Variances*. Mimeo series no 282. 26 p. West Lafayette, University of Purdue.

Carmo, E.L., Bueno, A.F. & Bueno, R.C.O.F. (2010) Pesticide selectivity for the insect egg parasitoid *Telenomus remus*. *BioControl* **55**, 455–464.

Cave, R.D. (2000) Biology, ecology and use in pest management of *Telenomus remus*. *Biocontrol News and Information* **21**, 21–26.

Chapman, J.W., Williams, T., Martinez, A.M., Cisneros, J., Caballero, P., Cave, R.D. & Goulson, D. (2000) Does cannibalism in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) reduce the risk of predation? *Behavioral Ecology and Sociobiology* **48**, 321–327.

Chau, A. & Mackauer, M. (2001) Preference of the aphid parasitoid *Monoctonus paulensis* (Hymenoptera: Braconidae, Aphidiinae) for different aphid species: female choice and offspring survival. *BiolControl* **20**, 30–38.

Clarke, G.M. & McKenzie, L.J. (1992) Fluctuating asymmetry as a quality control indicator for insect mass rearing processes. *Journal of Economic Entomology* **85**, 2045–2050.

Cônsoli, F.L., Kitajima, E.W. & Parra, J.R.P. (1999) Ultrastructure of the natural and factitious host eggs of *Trichogramma galloi* Zucchi and *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae). *International Journal of Insect Morphology and Embryology* **28**, 211–229.

Denis, D., Pierre, J.S., Van Baaren, J. & Van Alphen, J.J. (2011) How temperature and habitat quality affect parasitoid lifetime reproductive success – a simulation study. *Ecological Modelling* **222**, 1604–1613.

Dias, N.S., Parra, J.R.P. & da Costa Lima, T.C. (2008) Seleção de hospedeiro alternativo para três espécies de Tricogramatídeos neotropicais. *Pesquisa Agropecuária Brasileira* **43**, 1467–1473.

Dutton, A. & Bigler, F. (1995) Flight activity assessment of the egg parasitoid *Trichogramma brassicae* (Hym.: Trichogrammatidae) in laboratory and field conditions. *Entomophaga* **40**, 223–233.

Ferrer, F. (2001) Biological control of agricultural insect pests in Venezuela; advances, achievements, and future perspectives. *Biocontrol News and Information* **22**, 67–74.

Gardner, S.M. & Van Lenteren, J.C. (1986) Characterisation of the arrestment responses of *Trichogramma evanescens*. *Oecologia* **68**, 265–270.

Greene, G.L., Leppla, N.C. & Dickerson, W.A. (1976) Velvetbean caterpillar: a rearing procedure and artificial medium. *Journal of Economic Entomology* **69**, 487–488.

Kumar, D.A., Pawar, A.D. & Divakar, B.J. (1986) Mass multiplication of *Telenomus remus* Nixon (Hymenoptera: Scelionidae) on *Corcyra cephalonica* Stainton (Lepidoptera: Galleridae). *Journal of Advanced Zoology* **7**, 21–23.

Jones, T.S., Bilton, A.R., Mak, L. & Sait, S.M. (2015) Host switching in a generalist parasitoid: contrasting transient and transgenerational costs associated with novel and original host species. *Ecology and Evolution* **5**, 459–465.

Mills, N.J. & Kuhlmann, U. (2000) The relationship between egg load and fecundity among *Trichogramma* parasitoids. *Ecological Entomology* **25**, 315–324.

Parra, J.R.P. (1997) Técnicas de criação de *Anagasta kuehniella*, hospedeiro alternativo para produção de *Trichogramma*. p. 324 *in* Parra, J.R.P. & Zucchi, R.A. (*Eds*) *Trichogramma e o controle biológico aplicado*. Piracicaba, FEALQ.

Parra, J.R.P. (2001) *Técnicas de criação de insetos para programas de controle biológico*. 137 p. Piracicaba, ESALQ/FEALQ.

Perkins, W.D. (1979) Laboratory rearing of the fall armyworm. *Florida Entomologist* **62**, 87–91.

Pizzol, J., Pintureau, B., Khoualdia, O. & Desneux, N. (2010). Temperature-dependent differences in biological traits between two strains of *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae). *Journal of Pest Science* **83**, 447–452.

Pomari, A.F., Bueno, A.F., Bueno, R.C.O.F. & Menezes Junior, A. O. (2012) Biological characteristics and thermal requirements of the biological control agent *Telenomus remus* (Hymenoptera: Platygastridae) reared on eggs of different species of the genus *Spodoptera* (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America* **105**, 73–81.

Pomari-Fernandes, A., Bueno, A.F., Queiroz, A.P. & De Bortoli, S.A. (2015) Biological parameters and parasitism capacity of *Telenomus remus* Nixon (Hymenoptera: Platygastridae) reared on natural and factitious hosts for successive generations. *African Journal of Agricultural Research* **33**, 3225–3233.

Pomari-Fernandes, A., Bueno, A.F. & Bortoli, S.A.D. (2016). Size and flight ability of *Telenomus remus* parasitoids reared on eggs of the factitious host *Corcyra cephalonica*. *Revista Brasileira de Entomologia* **60**, 177–181.

Pratissoli, D. & Parra, J.R.P. (2000). Desenvolvimento e exigências térmicas de *Trichogramma pretiosum* Riley, criados em duas traças do tomateiro. *Pesquisa Agropecuária Brasileira* **35**, 1281–1288.

https://doi.org/10.1017/S000748531700030X Published online by Cambridge University Press

Prezotti, L. & Parra, J.R.P. (2002) Controle de qualidade em criações massais de parasitoides e predadores. p. 635 *in* Parra, J. R.P., Botelho, S.M., Ferreira, B.S.C. & Bento, J.M.S. (*Eds*) *Controle biológico no Brasil: Parasitóides e predadores*. São Paulo, Manole.

Prezotti, L., Parra, J.R., Vencovsky, R., Dias, C.T.D.S., Cruz, I. & Chagas, M. (2002) Teste de vôo como critério de avaliação da qualidade de *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae): Adaptação de metodologia. *Neotropical Entomology* **31**, 411–417.

Reznik, S.YA., Umarova, T.YA. & Voinovich, N.D. (2001) Long-term egg retention and parasitization in *Trichogramma principium* (Hymenoptera, Trichogrammatidae). *Journal of Applied Entomology* **125**, 169–175.

Rodrigues, S.M.M., Sampaio, M.V. & Miranda, J.E. (2009) Avaliação da capacidade de voo, parasitismo e emergência de linhagens de *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae). *Arquivos do Instituto Biológico* **76**, 749–753.

SAS Institute. (2009) SAS User's Guide: Statistics, 6 edn. Version 8.2. Cary.

Sequeira, R. & Mackauer, M. (1992*a*) Covariance of adult size and development time in the parasitoid wasp *Aphidius ervi* in relation to the size of its host, *Acyrthosiphon pisum*. *Evolutionary Ecology* **6**, 34–44.

Sequeira, R. & Mackauer, M. (1992*b*) Nutritional ecology of an insect host-parasitoid association: the pea aphid-*Aphidius ervi* system. *Ecology* **73**, 183–189.

Shapiro, S.S. & Wilk, M.B. (1965) An analysis of variance test for normality. *Biometrika* **52**, 591–611.

Smith, S.M. (1996) Biological control with *Trichogramma*: advances, successes, and potencial of their use. *Annual Review of Entomology* **41**, 375–406.

van Lenteren, J.C. (1992) Quality control for natural enemies used in greenhouses. *Bull OILB-SROP* **16**, 89–92.

van Lenteren, J.C. (2003) *Quality Control and Production of Biological Control Agents: Theory and Testing Procedures*. 327 p. Wallingford, CABI.

Vaz, L.A., Tavares, M.T. & Lomônaco, C. (2004) Diversidade e tamanho de himenópteros parasitóides de *Brevicoryne brassicae* L. e Aphis nerii Boyer de Fonscolombe (Hemiptera: Aphididae). *Neotropical Entomology* **33**, 225–230.

Wajnberg, E. & Hassan, S.A. (1994) *Biological Control with Egg Parasitoids*. pp. 286. British Library, Wallingford.