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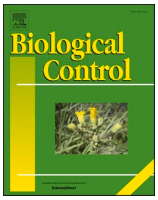

Isofemale line as an alternative to maintain quality of *Telenomus remus* Nixon, a biological-control agent for fall armyworm

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HIGHLIGHTS

- *Telenomus remus* regular line, lost its quality over generation at laboratory rearing.
- Flight capacity and parasitism decrease over the generations.
- Isofemale line appear to overcome the loss of quality.
- Flight capacity and parasitism still similar over 20 generations on isofemale line.

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ABSTRACT

This study evaluated whether rearing a regular line and an isofemale line of *Telenomus remus* Nixon for successive generations in laboratory conditions changed its flight capacity, parasitism, and longevity, desirable characteristics for a biological-control agent. The regular line of *T. remus* was re-introduced to laboratory rearing conditions from the field. The isofemale line was selected through inbreeding for nine generations, reducing its genetic variability. Flight capacity, parasitism, and longevity were evaluated over 11 generations in the regular strain, adding more two evaluations of parasitism and longevity (F17 and F19) and 20 generations in the isofemale line. Flight capacity was evaluated in flight-test units. Parasitism and lifespan were evaluated by offering egg-masses of *S. frugiperda* daily to individual females and counting the number of parasitized eggs, until the wasps died. The regular line of *T. remus* was affected by laboratory rearing over time. The proportion of flying individuals decreased by around 20 % between generations F1 and F6; the parasitism capacity was similar across generations; and longevity was similar in F1 and F6 (around 12 days), differing from F11 (8 days). Parasitism did not differ significantly in the *T. remus* isofemale line in the three generations evaluated, with no large variations in the parasitism potential in recently emerged female in any generation. The longevity of the parasitoid females was similar in generations F1 and F20 (7.3 and 8.1 days respectively) but differed from the F10 generation (15.2 days). Laboratory rearing of the regular line of *T. remus* over successive generations changed its flight capacity, parasitism, and longevity. Use of a *T. remus* isofemale line may be an alternative to maintain flight capacity and parasitism of this *S. frugiperda* biological-control agent over generations.

1. Introduction

The fall armyworm *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae) causes serious economic damage to crops, especially corn (maize), worldwide (Overton et al., 2021). In recent years, this pest has invaded several countries in Africa, Asia, and Oceania (Suby et al., 2020; Tambo et al., 2020; Sun et al., 2021). In Europe, *S. frugiperda* has been found in the Canary Islands, Spain, where emergency control measures were used, and on Cyprus (EPPO, 2023). These invasions are concerning to local researchers, farmers, and authorities because of the potentially severe socio-economic impact (Goergen et al., 2016; Sharanabasappa et al., 2018). This polyphagous species shows resistance to agrochemicals and transgenic crops, reducing the efficiency of these control methods (Diez-Rodríguez and Omoto, 2001; Yu

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et al., 2003; Storer et al., 2010; Carvalho et al., 2013; Paredes-Sánchez et al., 2021).

A potential natural enemy for use in biological control of *S. frugiperda* is *Telenomus remus* Nixon (Hymenoptera.: Scelionidae), a highly specific egg parasitoid (Figueiredo et al., 1999; Cave, 2000; de Bueno et al., 2008). Researchers in Venezuela (Ferrer, 2001) and Colombia (García-Roa et al., 2002) have reported successful cases of its use in corn crops in semi-field and field experiments. In Africa and Asia, *T. remus* has been found occurring naturally (Kenis et al., 2019; Liao et al., 2019). Biological-control programs have released different numbers of parasitoids, in different crops, and reaching different parasitism percentages (Colmenarez et al., 2022). Results from laboratory experiments diverge from those in the field, where *T. remus* has been less effective on parasitize *S. frugiperda* eggs (de Bueno et al., 2010; Colmenarez et al., 2022).

This divergence in results could be related to the loss of quality in laboratory rearing, resulting, for example, from inbreeding depression. However, the haplodiploid arrhenotokous *T. remus* undergoes little or no inbreeding depression, since haploid males, generated by parthenogenesis, express the deleterious alleles and die, "purifying the population" (Henter, 2003; West et al., 2005). On the other hand, even if inbreeding depression does not occur, laboratory conditions may impose selection pressure on a population, favoring individuals that are better adapted to laboratory conditions and less so to the field (Coelho et al., 2016). One way to maintain *T. remus* population quality may be through isofemale lines with desirable characteristics (Coelho et al., 2016; Nunney, 2003). This technique has been discussed as an alternative to maintain the quality of natural enemies, including members of the genus *Trichogramma* (Wajnberg and Colazza, 1998; Thomson and Hoffmann, 2002; Prezotti et al., 2004; Coelho et al., 2016) and the family Scelionidae, genus *Trissolcus*, as a way to evaluate patch-time allocation (Wajnberg et al., 2004).

In this study, we evaluated whether rearing *T. remus* for successive generations in the laboratory would change its characteristics as a biological-control agent of *S. frugiperda*, and whether an isofemale line could be used as an alternative to maintain parasitoid quality over generations.

2. Material and methods

2.1. Obtaining a regular line of T. remus

In order to obtain a *T. remus* line with characteristics close to wild insects, two regular lines reared in laboratory conditions, one since 1996 and another since 2011, were combined (Naranjo-Guevara et al., 2020). This mixed population was released in a cornfield in the experimental area of the Genetics Department of the Luiz de Queiroz College of Agriculture (ESALQ), University of São Paulo (USP) in Piracicaba, São Paulo, Brazil (22°42′14.2″S 47°38′15.6″W). The cornfield consisted of a 30 × 30 m plot, the plants were in vegetative stage V-10, 42 days old. To homogeneously distribute the host in the field, 50 *S. frugiperda* egg-masses (<24 h old) from a laboratory colony were distributed in five rows, distanced six meters from each other, in the rows the distance among the egg-masses was three meters, placing 10 egg-masses per row. After the egg-masses were set up in the cornfield, *T. remus* (24 h old) were released in the same day in the center of the plot, and the parasitism was allowed for 24 h under field conditions of temperature (average 23.5 ± 2.6 °C) and relative humidity (average 77 ± 10.3 %). After the egg-masses were collected from the field, they were kept at controlled conditions: 25 ± 1 °C, RH 60 ± 10 %, and a photophase of 14 h. The parasitoids that emerged from these parasitized egg-masses in the field were used to rear a new laboratory line, here termed the "regular line", that was assumed to have characteristics close to wild insects, with unknown genetic variation. This line was maintained under the same laboratory conditions of the maternal generation, fed with pure honey, and offered *S. frugiperda* eggs for parasitism.

2.2. Selection of T. remus isofemale line

To obtain a *T. remus* isofemale line, a wild population collected in 2019 and reported as the first natural occurrence of *T. remus* in Brazil was used as a genetically variable matrix, termed a "pool" (Wengrat et al., 2021). This population was collected at the Fazenda Areão experimental station of ESALQ/USP, Piracicaba (22°41′53″S, 47°38′30″W). In order to select a population with desirable attributes, a flight-test unit adapted from Prezotti et al. (2002) was used to select *T. remus* individuals with good flight capacity, over six generations. The flight-test unit, here termed the "flight-selector unit", consists of a 15 cm-diameter (Ø) and 20 cm-high (*h*) polyvinyl chloride (PVC) tube. And the top of the flight-selector was closed with a transparent Petri dish lid, without entomological glue. Egg-masses of *S. frugiperda* were attached with adhesive tape to the inner surface of the lid, in order to attract and select, through parasitism of eggs, only flying individuals from the population. The bottom of the flight-selector was closed with a plywood board and the edge was sealed with non-toxic playdough to prevent the wasps from escaping. The inner surface of the PVC tube was covered with black paper. On the black paper, a 0.5 cm-wide ring of entomological glue (Colly®) was applied around the inner surface of the tube 3.5 cm above the base, to form a barrier to capture insects that walked upward, ensuring that only the flying individuals would reach the eggs on the upper lid of the flight unit (Fig. 1).

From the flying insects selected in these units, the *T. remus* individuals that reached the *S. frugiperda* egg-masses in the top of the flight-unit, the isofemale line was obtained by isolating one female and offering a paper card containing *S. frugiperda* eggs for parasitism. After the offspring emerged, a single female that had copulated with her brother was again isolated and a new *S. frugiperda* egg card offered. This procedure of consanguineous mating was performed for nine generations to select an inbred line that, in the present work termed as isofemale lines with an expected inbreeding coefficient of at least 86 % (Li, 1955). This inbreeding protocol will have removed most of the genetic variation from the isoline, with less than 14 % of the initial heterozygosity remaining. Regarding the nuclear DNA, the backcrossing protocol repeated for nine generations created on average $(1\text{-}(0.5)^{9}) = 0.998$ identical nuclear genomic composition in the isoline (Coelho et al., 2016).

2.3. Biological parameters of T. remus regular line over generations

The potential of *T. remus* for *S. frugiperda* biological control was evaluated based on its parasitism and longevity in five generations (F1, F6, F11, F17, and F19), and the ability to fly in the F1, F6, and F11 generations. The *T. remus* population was maintained at environmental controlled conditions of 25 °C, 75 % RH, and 14 h photophase. The same conditions were used for all the experiments.

To evaluate the flight capacity of the *T. remus* regular line, the flight-test unit composed by a PVC tube 15 cm diameter was used (Dutton and Bigler, 1995; Prezotti et al., 2002). The unit was prepared as above, except the lid of the Petri dish on the top was covered inside with transparent entomological glue to capture and kill flying adults. Each experiment used 20 repetitions (flight-test units), each with a glass vial (8 cm *h* × 2 cm Ø) containing a parasitized *S. frugiperda* egg-mass attached at the base of the unit. At the 14th day of *T. remus* immature development, the vials containing the first emerging adults were placed in the flight-test units. The flight-test units were maintained in climate-controlled chambers for two days, to allow all *T. remus* individuals to emerge from the *S. frugiperda* eggs if they were parasitized, as well as allowing the emerged adults to fed on pure honey and to copulate. Then, differently from the above-mentioned procedure, the flight units were transferred to a freezer (–20 °C) to kill the wasps and facilitate counting and discrimination of insects emerged from the eggs, as flying insects trapped on the plastic Petri lid, from non-flying insects at the base and walkers stuck to the glue ring on the black paper on the inner surface of

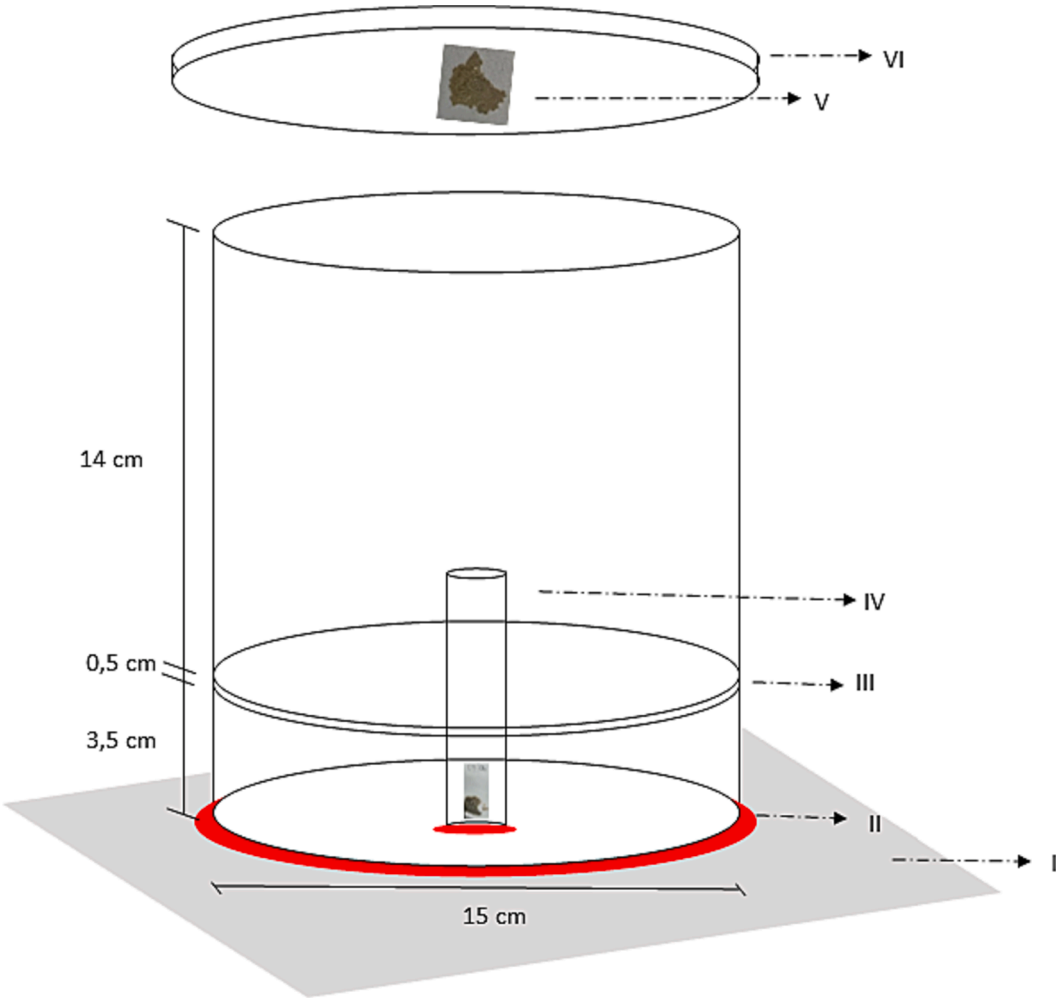

Fig. 1. Flight-selector unit based on flight-test unit used; to select flying individual. I. Plywood board; II. Non-toxic playdough; III. Ring of entomological glue; IV. Test tube containing a parasitized egg mass; V. *Spodoptera frugiperda* egg-mass to be parasitized by flying *Telenomus remus*; VI- transparent Petri dish lid.

the PVC tube.

For the parasitism evaluation, 25 *T. remus* females, ≤ 2 days old, were placed in individual glass vials (8 cm *h* × 2 cm Ø) closed with plastic film (Rolopac®). The tubes containing the females were kept in a climate-controlled chamber. Egg-cards prepared with *S. frugiperda* eggs (<24 h old) laid on bond paper and glued on cardboard sheets (7 cm *h* × 1.7 cm *w*) were offered daily. The number of eggs was standardized by using egg-masses with an area larger than 1.5 cm^2, to make sure that more than 150 eggs were offered to *T. remus*. The egg-masses were attached to the cardboard sheets with school glue (polyvinyl acetate), applying the glue only on the tip of the paper containing the egg-masses, along with droplets of pure honey placed on the egg-cards, as a food source. On successive days, the egg-cards were removed from the test tubes, kept in the same chamber, and replaced with new cards bearing *S. frugiperda* eggs until the females died. The egg-cards containing the parasitized egg-masses were isolated for 5 days or until the eggs darkened, facilitating discrimination of parasitized and non-parasitized eggs. The daily parasitism capacity of each female and the total parasitism in each generation were calculated by counting the number of parasitized (darkened) eggs.

Longevity was evaluated by recording the number of live *T. remus* females in the glass vials daily, the female was isolated two days after the emergence begging, once the males emerge in the first day. In this way the females were considered one day old when it was isolated.

2.4. Biological parameters of T. remus isofemale line over generations

The same biological parameters studied in the regular line were evaluated in the *T. remus* isoline. These parameters were evaluated in isoline individuals of the F1, F10, and F20 generations, using the same procedures for flight capacity, parasitism, and longevity described in item 2.3.

2.5. Statistical analyses

The means of parasitism in the two populations were compared using generalized linear models (GLM) (McCullagh and Nelder, 1989; Demétrio et al., 2014). The goodness-of-fit of the models was assessed through a half-normal plot with a simulated envelope, using the half-normal plot (HNP) function (Moral et al., 2017). The means were compared with the Tukey test ($P < 0.05$), especially designed for GLM, using the glht Multcomp package function with *P* value adjustment (Hothorn et al., 2008). Longevity data were analyzed with a survival curve, in which the means and standard errors were computed with the Kaplan-Meier estimator of the corresponding survival function (Therneau, 2015). Differences and means were compared using the log-rank test ($P < 0.05$).

For the flight test, multinomial logit models (Agresti and Min, 2004) were fitted to the data, including the effect of the treatment in the linear predictor. The significance of the treatment effect was assessed using likelihood-ratio (LR) tests between nested models. To compare flying vs.

other categories between generations, quasi-binomial models were fitted and the effect of generation was assessed using F-tests for nested models. All analyses were performed using the R statistical software, version 3.6.1 (R Development Core Team, 2019).

3. Results

3.1. Biological parameters of T. remus regular line over generations

For flight capacity, the proportion of flying individuals decreased over generations under laboratory conditions (LR = 311.07, df = 4, $P < 0.01$). The proportion of flying insects was 60 % in F1, falling to 45 % in the F6 and F11 generations (Fig. 2), with significant differences among these values ($F_{2,57} = 8.91$, $P < 0.01$). The proportion of walking and non-flying individuals increased in generations F6 and F11 (Fig. 2).

The parasitism of *T. remus* in *S. frugiperda* eggs was affected over generations, decreasing in 17 and 19 generations (F = 29.27; df = 4.110; $P = 0.001$). Females from the F1, F6, and F11 generations parasitized significantly more eggs (231.2, 261.6, and 263.5 eggs, respectively) than females from the F17 and F19 generations (97.8 and 141.0 eggs, respectively, Table 1). Parasitism was higher in the first days in the last generations; nearly 60 % of the eggs were parasitized in the first two days (Table 2).

The longevity of the F1 and F6 generations was similar, 12.4 and 11.7 days respectively (Table 1). However, the longevity of the F11 generation decreased to 8.2 days, and even more in the F17 and F19 generations, showing that laboratory rearing significantly affected the wasps' biology ($X^2 = 132$; df = 4; $P < 0.001$) (Table 1).

Table 1
Mean parasitism (number of eggs parasitized) and longevity of the genetically variable population of *Telenomus remus* in *Spodoptera frugiperda* eggs over generations at 25 °C, 75 % RH, and 14 h photophase.

Generation	Parasitism[1] ± SE*				Longevity[2] ± SE*			
F1	231.2	±	12.2	a	12.4	±	0.9	a
F6	261.6	±	11.2	a	11.7	±	0.8	a
F11	263.5	±	20.2	a	8.2	±	0.7	b
F17	97.8	±	11.4	b	2.1	±	0.2	c
F19	141.0	±	16.0	b	2.0	±	0.3	c

[1] Means followed by the same letter in the same column are not significantly different (P < 0.05; Tukey[1] and Kaplan-Meier[2] test P < 0.05).

3.2. Biological parameters of T. remus isofemale line over generations

The flight capacity of the F1 generation was significantly lower than in generations F10 and F20 (LR = 100.75, df = 4, $P < 0.01$), but was higher than 80 % in all generations tested (Fig. 3). The numbers of non-flying individuals of *T. remus* decreased, 5 % at generations F10 and F20 ($F_{2,52} = 8.00$, $P < 0.01$).

Parasitism by the *T. remus* isofemale line in *S. frugiperda* eggs did not differ significantly among the three generations evaluated (F1, F10, and F20), with 128.6, 163.7, and 129.0 parasitized eggs respectively (F = 2.6, df = 2; 69, $P = 0.08$) (Table 3). There were no large variations in the parasitism percentage in the first days in these three generations

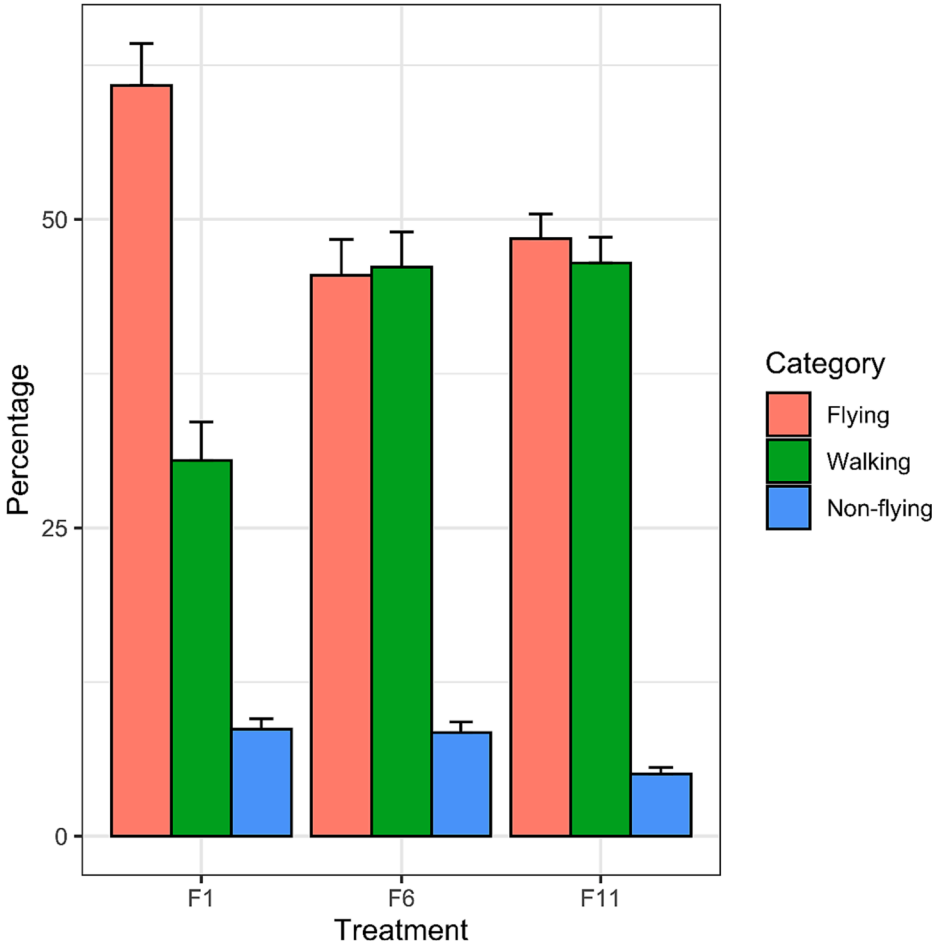

Fig. 2. Proportion of flying, walking and non-flying individual of the genetically variable *Telenomus remus* population in *Spodoptera frugiperda* eggs in F1, F6, and F11 generations at 25 °C, 75 % RH, and 14 h photophase. Different letters indicate significant differences among categories within treatments at 5 %, based on 95 % confidence intervals computed for each category probability.

Table 2
Daily parasitism percentage of the genetically variable population of *Telenomus remus* in *Spodoptera frugiperda* eggs over generations at 25 °C, 75 % RH, and 14 h photophase.

Generation	Daily parasitism (%) ± SE[1]														
	1			2			3			4			5		
F1	12.2	±	1.3	18.0	±	1.7	12.1	±	1.4	13.2	±	2.0	7.4	±	0.9
F6	10.0	±	0.9	11.1	±	1.3	13.4	±	1.3	24.5	±	2.4	8.9	±	0.9
F11	12.2	±	1.9	15.3	±	1.4	9.3	±	1.3	15.4	±	2.5	8.9	±	1.5
F17	26.8	±	4.9	16.7	±	6.2	25.5	±	4.4	5.7	±	0	25.5	±	0
F19	32.3	±	4.4	40.8	±	9.0	18.5	±	3.3	8.1	±	1.4	0.4	±	0

[1] Standard error.

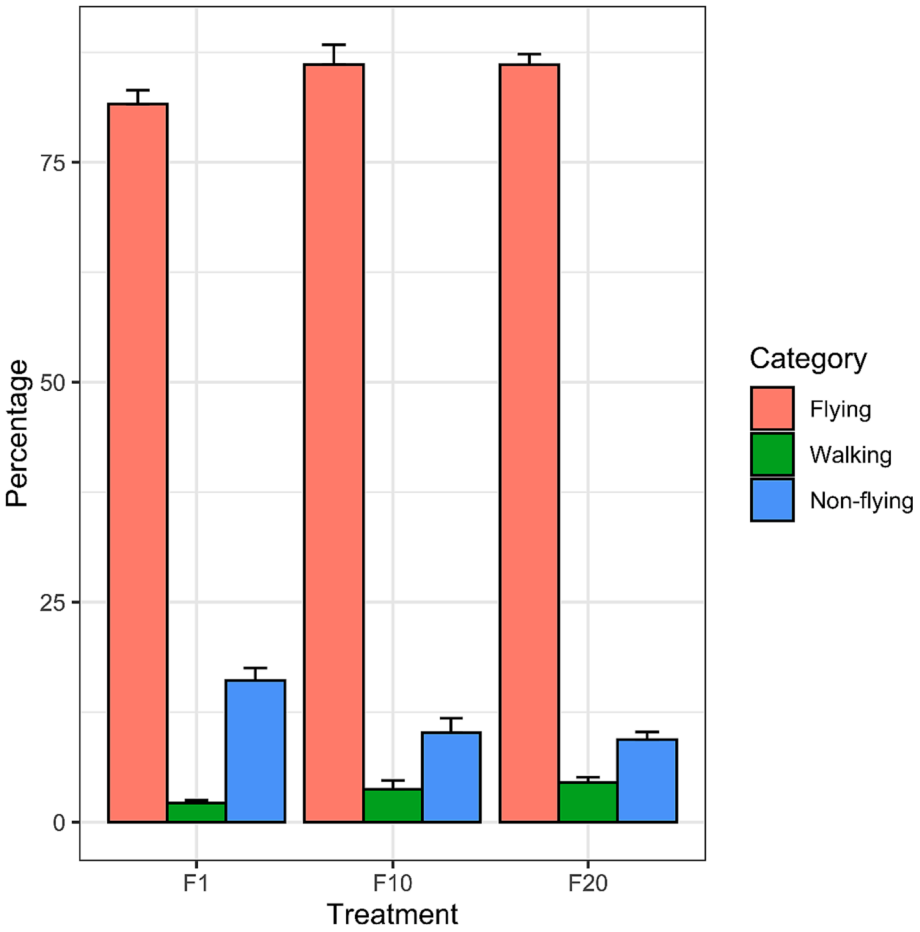

Fig. 3. Proportion of flying, walking and non-flying individual of the isofemale line of *Telenomus remus* population in *Spodoptera frugiperda* eggs in F1, F6, and F11 generations at 25 °C, 75 % RH, and 14 h photophase. Different letters indicate significant differences among categories within treatments at 5 %, based on 95 % confidence intervals computed for each category probability.

Table 3
Mean parasitism and longevity of the *Telenomus remus* isofemale line emerged from *Spodoptera frugiperda* eggs over generations at 25 °C, 75 % RH, and 14 h photophase.

Generation	Parasitism[1] ± SE*	Longevity[2] ± SE* (days)						
F1	128.6	±	15.0	a	7.3	±	0.93	a
F10	163.7	±	10.0	a	15.2	±	0.77	b
F20	129.0	±	11.0	a	8.1	±	0.66	a

[1] Means followed by the same letter in the same column are not significantly different ($P < 0.05$; Tukey[1] and Kaplan-Meier[2] test $P < 0.05$).

(Table 4).

Longevity was similar in the F1 and F20 generations, 7.3 and 8.1 days respectively, differing significantly from the F10 generation with 15.2 days ($X2 = 42.6$; $df = 2$; $P < 0.001$) (Table 3).

4. Discussion

The regular line of *T. remus* brought from the field showed reductions in flight capacity, parasitism, and longevity over generations of laboratory rearing. The loss of parasitism capacity may be related to the

Table 4
Daily parasitism percentage by *Telenomus remus* isofemale line in *Spodoptera frugiperda* eggs over generations at 25 °C, 75 % RH, and 14 h photophase.

Generation	Daily parasitism (%) ± SE[1]														
	1			2			3			4			5		
F1	36.5	±	4.1	6.0	±	1.3	12.2	±	2.8	7.3	±	2.0	11.1	±	2.6
F10	22.8	±	1.9	14.8	±	1.5	14.5	±	2.4	11.9	±	1.7	6.3	±	1.0
F20	31.0	±	2.8	21.9	±	2.5	17.2	±	2.1	18.7	±	2.1	8.4	±	1.4

[1] Standard error.

selection of individuals (haplotypes) that were better adapted to the rearing conditions. The reduction in parasitism in generations 17 and 19 may also be related to a reduction in longevity, as over the generations, a decrease in the *T. remus* parasitism period and an increase in parasitism percentage in the first days were recorded. In the F1 generation, the females reached 39 % of the total parasitism potential by day 3; on the other hand, F19 females parasitized 79 % of the eggs by day 3. Each laboratory has different conditions and methods for rearing insects, which can affect insect quality in different ways (Bartlett, 1984; 1985). Naranjo-Guevara et al. (2020) found that a *T. remus* population reared in the laboratory for approximately 600 generations showed a lower parasitism capacity than a *T. remus* population introduced from the field (<10 generations in laboratory conditions) and classified as wild.

Naranjo-Guevara et al. (2020) observed high parasitism and reduced flight capacity and fertility in a population of *T. remus* reared in the laboratory for more than 15 years (600 generations), compared to a wild-related population. The hypothesis raised here is that selection of individuals (haplotypes) more related (adapted) to laboratory conditions would lead to a decrease in performance, mainly in field conditions. This hypothesis is supported by the fact that *T. remus* has haplodiploid reproduction and is not subject to inbreeding depression (Legner, 1979; Fabritius, 1984; Sorati et al., 1996; Antolin, 1999; Luna and Hawkins, 2004; Prezotti et al., 2004; Coelho et al., 2016). The decreases in longevity and proportion of fliers, as well as high oviposition rates in the first days after emergence indicated a haplotype selection, where the adaptive cost (trade-off) is apparent. Over generations, abiotic factors such as constant temperature, relative humidity, and light, for example, could lead to the selection of certain biological characteristics of insects, increasing or decreasing their efficiency, as reported by Nunney et al. (2002) and Sørensen et al. (2012).

The *T. remus* isofemale line reared in laboratory conditions maintained its quality as a natural enemy during the 20 generations evaluated (280 days), with little change in biological characteristics over the generations. The flight capacity was lower in the F1 generation and increased in the F10 and F20 generations, but the percentage of flying insects remained higher than 80 %. The isofemale line is selected by consecutive consanguineous mating until the genetic variability of a population is strongly reduced, obtaining a parasitoid population with little or no influence from haplotype selection (Coelho et al., 2016). After inbreeding for nine generations, an isofemale line of *T. remus* should have at least an 86 % inbreeding coefficient and 99 % identical nuclear DNA (Li, 1955). The high flight capacity and parasitism shown by the *T. remus* isofemale line, in addition to the lack of major changes over generations, are quality indicators for natural enemies (van Lenteren, 2003). Longevity differed among the generations, being lower in the F1 and F20 generations than in F10. We believe that the fluctuation in the longevity of this *T. remus* isofemale line may be related to atmospheric pressure (Marchand and McNeil, 2000; Fournier et al., 2005). The annual rainy season causes variations in atmospheric pressure. Generations F1 and F20 were evaluated in rainy periods (summer), while generation F10 was evaluated in the dry period (winter) in Piracicaba, with 140 days (10 generations) between each assessment (Kottek et al., 2006).

The data presented here indicate that the *T. remus* isofemale line is more suitable for biological-control programs than a regular line. The *T. remus* isofemale line was selected from a population collected in Brazil in 2019, while the regular line was selected from a population recollected from the fields for the experiments, having as matrix a line brought from Venezuela and maintained under laboratory conditions since 1996 and 2010 (Naranjo-Guevara et al., 2020; Wengrat et al., 2021). It is possible that the population collected in Brazil in 2019 had superior natural biological attributes to the Venezuelan population, but, considering that the Brazilian strain has been maintained in the laboratory for less than a year, we assume that this strain has undergone little haplotype selection, and instead shows higher *in-nato* performance (Wengrat et al., 2021). This assumption is based on the finding by Naranjo-Guevara et al. (2020) that a *T. remus* population introduced into Brazil from Venezuela in 2010 showed higher fertility and flight capacity than a population also introduced from Venezuela in 1996. Both of the lines used by Naranjo-Guevara et al. (2020) were used in the present study as the regular line. Also, it should be considered that the Brazilian *T. remus* line showed little genetic variability, and is related to introductions of specimens from Venezuela and the Dominican Republic, which originated in Papua-New Guinea (Cock, 1985; Wengrat et al., 2021).

Colmenarez et al. (2022) compiled information regarding *T. remus* field experiments, aiming toward *S. frugiperda* control, and found diverging information. Varella et al. (2015) conducted a single release of 200,000 parasitoids per hectare and reported parasitism of 1.4 to 9 %; while Hernandez et al. (1989) released 5,000 individuals per hectare over three weeks and reported 60 to 100 % parasitism of *S. frugiperda* eggs. This divergence in results could be related to a lack of quality, mainly flight ability, of *T. remus* individuals reared under laboratory conditions. We note that the population used by Varella et al. (2015) was a *T. remus* regular line, introduced into Brazil from Venezuela in 1996.

Quality control of natural enemies is essential for the success of biological-control programs (Parra and Coelho, 2022). Laboratory rearing of *T. remus* over successive generations appears to change a population's flight ability, longevity, and parasitism in relation to wild-related populations. In the context of use of isolines, Nunney (2003) recommended that in an ideal situation, several isolines should be used in order to maintain the variation (genotypes) of the population. However, this is impractical in mass-rearing facilities; and in the present study, *T. remus* collected in Brazil showed only 6 haplotypes (Wengrat et al., 2021). As an alternative, individuals with specific traits, such as good flight capacity and parasitism, can be selected before starting the isoline (Nunney, 2003). This pre-selection should ensure that the traits selected for the isoline are suitable for an augmentative biological-control program. The findings of the present study suggest that use of an isofemale line can maintain the quality of *T. remus* as a biological-control agent for *S. frugiperda* over generations.

Declaration of Competing Interest

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.

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