




Trichogramma pretiosum in field release and under different temperatures in laboratory

Trichogramma pretiosum em liberação no campo e sob diferentes temperaturas em laboratório

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ABSTRACT: This study aimed to evaluate a strain of *Trichogramma pretiosum* native to the central region of the state Rio Grande do Sul, Brazil, under different temperatures in laboratory and the parasitism of *Helicoverpa zea* after releases in corn field. *Trichogramma pretiosum* females were kept at 18, 25 and 30 °C and had their parasitism potential evaluated. Nine plots (400 m²) of maize were installed in randomized blocks under the treatments: 1) staggered release of parasitoids in three different occasions, at three-day intervals; 2) a single release of parasitoids; 3) no release of parasitoids. Ears were collected every four days and the number of caterpillars and eggs of *H. zea* in the maize silk and the number of damaged ears were estimated. Evaluation started after the tassels growth and ended with the drying of the styles-stigmas. To estimate the grain mass and the ear number and mass, two lines in each plot were delimited and reserved at the end of the cultivation cycle. In laboratory, the highest parasitism rate occurred at 25 °C (35.54%) and the lowest longevity at 30 °C (6.8 days). A total of 1,063 *H. zea* eggs were collected in the field, 69.52% were parasitized. The mean parasitism rate was higher in T1 (staggered release, 82.77%) than in control (51.87%), but there was no difference in grain yield. Based on the parasitism capacity of the studied strain, it can be potentially used in programs of biological control of *H. zea* in corn crops of Rio Grande do Sul.

KEYWORDS: *Helicoverpa zea*; strain; corn; parasitism.

RESUMO: O estudo objetivou avaliar uma linhagem de *Trichogramma pretiosum* nativa da região Central do Rio Grande do Sul (Brasil) sob diferentes temperaturas em laboratório, e no parasitismo de *Helicoverpa zea* após liberações em área de milho. Fêmeas de *T. pretiosum* foram mantidas a 18, 25 e 30 °C e avaliadas quanto ao potencial de parasitismo. No campo, foram instaladas nove parcelas (400 m²) de milho híbrido em blocos ao acaso, com os seguintes tratamentos: 1) liberação de parasitoides fracionada em três vezes, em intervalos de três dias; 2) liberação em apenas uma ocasião; 3) sem liberação. A cada quatro dias foram coletadas espigas e registrados os danos e o número de lagartas e ovos de *H. zea* dos estilos-estigmas. A avaliação iniciou durante o pendramento e estendeu-se até a fase de secagem dos estilos-estigma. Duas linhas em cada parcela foram reservadas para avaliação do número e da massa de espigas e grãos. Em laboratório, o maior percentual de parasitismo foi aos 25 °C (35,54%) e a menor longevidade aos 30 °C (6,8 dias). No campo, foram coletados 1.063 ovos de *H. zea*, dos quais 69,52% estavam parasitados. O percentual médio de parasitismo foi maior com liberação escalonada de *T. pretiosum* (82,77%), comparativamente ao controle (51,87%), porém, não houve diferença na produção de grãos. Tendo em vista a capacidade de parasitismo da linhagem avaliada, sugere-se que ela possui potencial para ser utilizada em programas de controle biológico da lagarta da espiga no cultivo de milho no Rio Grande do Sul.

PALAVRAS-CHAVE: *Helicoverpa zea*; linhagem; milho; parasitismo.

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INTRODUCTION

Parasitoids of the genus *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) are an important group of natural enemies and comprise the most numerous genus within the Trichogrammatidae family. *Trichogramma pretiosum* Ryley has a generalist habit, wide geographic distribution, and was recorded in over 240 hosts, being commonly found in lepidopteran eggs (SOUZA et al., 2016; ZUCCHI et al., 2010).

The natural parasitism of *Helicoverpa zea* (Boddie) eggs (earworm) (Lepidoptera: Noctuidae) by *T. pretiosum* in corn crops often reaches 50%, but can occasionally reach over 90% of eggs (SÁ; PARRA, 1994). According to FORESTI et al. (2012), the parasitism rate can be increased with releases of the agent in the field.

The use of commercial strains of *T. pretiosum* for caterpillar control is a widespread technique among farmers since, compared with chemical insecticides, it is an efficient and low-price method that aims to restrict pesticide use (PARRA, 2010; FIGUEIREDO et al., 2015). However, the lack of information regarding the field release of these strains often results in unsuccessful outcomes and the technique is frequently disbelieved. It is crucial to establish the most appropriate release interval depending on the locality and infestation degree (SÁ; PARRA, 1993).

Although there are evidences of the association between parasitoids and their hosts and the generalist habit of *T. pretiosum*, the correct use of specific strains is crucial for the success of control programs. Parasitoids have widely variation in their search behavior, host preference, and responses to environmental conditions (HASSAN, 1997) and the correct parasitoid choice improves the chances of success. Several abiotic factors can affect the strain performance and parasitoids of the genus *Trichogramma* can have their parasitism performance altered at certain thermal limits (PRATISSOLI et al., 2004; BUENO et al., 2010). Variations in microclimate conditions between different regions can also affect the reproductive capacity of different strains (PRATISSOLI et al., 2007).

The state of Rio Grande do Sul (RS) has high thermal amplitude along the corn crop year. The sowing occurs in winter (August) and the second season in summer (January) when temperatures can reach up to 30 °C (RODRIGUES; SILVA, 2011). Considering that different insect strains can have different reproductive capacity and longevity at different temperatures (PRATISSOLI et al., 2006), the investigation of strains from specific climatic regions can provide crucial information for the augmentative biological control.

This study aims to evaluate the reproductive capacity of a *Trichogramma pretiosum* strain from the Central region of RS (Brazil) under different temperatures in laboratory conditions. The reproductive capacity was also evaluated during parasitism of *Helicoverpa zea* after single and staggered releases in experimental corn fields.

MATERIAL AND METHODS

The *T. pretiosum* strain was obtained from *H. zea* eggs collected in Santa Maria (RS) in a previous second-season corn crop. All parasitoids were identified at the specific level following the QUERINO; ZUCCHI (2011) methodology. Since all individuals were collected from the same site, they were all considered a single strain. The parasitoids were kept under controlled conditions (25 °C and 12 h of light) and multiplied on eggs of the alternative host *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) following the methodology proposed by PARRA (1997).

Laboratory

The bioassays were conducted with *T. pretiosum* females under three different temperatures (18, 25, and 30 °C) and parasitism rates throughout the females' lifetime (25 °C). Twenty 24h-old females (at each temperature) were isolated in glass test tubes (0.8 × 15 cm). Every two days females received one card (1 cm²) with unviable eggs from the alternative host. After the exposure period, the cards were isolated in plastic microtubes and maintained at the respective temperatures. The total number of parasitized eggs and their viability rate, females' longevity, and offspring sex ratio (RS = number of females/number of females + number of males) were estimated for each treatment. The mean values of each variable were analyzed with an ANOVA and results between treatments were compared with a Tukey's test with 5% significance. The mean number of parasitized eggs per day of life of females at 25 °C was also compared with a Tukey's test. All analyses were performed in BioEstat 5.0 (AYRES et al., 2007).

Field

The field work was conducted in the Forestry Research Secretaria da Agricultura, Pecuária e Desenvolvimento Rural, in Santa Maria (29° 41' 24"S; 53° 48' 42"O), in the Central Depression region of RS. On January 22, 2015 the experimental plots (nine 400 m² areas) were sown with maize from a nontransgenic hybrid cultivar (Fepagro RS 22) using a 0.45 m distance between lines and 0.66 m between plants. Plots were separated from each other by at least 20 m of a same crop. Previous soil analyses were conducted to perform the soil pH correction and fertilization. Except for the use of Roundup applied prior sowing, planting was carried out without pesticides throughout the entire cycle.

Three treatments were established: 1) Staggered release of parasitoids in three occasions, at three-day intervals; 2) a single release of parasitoids; 3) no release of parasitoids (control). Each treatment was conducted in three plots distributed in random blocks.

Releases of T1 and T2 were performed at the central point of each plot. Ripe eggs of *A. kuehniella* were glued with Arabic gum on a blue card (10 cm²) fixed on a wooden rod at the center of each plot. The cards were placed in the field on March 24th, 2015, after 50% of the plants had styles-stigmas emission. This procedure was repeated three and six days after the first release in T1. The total number of insects released in each plot of T1 and T2 was proportionally equivalent to 200,000 per hectare.

Nearly 65 corn ears were collected every four days. The number of caterpillars and eggs on the styles-stigmas of each corn ear, as well as damaged corn ears, was estimated. The eggs were removed from the styles-stigmas, packed in glass tubes sealed with plastic film, kept in controlled conditions (25 °C, 12 h of light), and monitored daily for the presence of parasitism. The sampling period started immediately after the tassels growth and ended with the complete drying of the styles-stigmas, totalizing 13 sampling occasions.

To estimate the grain mass and the ear number and mass, two lines in each plot were delimited and reserved at the end of the cultivation cycle.

The mean parasitism rate, the mean number of eggs, caterpillars, damaged ears, produced ears, and the mass of ears and grains were compared between treatments with a Kruskal–Wallis test with 5% significance level. All statistical analyzes were performed in BioEstat 5.0 (AYRES et al., 2007).

RESULTS

Laboratory

The mean number of eggs parasitized by females of *T. pretiosum* kept at 25 °C was higher than those submitted to temperatures of 18 and 30 °C (F = 4.96; *p* < 0.05). The mean longevity was similar in insects exposed to 18 and 25 °C and lower in those kept at 30 °C (F = 11.34; *p* < 0.01). The sex ratio and eggs viability were similar in all temperatures (Table 1).

The parasitism rate was higher in 3-day-old (23.59 ± 2.9) and 5-day-old (17.28 ± 2.35) females kept at 25 °C. Females older than five days had lower parasitism rates (F = 5.81; *p* < 0.05).

Table 1. Average number (± standard error) of parasitized eggs and females' longevity, percentage of egg viability and offspring sex ratio of *Trichogramma pretiosum* under different temperatures.

| Temperature (°C) | N° of parasitized eggs | Longevity (days) | Eggs viability (%) | Sex ratio (%) | |
|------------------|------------------------|------------------|---------------------|---------------|-------|
| | | | | ♀ | ♂ |
| 18 | 35.54 ± 2.45 b* | 12.82 ± 0.72 a | 96.00 ^{ns} | 53.01 | 47.88 |
| 25 | 50.10 ± 5.43 a | 12.96 ± 1.16 a | 97.75 | 46.47 | 53.52 |
| 30 | 32.05 ± 4.82 b | 6.8 ± 0.71 b | 97.00 | 47.81 | 51.92 |

*Means followed by different letters in the same column are significantly different between treatments (Tukey's test *p* < 0.05). ^{ns}There wasn't statistical difference between the mean values in the columns without letters.

Field

A total of 1,063 eggs of *H. zea* were collected, 739 (69.52%) were parasitized. The highest number of collected and parasitized eggs was obtained in the first sampling (Fig. 1).

The parasitism rate in T1 (staggered release) was higher than in T3 (control) (H = 6.48; *p* = 0.039) (Table 2).

The mean number of eggs (H = 5.74; *p* = 0.057), caterpillars (H = 1.41; *p* = 0.49), damaged ears (H = 0.42; *p* = 0.80), and ears produced (H = 4.50; *p* = 0.10), and the mass of produced ears (H = 3.59, *p* = 0.30) and grains (H = 0.80; *p* = 0.67) (Table 2) were similar in all treatments.

DISCUSSION

The evaluated strain demonstrated low parasitism rates in extreme temperatures. This is a behavior different from what has been recorded for strains from southeast region of Brazil, where the parasitism rate is kept stable in temperatures up to 28 °C (OLIVEIRA et al., 2017) and 30 °C (PRATISSOLI et al., 2004). However, the finding corroborates the results of PRATISSOLI et al. (2007) that stated that strains from high-temperatures regions had an altered development after being kept at 20 °C. The authors also found that strains from low-temperature regions had low development rates when subjected to a temperature of 30 °C. Since corn seeding in RS occurs twice a year, in August (low temperatures) and January (high temperatures), comprising a wide temperature range,

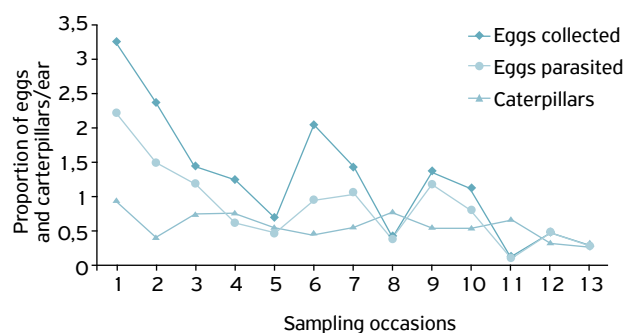


Figure 1. Proportion of eggs of *Helicoverpa zea*, parasitized eggs, and caterpillars collected per ear in different sampling occasions.

Table 2. Mean parasitism rate (\pm standard error), number of *Helicoverpa zea* eggs and caterpillars, damaged ears, ears produced, and ears (g) and grains (g) mass in different treatments of experimental corn fields in Santa Maria, RS, Brazil.

| | T1* | T2 | T3 |
|----------------------|---------------------------------|---------------------|--------------------|
| Parasitism (%) | 82.77 \pm 2.46 a** | 71.71 \pm 6.04 ab | 51.87 \pm 2.22 b |
| Eggs number | 116.00 \pm 8.97 ^{ns} | 136.33 \pm 8.82 | 102.00 \pm 3.00 |
| Caterpillar number | 50.66 \pm 5.46 | 55.66 \pm 11.57 | 58.33 \pm 2.02 |
| Ear damages number | 26.66 \pm 5.61 | 30.00 \pm 4.73 | 31.33 \pm 3.18 |
| Ears produced number | 50 \pm 6.60 | 48 \pm 8.19 | 32.33 \pm 6.53 |
| Mean ear mass (g) | 101.68 \pm 24.15 | 96.34 \pm 5.39 | 94.88 \pm 7.91 |
| Mean grain mass (g) | 74.73 \pm 17.79 | 71.20 \pm 3.61 | 65.83 \pm 10.28 |

*T1 = Staggered parasitoid release in three different occasions, in three-day intervals; T2 = a single release of parasitoids; T3 = No release of parasitoids. **Means followed by different letters in the same line are significantly different between treatments (Kruskal-Wallis test $p < 0.05$).

^{ns}There wasn't statistical difference between the mean values in the lines without letters.

it is suggested that the studied *T. pretiosum* strain should be released in months with mild temperatures.

The lower longevity of females at elevated temperatures corroborates the study by PRATISSOLI et al. (2006), in which the mean longevity of *T. pretiosum* on *T. absoluta* eggs varied from 1.1 to 11.3 days at temperatures of 30 and 15 °C, respectively. According to the authors, these differences in longevity are related to a physiological response in which the metabolic activity is reduced in low temperatures. The sex ratio was similar in all experimental temperatures, corroborating previous experiments with the same species at temperatures ranging from 18 to 33 °C (OLIVEIRA et al., 2017).

When monitoring the number of parasitized eggs throughout the females' lifetime, the highest reproductive capacity occurred in the first three days, suggesting that insects released in crops would have to be replaced after this period. This feature was also recorded to five other strains of *T. pretiosum* kept at 25 °C which reached over 80% of parasitism by the fourth day of life (PRATISSOLI et al., 2006).

The highest number of eggs of *H. zea* was collected at the beginning of the sampling period when approximately 50% of plants had styles-stigmas. In the first two sampling occasions, near 70% of the collected ears had eggs on them. FORESTI et al. (2013) detected that, when 50% of the crop had styles-stigmas, only 15% of those had eggs infestation in the northwest region of RS. Although the infestation rates detected in this study and in the study by FORESTI et al. (2013) were different, there was a similarity related to the gradual reduction in the number of eggs throughout samplings. This corroborates the authors' statement that senescent styles-stigmas become unattractive to *H. zea* oviposition.

Although there were peaks in the eggs rate per ear over the sampling period, the number of caterpillars remained constant. This may be related to the high mortality rates during the first development phase of insects (TOWNSEND et al., 2010). SÁ; PARRA (1993) recorded a predation rate of over 70% of eggs of *H. zea* in corn crops and predators were mainly comprised by coccinellids, crysopids, and forficulids.

The staggered release of parasitoids provided an increase in the parasitism rate on *H. zea* eggs when compared with control plots. The results showed that, during the first days of females' lifetime, the parasitism efficiency is higher. So, a good strategy would be releasing young and reproductive females in the field in three occasions, at three-day intervals. The two subsequent releases would add parasitoids in the field until those from the first release complete their biological cycle which in mild temperatures is approximately 10 days (PARRA, 1997).

The similar number of *H. zea* eggs between treatments was an expected outcome since the homogeneous oviposition behavior has been already recorded for the species. Studies performed in crops with plants of different varieties, such as landrace, conventional and transgenic hybrids, demonstrated the occurrence of homogeneous oviposition (LUONG et al., 2016; VARGAS et al., 2017).

Although there was a higher parasitism rate in plots with staggered release, there were no differences in the rate of damaged ears between treatments. The expected result of reducing ear damage by adding parasitoids into the crops may not directly occur and depends on several factors such as the density of host eggs in relation to the rate of insects released (PARON et al., 1998). SÁ; PARRA (1993) recorded a 26% reduction in the damage of *H. zea* after three releases of 100,000 insects per hectare in one experimental area and a 16% reduction after four releases in other areas. According to the authors, comparative assessments should be conducted in neighboring areas, changing the number of insects and the release time, to complement the number of releases.

The similar ear damage rate between treatments may have impacted the ears and grains mass. In addition, the homogeneous injury caused by fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), in the experimental plots in the beginning of the vegetative stage may also have compromised the corn production. According to FIGUEIREDO et al. (2006), severe injuries of young plants caused by fall armyworm can result in losses of over 50% of corn crops.

The *T. pretiosum* strain from the Central region of RS can be potentially used to control *H. zea* infestations in corn crops due to its high parasitism capacity in field, especially in staggered releases. However, the number of released insects, infestation level monitoring, and the best release period should be better investigated to enable the use of the strain in biological control of corn earworm in crops of south Brazil.

CONCLUSIONS

The staggered release of *T. Pretiosum* strain in three occasions, at three-day intervals, increases the parasitism rate on *H. zea* eggs in corn crops.

The *T. pretiosum* strain from the central region of the state Rio Grande do Sul has higher reproductive potential at 25 °C.

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