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Research Article

Comparative field performance of pupal and adult releases of *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) for control of *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae) in maize

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Abstract. The fall armyworm, *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae), is an important pest of maize, particularly during the early vegetative stages of the crop. The egg parasitoid *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) is used for its biological control, but information comparing different field release forms remains limited. This study compared pupal and adult releases of *T. pretiosum* at two release rates for suppression of *S. frugiperda* damage in second-crop maize under field conditions. The experiment was conducted in Ribeirão Preto, São Paulo State, Brazil, in a randomized block design with five treatments and six replicates. Treatments consisted of three consecutive weekly releases of 100,000 or 200,000 pupae or adults ha^{-1} and an untreated control. Leaf damage was assessed weekly on ten plants per plot using a 0–9 visual scale. The overall treatment effect was significant (P = 0.00071), and all *T. pretiosum* treatments had significantly lower overall damage than the untreated control. Mean damage scores were 5.30 in the control and 3.79, 3.55, 3.85, and 3.98 for adult releases at 100,000 and 200,000 ha^{-1} and pupal releases at 100,000 and 200,000 ha^{-1}, respectively, corresponding to reductions of 25.0–33.1% relative to the control. No overall significant differences were detected between pupal and adult release forms or between the two release rates. These results demonstrate that inundative releases of *T. pretiosum* can reduce *S. frugiperda* injury in maize, while indicating that, under the conditions of this study, pupal and adult releases provided comparable levels of damage suppression.

Keywords: biological control, parasitoid release technology, egg parasitoids, inundative release, maize pest management.

Introduction

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae), is one of the major pests of maize and remains an important challenge for integrated pest management. Biological control has an increasingly important role in its management, and egg parasitoids of the genus *Trichogramma* Westwood, 1833 are among the natural enemies considered for augmentative release against this pest (Bueno et al. 2023; Navik et al. 2023; Kenis et al. 2024; Wyckhuys et al. 2024).

Augmentative releases of *Trichogramma* can be performed using adults or parasitized host eggs containing immature parasitoids. Previous field research with *Trichogramma galloi* Zucchi, 1988 (Hymenoptera: Trichogrammatidae) in sugarcane showed that release technique can interact with environmental conditions. Protected pupal releases provided greater parasitism than adult releases under adverse weather conditions, whereas differences among release techniques were not evident when environmental conditions were favorable (Pinto et al. 2003). These findings suggest that releasing *Trichogramma* before adult emergence may provide advantages under some field conditions. However, whether pupal releases of *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) improve the suppression of *S. frugiperda* damage in maize compared with adult releases remains unclear.

Therefore, this study compared the field performance of pupal and adult releases of *T. pretiosum* at two release rates for suppression of *S. frugiperda* damage in second-crop maize. Based on previous evidence from another *Trichogramma*–host system, we hypothesized that pupal releases would provide more consistent suppression of *S. frugiperda* damage over time than adult releases under field conditions.

Material and Methods

The field experiment was conducted during the second-crop maize season in Ribeirão Preto, São Paulo State, Brazil. Maize was sown in early March using a commercial hybrid under standard agronomic practices, without insecticide applications throughout the experimental period. The experimental design was a randomized block with five treatments and six replicates. Each plot measured 20 × 20 m and was surrounded by buffer areas to minimize parasitoid movement between treatments.

Trichogramma pretiosum used in the experiment was obtained from BUG Agentes Biológicos (Piracicaba, São Paulo State, Brazil). Parasitoids belonged to the RV strain and were reared on eggs of the factitious host *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae). Parasitoid quality was assessed from ten samples containing approximately 80 parasitized host eggs each, maintained under ambient conditions, and adult emergence averaged 87.3%.

Treatments consisted of inundative releases of *T. pretiosum* as either pupae or adults at rates of 100,000 and 200,000 individuals ha^{-1} and an untreated control. Three consecutive weekly releases were performed on 16, 23, and 30 March 2016, shortly after plant emergence. Adults were 4–16 h old at the time of release, provided with pure honey droplets, and released early in the morning at the center of each plot. For pupal releases, parasitized *E. kuehniella* eggs containing *T. pretiosum* pupae expected to emerge as adults approximately one day later were released directly onto the soil without protection.

Meteorological data for the release period were obtained from the INMET automatic meteorological station in Pradópolis, São Paulo State

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(station A747), the nearest station with available records. From 16 to 30 March 2016, cumulative rainfall was 1.0 mm, mean air temperature was 24.4 °C, and mean relative humidity was 74.8%. Rainfall was negligible during the release period.

Damage caused by *S. frugiperda* larvae was evaluated weekly by visually assessing ten plants randomly selected within each plot at each evaluation date using the standardized 0–9 visual damage scale proposed by Davis et al. (1992). A new random sample of ten plants was selected at each evaluation date; thus, individual plants were not followed repeatedly over time. No pre-release damage assessment was performed because maize plants were still at an early developmental stage at the time of the first parasitoid release.

Statistical analysis. Leaf-damage scores were first averaged across the ten plants sampled within each plot at each evaluation date, with the plot considered the experimental unit. Data were analyzed using linear mixed-effects models in R version 4.6.1 (R Core Team 2026). In the first analysis, treatment, evaluation date, and their interaction were included as fixed effects, block was included as a fixed effect, and plot was included as a random effect to account for repeated evaluations of the same experimental units over time. When significant effects were detected, estimated marginal means were compared using multiplicity-adjusted pairwise comparisons.

A complementary factorial analysis was performed using only the four *T. pretiosum* release treatments to specifically test the effects of release form (adult or pupa), release rate (100,000 or 200,000 parasitoids ha^{-1}), evaluation date, and their interactions. Block was included as a fixed effect and plot as a random effect. Degrees of freedom and *P*-values for fixed effects were obtained using Satterthwaite's approximation. Following a significant release form × evaluation date interaction, adult and pupal releases were compared within each evaluation date using estimated marginal means, averaging over release rates, with *P*-values adjusted by the Holm method. Model assumptions were assessed by graphical inspection of residuals and normal Q–Q plots and by the Shapiro–Wilk test. No evidence of departure from residual normality was detected (W = 0.99, P = 0.70), and no singular fit was detected. Analyses were performed using the lme4, lmerTest, and emmeans packages. Statistical significance was established at $\alpha = 0.05$.

Results and Discussion

All release treatments of *T. pretiosum* significantly reduced leaf damage caused by *S. frugiperda* compared with the untreated control over the experimental period (Tab. 1). Damage was significantly affected by treatment ($F4,20 = 7.54$, $P = 0.00071$), evaluation date ($F6,150 = 11.37$, $P < 0.0001$), and the treatment × evaluation date interaction ($F24,150 = 1.94$, $P = 0.00884$). Across the evaluation period, mean damage scores were 5.30 in the untreated control and 3.79, 3.55, 3.85, and 3.98 following releases of 100,000 adults, 200,000 adults, 100,000 pupae, and 200,000 pupae ha^{-1}, respectively, corresponding to overall reductions of 25.0–33.1% relative to the control (Tab. 1).

When the four parasitoid-release treatments were analyzed factorially, neither release form (adult vs. pupa; $F1,15 = 0.98$, $P = 0.337$) nor release rate (100,000 vs. 200,000 parasitoids ha^{-1}; $F1,15 = 0.05$, $P = 0.823$) significantly affected damage overall. The release form × rate interaction was also not significant ($F1,15 = 0.54$, $P = 0.472$). However, the release form × evaluation date interaction was significant ($F6,120 = 2.46$, $P = 0.028$), whereas the rate × evaluation date ($P = 0.600$) and release form × rate × evaluation date ($P = 0.521$) interactions were not significant.

At the first evaluation on 26 March, mean damage was 1.82 scale units lower in the adult-release treatments than in the pupal-release treatments when release rates were pooled ($P = 0.00025$). However, comparisons among the five individual treatments showed that the untreated control did not differ significantly from either adult or pupal release treatments at this first evaluation (Fig. 1). No differences between release forms were detected at any subsequent evaluation when release rates were pooled ($P \geq 0.782$). Because no pretreatment damage assessment was available, the difference observed at the first evaluation should be interpreted cautiously and cannot be unequivocally attributed to release form.

The temporal pattern of treatment effects also varied among evaluation dates (Fig. 1). On 2 and 16 April, all four parasitoid-release treatments had significantly lower damage than the untreated control. On 9 April, damage was significantly lower than the control following releases of 200,000 adults and 100,000 pupae ha^{-1}, whereas the other two release treatments were intermediate and did not differ from either group. From 23 April onward, no significant differences among treatments were detected within individual evaluation dates.

Although *T. pretiosum* releases significantly reduced foliar injury over the experimental period, damage suppression was moderate, with overall reductions of approximately 25–33% relative to the untreated control. This level of suppression may partly reflect characteristics of *S. frugiperda* egg masses, which are frequently deposited in multiple layers and covered with scales, potentially restricting parasitoid access to eggs within the mass. Such characteristics are recognized as important constraints on the effectiveness of egg parasitoids against fall armyworm (Bueno et al. 2023; Navik et al. 2023).

The absence of an overall advantage of pupal over adult releases contrasts with the initial hypothesis but is consistent with important aspects of previous field observations with *Trichogramma*. Pinto et al. (2003), working with *T. galloi* in sugarcane, found that protected pupal releases performed better under adverse environmental conditions, whereas differences among release techniques were not evident under favorable conditions. In the present study, pupae were released directly onto the soil without protection, and weather conditions during the release period were relatively mild and dry, with only 1.0 mm of cumulative rainfall from 16 to 30 March, a mean temperature of 24.4 °C, and mean relative humidity of 74.8%. Thus, the absence of a consistent advantage of pupal releases under the relatively mild and dry conditions observed in this study does not necessarily conflict with the earlier findings.

From an operational perspective, the comparable biological performance of adult and pupal releases may be relevant for implementation of augmentative biological control. Rather than indicating a biologically superior release form, the present results suggest that the choice between adults and pupae may depend on practical considerations such as production, transport, storage, release technology, labor requirements, and exposure to environmental conditions. These operational aspects were not evaluated in the present study and should therefore be addressed in future field comparisons.

Likewise, the absence of a significant rate effect indicates that

Table 1. Overall mean leaf damage scores (± SE) caused by *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae) on maize plants following adult or pupal releases of *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) under field conditions. Damage was assessed using a visual scale ranging from 0 (no damage) to 9 (severe damage).

Treatment	Rate (individuals ha^{-1})	Mean damage score (overall ± SE)
Control	-	5.30 ± 0.34 a
Adult	100,000	3.79 ± 0.18 b
Adult	200,000	3.55 ± 0.22 b
Pupa	100,000	3.85 ± 0.28 b
Pupa	200,000	3.98 ± 0.44 b

Note: Means followed by the same letter do not differ significantly based on Tukey-adjusted pairwise comparisons of estimated marginal means obtained from the linear mixed-effects model ($P \leq 0.05$).

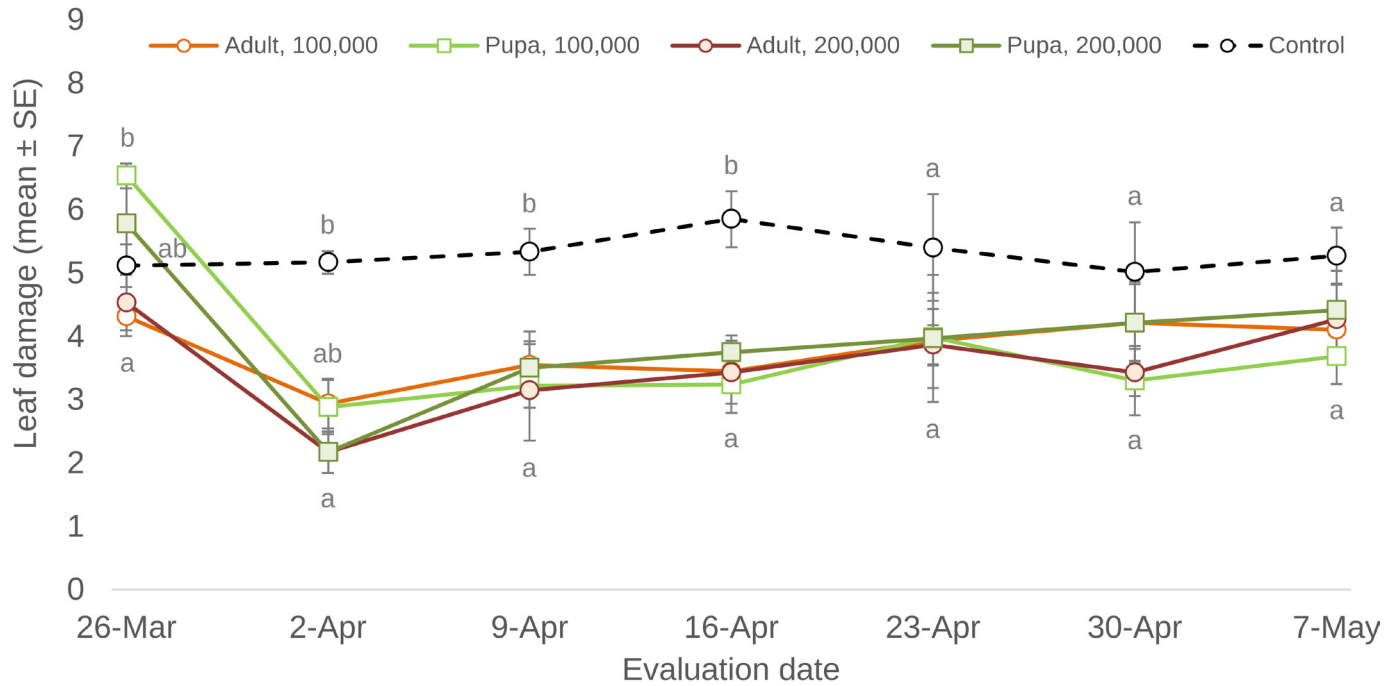


Figure 1. Mean leaf damage scores (± SE) caused by *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae) on maize plants over time following adult or pupal releases of *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae). Evaluations were performed weekly using a visual damage scale ranging from 0 (no damage) to 9 (severe damage). Within each evaluation date, means followed by the same letter do not differ significantly based on Tukey-adjusted pairwise comparisons of estimated marginal means obtained from the linear mixed-effects model ($P \leq 0.05$).

increasing the release rate from 100,000 to 200,000 parasitoids ha^{-1} did not improve damage suppression under the conditions of this experiment. This finding warrants further investigation because optimization of release rates is important for the economic feasibility of augmentative biological control programs.

Conclusion

Inundative releases of *T. pretiosum* reduced foliar damage caused by *S. frugiperda* in maize, with overall damage reductions of 25.0–33.1% relative to the untreated control. Contrary to the initial hypothesis, pupal releases did not provide greater overall damage suppression than adult releases, and increasing the release rate from 100,000 to 200,000 parasitoids ha^{-1} did not significantly improve damage suppression. Under the conditions of this study, adult and pupal releases therefore provided comparable biological performance. Further studies across environments and cropping seasons, including direct measurements of egg parasitism and operational costs, are needed to determine whether particular release forms or rates offer advantages under specific field conditions.

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Authors' Contributions

ASP: Conceptualization, Methodology, Formal analysis, Supervision, Validation, Writing – original draft, Writing – review & editing; MGL: Investigation, Methodology, Data curation, Validation, Writing – review & editing.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethical Approval

This study did not involve humans or vertebrate animals and therefore did not require ethical approval.

References

Bueno, A. F.; Sutil, W. P.; Maciel, R. M. A.; Roswadoski, L.; Colmenarez, Y. C.; Colombo, F. C. (2023) Challenges and opportunities of using egg parasitoids in FAW augmentative biological control in Brazil. *Biological Control*, 186: 105344. doi: 10.1016/j.biocontrol.2023.105344

Davis, F. M.; Ng, S. S.; Williams, W. P. (1992) Visual rating scales for screening whorl-stage corn for resistance to fall armyworm. *Mississippi Agricultural and Forestry Experiment Station Technical Bulletin*, 186: 1-9.

Kenis, M.; Zhong, Y.; Fontes, J.; Kenis, J.; Herz, A.; Babendreier, D. (2024) Pre-emptive augmentative biological control of *Spodoptera frugiperda* in Europe using *Trichogramma* spp. *CABI Agriculture and Bioscience*, 5(96): 1-8. doi: 10.1186/s43170-024-00296-1

Navik, O.; Yele, Y.; Kedar, S. C.; Sushil, S. N. (2023) Biological control of fall armyworm *Spodoptera frugiperda* (JE Smith) using egg parasitoids, *Trichogramma* species (Hymenoptera: Trichogrammatidae): a review. *Egyptian Journal of Biological Pest Control*, 33(1): 118. doi: 10.1186/s41938-023-00759-z

Pinto, A. S.; Parra, J. R. P.; Oliveira, H. N.; Arrigoni, E. B. (2003) Comparação de técnicas de liberação de *Trichogramma galloi* Zucchi (Hymenoptera: Trichogrammatidae) para o controle

de *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae). *Neotropical Entomology*, 32(2): 311-318. doi: 10.1590/S1519-566X2003000200018

R Core Team (2026). R: A language and environment for statistical computing. R Foundation for Statistical Computing. https://www.R-project.org

Wyckhuys, K. A. G.; Akutse, K. S.; Amalin, D. M.; Araj, S. D.; Barrera, G.; Beltran, M. J. B.; Fekih, I. B.; Calatayud, P. A.; Cicero, L.; Cokola, M. C., et al. (2024) Global scientific progress and shortfalls in biological control of the fall armyworm *Spodoptera frugiperda*. *Biological Control*, 191: 105460. doi: 10.1016/j.biocontrol.2024.105460