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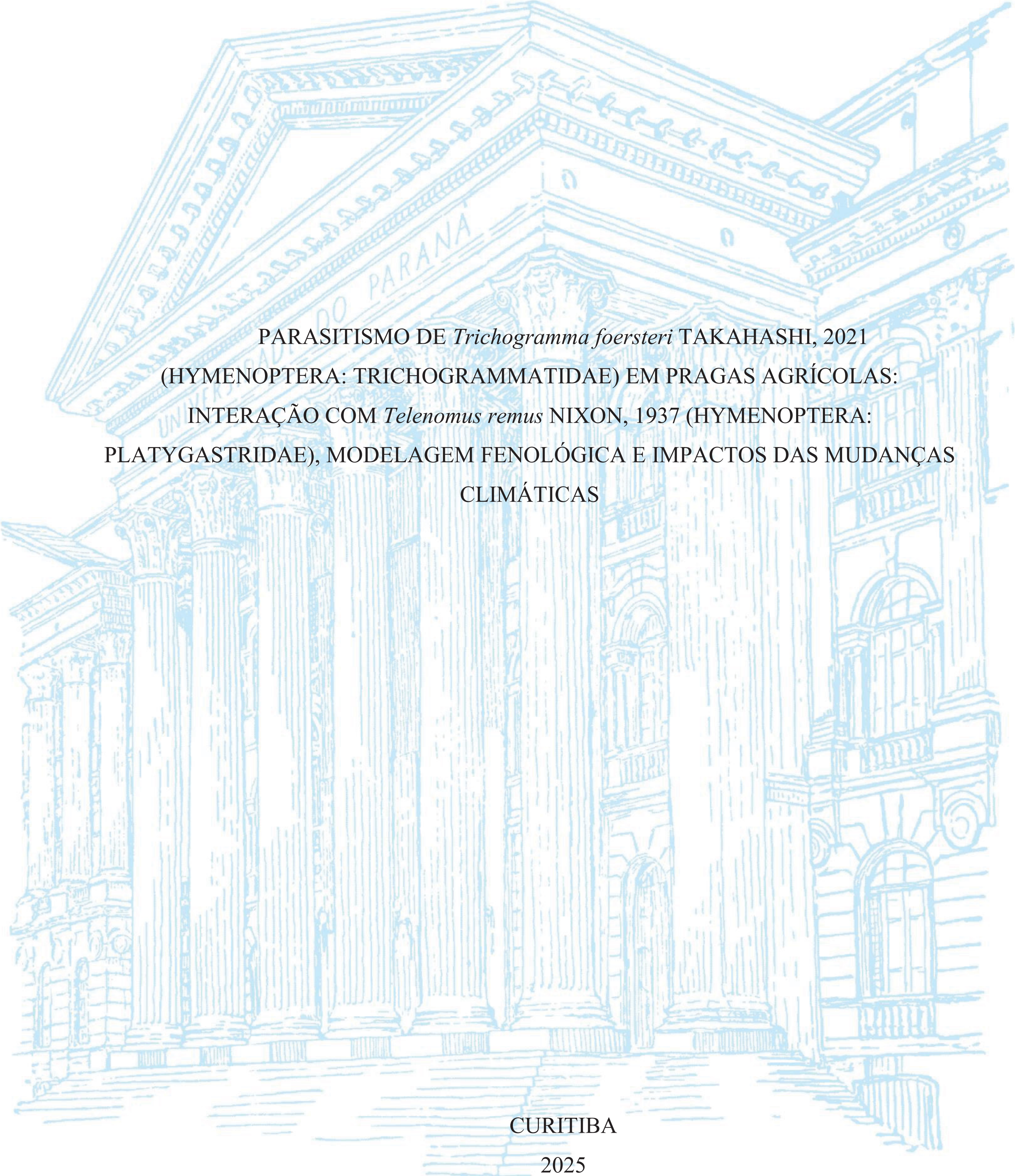
FÁBIO SAMPAIO



PARASITISMO DE *Trichogramma foersteri* TAKAHASHI, 2021 (HYMENOPTERA: TRICHOGRAMMATIDAE) EM PRAGAS AGRÍCOLAS: INTERAÇÃO COM *Telenomus remus* NIXON, 1937 (HYMENOPTERA: PLATYGASTRIDAE), MODELAGEM FENOLÓGICA E IMPACTOS DAS MUDANÇAS CLIMÁTICAS

CURITIBA

2025

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Tese apresentada ao curso de Pós-Graduação em Agronomia – Produção Vegetal, Setor de Ciências Agrárias, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutor em Agronomia (Produção Vegetal).

Orientador: Prof. Dr. Luis Amilton Foerster

Coorientador: Prof. Dr. Cesar Augusto Marchioro

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação AGRONOMIA (PRODUÇÃO VEGETAL) da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **FÁBIO SAMPAIO**, intitulada: **PARASITISMO DE *Trichogramma foersteri* TAKAHASHI, 2021 (HYMENOPTERA: TRICHOGRAMMATIDAE) EM PRAGAS AGRÍCOLAS: INTERAÇÃO COM *Telenomus remus* NIXON, 1937 (HYMENOPTERA: PLATYGASTRIDAE), MODELAGEM FENOLÓGICA E IMPACTOS DAS MUDANÇAS CLIMÁTICAS**, sob orientação do Prof. Dr. JOATAN MACHADO DA ROSA, que após terem inquirido o aluno e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 16 de Maio de 2025.

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Presidente da Banca Examinadora

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Biologicamente somos mortais. Tornamo-nos imortais apenas por meio de nossos atos sociais: a pequena contribuição que nosso percurso pessoal oferece à corrida de revezamento do progresso social, da geração passada à geração futura. (Florestan Fernandes)

RESUMO

Este estudo avaliou o potencial de *Trichogramma foersteri* Takahashi e *Telenomus remus* Nixon como agentes de controle biológico contra importantes pragas agrícolas, com foco na eficácia do parasitismo, nas interações competitivas e na influência de fatores abióticos, particularmente a temperatura. No Capítulo I, foi avaliado o parasitismo de *T. foersteri* em ovos de *Spodoptera frugiperda* J.E. Smith e *Spodoptera eridania* Stoll. Os resultados demonstraram sua eficácia, controlando 334 e 445 ovos de *S. frugiperda* e *S. eridania*, respectivamente, ao longo de três dias. O estudo destacou efeitos significativos de mortalidade não reprodutiva, reforçando o potencial de *T. foersteri* para o manejo integrado de pragas. O segundo capítulo analisou o parasitismo de *T. foersteri* e *Te. remus* em massas de ovos de *S. frugiperda* com uma e duas camadas, além da interação entre os parasitoides. Embora *Te. remus* tenha apresentado taxas de parasitismo mais altas, a competição com *Te. remus* aumentou a eficácia de *T. foersteri*. Ambas as espécies demonstraram potencial para uso individual ou combinado em programas de controle biológico. No Capítulo III avaliaram-se os efeitos da temperatura e das mudanças climáticas sobre o desenvolvimento e voltinismo de ambos os parasitoides usando modelos fenológicos. O tempo de desenvolvimento de *Te. remus* variou de 52,7 dias a 15 °C a 8,1 dias a 35 °C, com o parasitismo mais alto registrado a 35 °C. Os modelos Brière-2 e Shi foram identificados como os mais adequados para *Te. remus* e *T. foersteri*, respectivamente. Projeções climáticas indicaram redução do voltinismo em regiões mais quentes, mas aumento de gerações em áreas mais frias. Esta tese contribui com informações críticas sobre a biologia e o comportamento desses parasitoides, apoiando sua aplicação no manejo sustentável de pragas e enfrentando os desafios impostos pelas mudanças climáticas.

Palavras-chave: Controle biológico; parasitoides de ovos; Manejo Integrado de Pragas; taxa de desenvolvimento; aquecimento global.

ABSTRACT

This study evaluated the potential of *Trichogramma foersteri* Takahashi and *Telenomus remus* Nixon as biological control agents against major agricultural pests, focusing on parasitism efficacy, competitive interactions, and the influence of abiotic factors, particularly temperature. In Chapter I, the parasitism of *T. foersteri* was assessed on eggs of *Spodoptera frugiperda* J.E. Smith and *Spodoptera eridania* Stoll. The results demonstrated its effectiveness, with *T. foersteri* controlling 334 and 445 eggs of *S. frugiperda* and *S. eridania*, respectively, over three days. The study also highlighted significant non-reproductive mortality effects on the host, reinforcing the potential of *T. foersteri* for integrated pest management. Chapter II investigated the parasitism of *T. foersteri* and *Te. remus* on egg masses of *S. frugiperda* with one and two layers, as well as the interaction between parasitoids. While *Te. remus* exhibited higher parasitism rates, competition with *Te. remus* was found to enhance the efficacy of *T. foersteri*. Both species demonstrated potential for combined use in biological control programs. Chapter III investigated the effects of temperature and climate change on both parasitoids using phenological models. The development time of *Te. remus* ranged from 52.7 days at 15 °C to 8.1 days at 35 °C, with the highest parasitism observed at 35 °C. The Brière-2 model was identified as the most suitable for *Te. remus*, while the Shi model best represented the development of *T. foersteri*. Climate change projections suggested a reduction in voltinism in warmer regions but an increase in generations in colder areas. This thesis provides critical insights into the biology and behavior of these parasitoids, supporting their application in sustainable pest management strategies while addressing the challenges posed by climate change.

Keywords: Biological control; egg parasitoids; Integrated Pest Management; development rate; global warming.

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1 INTRODUÇÃO GERAL

O controle biológico é uma prática empregada a nível comercial há mais de um século, e tem colaborado no controle sustentável de diversas espécies causadoras de danos em plantas de interesse econômico (van Lenteren, 2012; Heimpel e Mills, 2017). No decorrer dos anos, essa área passou a ser um campo de interesse não apenas de pesquisadores, empresas e produtores, mas também de importantes discussões entre políticos e formuladores de políticas, organizações e consumidores, que demandam cada vez mais por produtos mais saudáveis e produzidos de uma maneira ambientalmente mais segura (van Lenteren et al., 2018). No Controle Biológico Aumentativo (CBA), inimigos naturais como parasitoides, predadores ou microrganismos, são produzidos em massa para liberação em grandes quantidade para controle imediato de pragas em culturas de ciclo curto (controle biológico inundativo), ou para controle de pragas por várias gerações em culturas com um longo ciclo de produção (controle biológico inoculativo sazonal) (van Lenteren, 2012). Nesse processo, pode haver inúmeros inimigos naturais da mesma praga (Boivin e Brodeur, 2006; Silva et al., 2015). Espécies mais promissoras são comparadas usando principalmente características como eficácia do controle de pragas, taxa de crescimento populacional, alcance do hospedeiro, adaptação à cultura e ao clima (van Lenteren et al., 2018).

Entre os inimigos naturais mais importantes dos insetos estão os parasitoides pertencentes à ordem Hymenoptera (Fei, Gols e Harvey, 2023). Parasitoides de ovos do gênero *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) são os inimigos naturais mais produzidos e utilizados para a supressão de lepidópteros-praga em programas de controle biológico em diferentes culturas e regiões do mundo (Parra et al., 2015), principalmente no Brasil (Parra et al., 2015; Oliveira et al., 2020). Uma nova espécie deste gênero, *Trichogramma foersteri* Takahashi, 2021 (Hymenoptera: Trichogrammatidae), foi coletada pela equipe do Laboratório de Controle Integrado de Insetos (LCII) da Universidade Federal do Paraná, parasitando ovos de *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Noctuidae) na cultura da soja na safra 2017/18 (Takahashi et al., 2021). A partir dessa descoberta, a biologia da espécie foi estudada em laboratório e duas características em sua reprodução se destacaram: (i) a elevada fecundidade e a (ii) capacidade de inviabilizar os ovos hospedeiros sem, no entanto, ocorrer a deposição de ovos (mortalidade não reprodutiva) (Nishimura et al., 2020). A mortalidade não reprodutiva, embora contribua para a redução populacional do hospedeiro, ainda é pouco considerada no processo de controle biológico (Marchioro; Krechemer e Foerster, 2015; Abram et al., 2019). Essas características, aliadas aos resultados superiores em comparação com outras espécies de *Trichogramma* (Bueno et al., 2009; Foerster, Marchioro e Foerster, 2014; Pereira et al., 2019), indicam o potencial de *T. foersteri* para aplicação em programas de controle biológico.

Telenomus remus Nixon, 1937 (Hymenoptera: Platygastridae) é outro eficiente parasitoide de ovos de vários lepidópteros (Liao et al., 2019; Kenis et al., 2019; Colmenarez et al., 2022). Considerado o principal parasitoide de ovos de *Spodoptera frugiperda* J.E. Smith, 1797 (Lepidoptera: Noctuidae), o parasitoide é utilizado com sucesso em programas de controle biológico aumentativo em diferentes partes do mundo (Kenis et al., 2019, Wengrat et al., 2021). Recentemente, a equipe do LCII coletou a espécie em campo parasitando ovos de *Spodoptera cosmioides* Walker, 1858 (Lepidoptera: Noctuidae). Além dessa coleta na região sul do país, *Te. remus* também foi coletado em campo em ovos de *S. frugiperda* na região sudeste (Wengrat et al., 2021). Estes registros configuram a primeira coleta de *Te. remus* em condições de campo, após mais de 30 anos de sua liberação no país (Wengrat et al., 2021).

A coleta de ambos os parasitoide em regiões e épocas próximas reforçam a ocorrência natural de mais de um inimigo natural sobre os hospedeiro (Silva et al., 2015; van Lenteren et al., 2018). Nesse cenário, a eficiência do parasitismo de insetos-pragas pode ser potencializada quando associadas diferentes espécies no seu controle (Boivin e Brodeur, 2006; Carneiro e Fernandes, 2012). O desempenho de espécies de *Trichogramma*, por exemplo, pode ser favorecido se combinado com espécies de *Telenomus* Haliday, uma vez que *Trichogramma* comumente parasita apenas uma camada de ovos (Goulart et al., 2011; Li et al., 2023); diferentemente de *Te. remus*, que é capaz de parasitar múltiplas camadas (Goulart et al., 2011; Carneiro e Fernandes, 2012; Liao et al., 2019). Nesse contexto, compreender a influência da associação ou competição interespecífica entre parasitoides na supressão de pragas, pode subsidiar o desenvolvimento de programas de controle biológico mais eficientes (Boivin e Brodeur, 2006).

O uso de agentes de controle biológico ganha mais importância diante de três grandes problemas atuais relacionados aos insetos-pragas: (i) diversos grupos destes insetos têm apresentando resistência à inseticidas químicos (Durigan et al., 2017; Yang et al., 2020; Samanta et., 2023; Thirawut et al., 2023); (ii) muitas espécies possuem tolerância a plantas geneticamente modificadas contendo proteínas tóxicas da bactéria *Bacillus thuringiensis* (Berliner, 1911) (Eubacteriales: Bacillaceae) (Storer et al., 2010; Yang et al., 2020; Jurat-Fuentes, Heckel e Ferré, 2021); e (iii) insetos-pragas estão migrando cada vez mais rápido para regiões onde até então não eram registrados problemas (Goergen et al., 2016; Goergen, 2018; Wu et al., 2019; EPPO, 2020). Dentro desse amplo grupo de insetos resistentes, tolerantes e com alta capacidade de migração, estão as espécies do complexo *Spodoptera* Guenée (Parra et al., 2022), como é o caso da lagarta-do-cartucho, *S. frugiperda* e a lagarta-das-vagens, *Spodoptera eridania* Stoll, 1782 (Lepidoptera: Noctuidae) (Parra et al., 2022). *Spodoptera frugiperda* é amplamente reconhecida como a principal praga do milho (Parra et al., 2022), enquanto que *S. eridania* se destaca pelos prejuízos causados em cultivos importantes, como soja e algodão (Montezano et al., 2014). Recentemente, essas espécies foram registradas pela primeira vez em vários países da África Ocidental e Central (Goergen et al., 2016; Goergen, 2018). Em 2019, *S. frugiperda* foi identificada também na China, onde se estabeleceu como uma praga invasiva altamente destrutiva (Wu et al., 2019). Na Europa, *S. eridania* foi classificada como praga quarentenária A1, devido ao risco significativo de dispersão pelo continente (EPPO, 2020). O comportamento polífago de ambas contribui para sua rápida adaptação em áreas invadidas. *Spodoptera frugiperda* é capaz de se alimentar de mais de 350 espécies vegetais (Kenis et al., 2023), enquanto *S. eridania* já foi relatada consumindo mais de 200 espécies de plantas pertencentes a 58 famílias diferentes (Montezano et al., 2014). Tais evidências destacam a ameaça que essas pragas representam às culturas agrícolas, bem como a urgência da tomada de medidas de controle alternativas e eficientes, respeitando os princípios do Manejo Integrado de Pragas (MIP).

Para o aprimoramento de estratégias de MIP, no entanto, é necessário também o conhecimento dos fatores que influenciam tanto a biologia do inseto-praga a ser controlado, como do seu inimigo natural. A ectotermia não permite que os insetos regulem a temperatura do seu corpo através de um mecanismo interno, sendo sua temperatura próxima à do meio onde está inserido, influenciando no seu desenvolvimento e os caracterizando como organismos altamente sensíveis à temperatura (Denlinger e Yocum, 1998; Bale et al., 2002; Marchioro, Krechemer e Foerster, 2017). A temperatura pode induzir mudanças nos ciclos biológicos dos insetos, como na duração do ciclo de vida, interferindo na taxa de desenvolvimento e no número de gerações (voltinismo) da espécie, colaborando para mudanças nas taxas de crescimento populacional (Porter, Parry e Carter, 1991; Bale et al., 2002). Um fator determinante na eficácia de programas de controle biológico, está relacionado às exigências térmicas dos organismos envolvidos (Bueno et al., 2009; Bari, Jahan e Islan, 2015). Para espécies do gênero *Trichogramma*, por exemplo, a baixa sobrevivência e reprodução, e consequentemente um parasitismo reduzido em campo, pode estar relacionado à sua incapacidade em se adaptar bem às condições naturais, principalmente quanto a fatores abióticos como temperatura (Cherif, Mansour e Grissa-Lebdi, 2021).

Para compreender melhor a influência da temperatura no desenvolvimento dos insetos, é necessário conhecer os limiares tolerados por uma determinada espécie. Nessas situações, podem ser utilizados modelos fenológicos (Sampaio, Marchioro e Krechemer, 2021), que permitem descrever a relação entre temperatura e taxa de desenvolvimento de insetos. Modelos fenológicos são considerados ferramentas importantes no MIP (Borchert et al., 2004), o que torna a seleção de modelos adequados para cada espécie uma etapa de grande importância (Quinn, 2017). Espécies de parasitoides apresentam comportamento distinto quando expostas a diferentes condições temperaturas, mesmo que ainda pertençam ao mesmo gênero, pois é comum uma diferença entre espécies na natureza (Bari, Jahan e Islan, 2015). Modelos fenológicos podem então estimar a probabilidade de sobrevivência e a ocorrência destes organismos em campo para regiões distintas (Nietschke et al., 2007), tanto no presente, quanto em cenários futuros de mudanças climáticas.

Mudanças na temperatura média e na frequência de eventos extremos, decorrentes das mudanças climáticas, exercem impactos significativos sobre espécies, interações ecológicas e redes tróficas (Ode, Vyas e Harvey, 2022). O metabolismo dos insetos depende do acúmulo de calor acima da temperatura base (Bale et al., 2002). Invernos e primaveras mais quentes encurtam a hibernação, aumentam a sobrevivência de pragas, prolongam a estação de crescimento e elevam o voltinismo. Embora temperaturas moderadamente flutuantes favoreçam o desempenho, eventos extremos, como geadas ou ondas de calor, podem impactar negativamente o desenvolvimento. Essas mudanças podem alterar a distribuição de pragas em diferentes latitudes e altitudes (Bale et al., 2022; Harvey et al., 2023). Esses efeitos tendem a ser ainda mais acentuados nos níveis tróficos superiores, que dependem diretamente da capacidade dos níveis inferiores em se adaptarem às transformações ambientais (Hance et al., 2007). Além disso, a exposição a temperaturas extremas causa danos letais e subletais aos insetos, reduzindo sua longevidade, fecundidade, mobilidade, capacidade de orientação por odores atrativos e habilidades de aprendizado, além de aumentar a proporção de machos na prole, desestabilizando a dinâmica populacional e podendo levar à extinção de parte do sistema (Hance et al., 2007). Nesse contexto, compreender os efeitos da variabilidade climática sobre os parasitoides de ovos é essencial, tanto do ponto de vista teórico quanto prático, especialmente para otimizar o uso de parasitoides em programas de controle biológico (Hance et al., 2007; Furlong e Zalucki, 2017).

Diante da coleta de ambos os parasitoides em campo, e dos resultados iniciais promissores envolvendo *T. foersteri* e o desempenho já conhecido de *Te. remus*, esse trabalho se estruturou em três capítulos com os objetivos de: (i) avaliar em laboratório as características biológicas e a capacidade de parasitismo de *T. foersteri* em ovos de *S. frugiperda* e *S. eridania*; (ii) investigar a possibilidade de associação entre *T. foersteri* e *Te. remus* para o controle de ovos de *S. frugiperda*; e (iii) obter informações sobre o efeito da temperatura no desenvolvimento e sobrevivência de *Te. remus*, a fim de selecionar modelos fenológicos adequados para *Te. remus* e *T. foersteri* para assim avaliar os impactos das mudanças climáticas sobre o desenvolvimento de ambos os parasitoides na região sul do Brasil.

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2 CAPÍTULO I - A NEW BIOCONTROL AGENT AGAINST OLD ENEMIES: THE POTENTIAL OF *Trichogramma foersteri* FOR THE CONTROL OF *Spodoptera frugiperda* AND *Spodoptera eridania*[1]

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ABSTRACT

Species of the genus *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) are widely used for pest control, highlighting the importance of exploring new species for the advancement of biological control programs. The study aimed to evaluate the efficacy of *Trichogramma foersteri* Takahashi, a recently discovered species, in controlling *Spodoptera frugiperda* (JE Smith) and *S. eridania* (Stoll), two significant pests of numerous cash crops. Over three days, the research examined parasitism, non-reproductive mortality, and emergence rates for both pest species. *Trichogramma foersteri* demonstrated effective parasitism, reducing 144.1 ± 6.0 eggs (one layer) and 55.4 ± 4.0 eggs (two layers) of *S. frugiperda*, as well as controlling 150.7 ± 5.6 eggs of *S. eridania*. Non-reproductive effects contributed significantly to mortality, causing the demise of 192.6 ± 4.7 and 118.6 ± 3.8 eggs in one and two layers of *S. frugiperda*, and 294.7 ± 7.0 eggs in *S. eridania*. Within three days, *T. foersteri* controlled approximately 334.2 ± 7.6 and 174.0 ± 4.0 eggs of *S. frugiperda* in one and two layers, respectively, and 445.4 ± 7.2 eggs of *S. eridania*. The highest emergence of *T. foersteri* occurred on the first day of evaluation, with 80.4% for *S. frugiperda* and 23.0% for *S. eridania*. These findings underscore the potential of *T. foersteri* as an effective control agent for both pests and provide valuable insights for the development of sustainable pest control strategies.

Keywords: Biological control, parasitoid, mortality factors, Trichogrammatidae, fall armyworm, southern armyworm.

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1 INTRODUCTION

Biological control has been implemented globally for over a century, providing sustainable management of numerous agricultural pests (van Lenteren et al., 2018). Among the more than 230 species documented worldwide, egg parasitoids of the genus *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) are extensively used against agricultural pests in both annual and perennial crops (Querino and Zucchi, 2019; Mills, 2010; Parra et al., 2015; Oliveira et al., 2020; Zang et al., 2021). Despite the widespread use of *Trichogramma*, the prospection of new species with the potential to effectively suppress insect pests remains crucial for the advancement of biological control programs (Zhang et al., 2013; Takahashi et al., 2021). Recently, field surveys carried out on soybean crops in southern Brazil revealed a new species of *Trichogramma*, (*Trichogramma foersteri* Takahashi) parasitizing eggs of the velvet bean caterpillar, *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae) (Takahashi et al., 2021). Screening tests showed that *T. foersteri* not only had a higher parasitism rate on eggs of *A. gemmatalis* compared to previous studies, but also effectively parasitized ten other lepidopteran pests, including *Spodoptera* species. Apart from its high fecundity, *T. foersteri* causes significant non-reproductive mortality, being capable of inviabilizing more than 200 eggs of *Anticarsia gemmatalis* during the female lifespan (Takahashi et al. 2021).

Research into new biocontrol agents is particularly important when dealing with species of the genus *Spodoptera* Guenée, as most studies report low parasitization rates of *Trichogramma* spp. on eggs of these species. This can be attributed to the behavior of certain female *Spodoptera* species, which usually lay their eggs in multiple layers and subsequently cover them with protective scales during oviposition (Beserra et al., 2005; Li et al., 2023), creating a barrier that hampers parasitism by *Trichogramma* (Greenberg et al., 1998). Female of *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) deposit egg masses with multiple layers covered by scales. Along with the southern armyworm, *Spodoptera eridania* (Stoll) (Lepidoptera: Noctuidae), both species are economically important pests that attack various crops. In the Neotropical region, *S. eridania* has gained increased attention due to the damage it causes to important and widely grown crops such as soybeans and cotton (Montezano et al., 2014), while *S. frugiperda* has long been recognized as the most prominent corn pest (Parra et al., 2022). Furthermore, both species were recently detected for the first time in several countries in Western and Central Africa (Goergen et al., 2016; Goergen, 2018). In 2019, *S. frugiperda* was identified in China, where it is considered a highly destructive invasive pest (Wu et al., 2019). Recently, *S. eridania* has been classified as an A1 quarantine pest in Europe due to a significant risk of spreading to other regions of the continent (EPPO, 2020). The highly polyphagous behavior of *S. eridania* and *S. frugiperda* facilitates their establishment and spread in the invaded regions. The fall armyworm is known to feed on more than 350 plant species (Kenis et al., 2023), while the southern armyworm has been observed feeding on more than 200 plant species belonging to 58 different families (Montezano et al., 2014).

In addition to their polyphagous behavior, the lack of effective control techniques against *S. eridania* and *S. frugiperda*, their wide temperature tolerance range, and the presence of resistant populations to various insecticides are the primary factors contributing to their widespread distribution and control failures (Sosa-Gómez and Omoto, 2012; Durigan et al., 2017; Yang et al., 2020; Wan et al., 2021). Furthermore, the *Spodoptera* species complex naturally exhibits tolerance to genetically modified plants expressing toxic proteins from *Bacillus thuringiensis* Berliner (Eubacteriales: Bacillaceae) (Storer et al., 2010; Yang et al., 2020; Nascimento et al., 2022). These conditions highlight the importance of employing different control practices to manage these pests (Wan et al., 2021; Colmenarez et al., 2022).

Screening tests performed in the laboratory have shown that *T. foresteri* can successfully parasitize the eggs of *S. eridania* and *S. frugiperda*. However, further studies are needed to fully understand the potential of this species in controlling these pests. Like other *Trichogramma* species, *T. foersteri* can negatively impact hosts without successful offspring development or direct feeding - a characteristic referred to as mortality due to non-reproductive effects (Cebolla et al., 2017; Abram et al., 2019. These effects do not offer clear benefits to parasitoids in terms of current or future reproduction but entail negative consequences for hosts, such as mortality (Abram et al., 2019). Despite that, host mortality by non-reproductive effects continues to be a factor often overlooked in many studies (Marchioro et al., 2015; Cebolla et al., 2017; Abram et al., 2019). Ideally, all factors contributing to host mortality should be considered in biological control programs (Cebolla et al., 2017). With this in mind and given the (1) recognized role of *Trichogramma* parasitoids in maintaining pest populations below the economic injury level, (2) the increasing significance of *Spodoptera* species as pests in various cultivated plants worldwide, and (3) the successful parasitism of *Spodoptera* eggs observed in screening tests, the objective of this study was to assess the potential of *T. foersteri* to control *S. frugiperda* and *S. eridania*. Our hypothesis is that *T. foersteri* can effectively kill eggs of *S. frugiperda* and *S. eridania*, indicating its potential to be used in biological control programs against these pests.

2 MATERIAL AND METHODS

2.1 LABORATORY REARING OF THE PARASITOID AND ITS HOSTS

Colonies of *T. foersteri*, its host (*A. gemmatalis*), as well as the target pests (*S. frugiperda* and *S. eridania*), were maintained in the Laboratory of Integrated Insect Control at the Federal University of Paraná under controlled temperature, humidity, and photophase conditions (25 ± 1 ºC, 70 ± 10% RH, and a photophase of 14:10 hours). Larvae of *A. gemmatalis* and *S. eridania* were collected from commercial soybean areas between the crop seasons 2017/18 and 2019/20 in São José dos Pinhais (25°36'49.0"S, 49°08'01"W), Paraná, Brazil, and *S. frugiperda* was collected during samplings conducted in the 2019 maize season at the Experimental Farm of Canguiri in Pinhais city (25°24'01"S, 49°07'01"W), Paraná, Brazil.

Anticarsia gemmatalis was kept in the laboratory following the methodology described by Hoffman-Campo et al. (1985), and *S. frugiperda* and *S. eridania* were reared using the methods outlined by Parra (2001) and fed an artificial diet proposed by Marchioro and Foerster (2012). The parasitoids used in the experiments were obtained from a population originally collected from *A. gemmatalis* eggs sampled on soybean during the 2017/18 season in São José dos Pinhais (Takahashi et al. 2021). The rearing of *T. foersteri* followed the methodology described by Parra (2010) with adaptations and was conducted using eggs of its natural host, *A. gemmatalis*.

2.2 PARASITISM CAPACITY OF *Trichogramma foersteri*

The experiment was conducted using a completely randomized design. Females *S. eridania* typically lay their eggs in a single layer (Sampaio et al., 2023); on the other hand, egg masses of *S. frugiperda* typically contain one to four layers in the field (Beserra et al., 2005; Li et al., 2023), and in this case, both one-layer and two-layer egg masses were offered for parasitism. Before the experiments, the parasitoids were reared for two consecutive generations in *S. frugiperda* and *S. eridania* eggs. Approximately 100 eggs of each species, less than 24 hours old, were provided for parasitism by *T. foersteri* females from the laboratory colony. After 24h, the females were removed and the eggs were kept in a climate-controlled chamber (25 ± 1 ºC, 70 ± 10% RH, and a photophase of 14:10 hours) until the parasitoids emerged. Following the emergence of the first generation, this procedure was replicated to obtain a second generation of parasitoids, and the adults emerged from each host species were used in the experiments. Parasitoids reared in *S. frugiperda* eggs for two generations were employed in experiments with *S. frugiperda*, and the same protocol was followed for experiments with *S. eridania*.

Parasitism was assessed over three consecutive days for both species, with 20 replicates for each treatment. For each host species, 20 copulated females of *T. foersteri*, less than 24h old, were individually placed in glass tubes measuring 7.5 cm height and 1.0 cm diameter. The tubes were closed with a cotton ball and placed in chambers at 25 ± 1 ºC, 70 ± 10% RH, and a photoperiod of 14:10 hours. Droplets of pure honey were provided as food inside the tubes. Approximately 250 eggs, less than 24h old, were offered for parasitism for 24 hours within each glass tube. After this period, the egg masses were transferred to new glass tubes. New egg masses were offered for parasitism for two additional consecutive days. In the experiments, the egg masses were handled with minimal manipulation to ensure the preservation of the existing scale, which varied in thickness from 0 to 71.6 µm, based on the classification introduced by Hou et al. (2022).

The number of eggs parasitized per female was recorded for each species by counting the darkened host eggs. During preliminary laboratory tests, it was observed that parasitism drastically decreases starting from the third day. Based on this information, we opted to evaluate parasitism during the first three days of the female's life. Larvae of *S. frugiperda* and *S. eridania* that hatched from non-parasitized eggs were counted and removed from the egg masses twice a day to avoid egg cannibalism.

2.3 NON-REPRODUCTIVE MORTALITY CAUSED BY *Trichogramma foersteri*

Because previous studies indicated that *T. foersteri* can cause host mortality through non-reproductive means (Takahashi et al., 2021), the methodology employed by Vásquez et al. (1997) and Marchioro et al. (2015), based on the procedure outlined by Abbot (1925), was used to assess the total mortality caused by the parasitoid, as follows:

$$\mathrm{TM} = \left(\frac{PVE - PHL}{PVE}\right) * 100$$

where, TM = total mortality, PVE = percentage of viable eggs, and PHL = percentage of hatched larvae in each treatment. To determine egg viability, 10 cards containing 50 eggs of each species were placed individually in glass tubes and maintained under the same controlled experimental conditions. These eggs had the same origin as those used in the experiments. Mortality caused by parasitism was directly assessed by the visual inspection of the host eggs offered to parasitism. Non-reproductive mortality was determined by subtracting the total number of eggs controlled by the parasitoid (TM) from the mortality caused by parasitism.

Five days after parasitism, photographs of the egg masses were taken using cameras connected to a stereoscopic microscope. This allowed the identification and counting of the number of eggs killed by parasitism (dark eggs) and by non-reproductive causes (greenish eggs). The counting of the egg masses was performed using the ImageJ software (Schneider et al., 2012). To ensure the accuracy of the results obtained from the photographs, each parasitized egg was individually evaluated under a stereoscope microscope after the emergence of the parasitoids. This additional evaluation served as confirmation and validation of the findings derived from the photographic analysis.

2.4 EMERGENCE, LONGEVITY, AND SURVIVAL OF *Trichogramma foersteri*

Following the emergence of the parasitoids, the parasitized egg masses were examined to determine the emergence rate. This was calculated by dividing the number of eggs with an exit hole by the total number of darkened eggs. To assess the longevity of *T. foersteri*, 20 newly emerged couples were kept in 7.5 x 1.0 cm glass tubes. Droplets of pure honey were provided as food source, while no eggs were offered in this setup. Daily observations were conducted to record the date of death for both females and male, enabling the calculation of the survival rate.

2.5 STATISTICAL ANALYSIS

The parameters evaluated in the study were analyzed using generalized linear models (GLM) in the R computing environment (R Core Team, 2022). The ability of *T. foersteri* to control *S. eridania* and *S. frugiperda* was analyzed separately, as our objective was not to compare parasitism between different hosts. For *S. frugiperda*, factors such as the day of parasitism and the number of egg layers were considered when analyzing parasitism rate, non-reproductive mortality, and the total number of eggs killed by the parasitoid. For *S. eridania*, since egg masses typically consist of only one layer, only the day of parasitism was considered as a factor. The number of eggs controlled by parasitism and by non-reproductive causes, as well as the total number of eggs killed by the parasitoid, were evaluated with a quasi-poisson distribution with a logarithmic link function. The emergence rate was evaluated using logistic regression with a quasi-binomial distribution and logit link function. Longevity was assessed using a Gaussian distribution with an identity link function. When significant differences were observed among treatments ($P < 0.05$), Tukey's test was employed to compare the means at a significance level of 5%. Additionally, survival curves were constructed for female and male of *T. foersteri* individuals developing on both hosts. These curves were compared using the non-parametric Kaplan-Meier method (Kaplan and Meier, 1958) implemented in the survival R package. All mentioned averages in the text are accompanied by their respective standard errors.

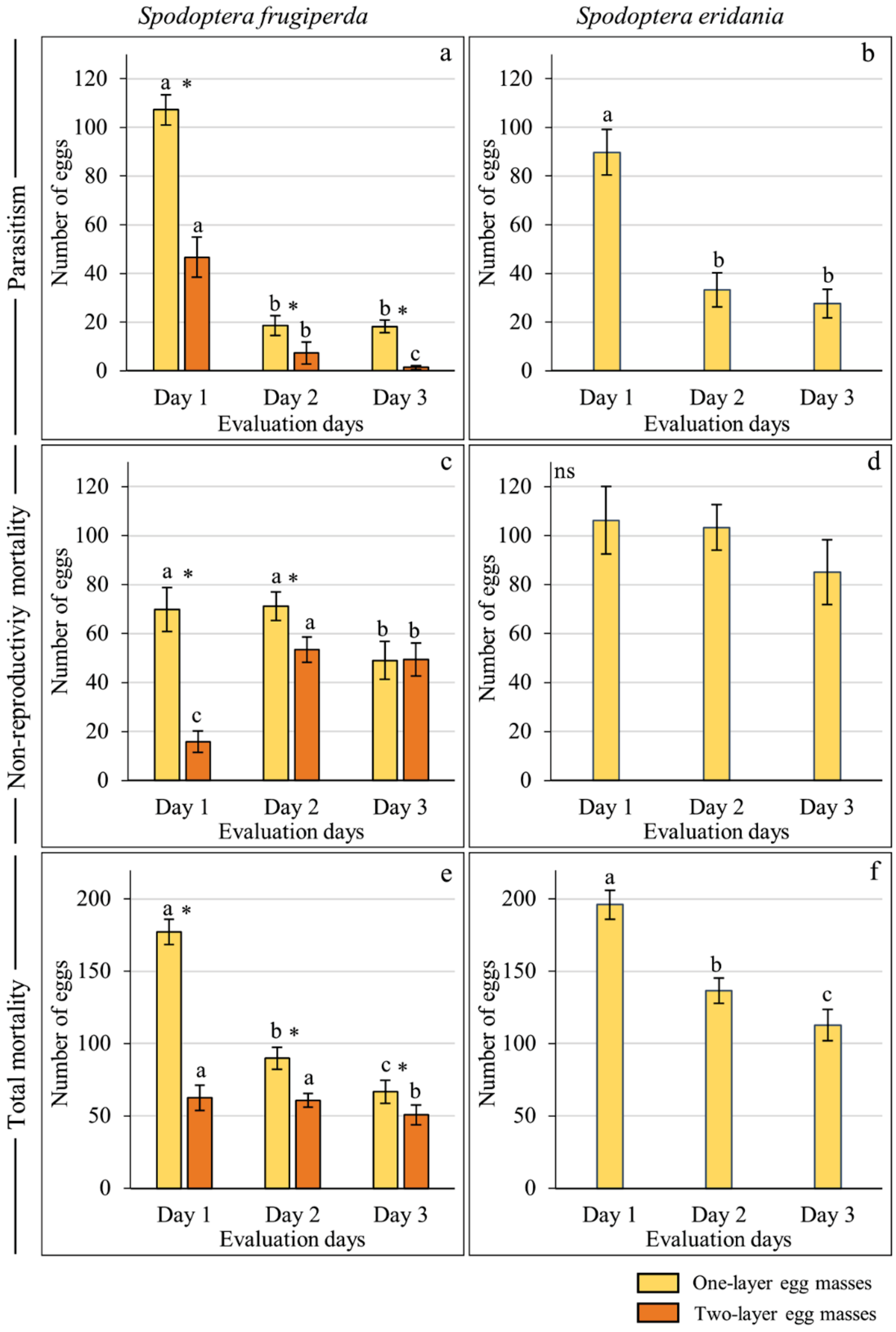
3 RESULTS

3.1 PARASITISM BY *Trichogramma foersteri*

Trichogramma foersteri was capable of parasitizing both host species throughout the three-day evaluation period. Parasitism of *S. frugiperda* eggs was significantly affected by the number of egg layers ($\chi^2 = 38.4$; df = 1; $p < 0.05$) and the parasitism day ($\chi^2 = 151.9$; df = 2; $p < 0.05$). However, the interaction between these two factors did not significantly influence parasitism ($\chi^2 = 5.5$; df = 2; $p = 0.06$). Parasitism levels were higher on egg masses with a single layer compared to two-layer egg masses. When considering data from all three evaluation days, *T. foersteri* parasitized an average of 144.1 ± 6.0 eggs on one-layer egg masses and 55.4 ± 4.0 eggs on egg masses with two layers (Figure 1a). Irrespective of the number of egg layers, the parasitism was notably higher on the first day of evaluation in comparison to the second and third days (Figure 1a).

Parasitism of *S. eridania* eggs was significantly influenced by the evaluation days ($\chi^2 = 35.7$; df = 2; $p < 0.05$). The average number of parasitized eggs was higher on the first day (89.8 ± 9.4 eggs), followed by the second (33.2 ± 7.0 eggs) and third evaluation days (27.6 ± 5.7 eggs) (Figure 1b), totalling an average of 150.7 ± 5.6 parasitized eggs of *S. eridania* over the three evaluated days.

FIGURE 1 – Parasitism, non-reproductive mortality and total number of eggs killed (parasitism + non-reproductive mortality) by *Trichogramma foersteri* in eggs of *Spodoptera frugiperda* (a, c, and e) and *Spodoptera eridania* (b, d, and f) over a period of three consecutive days. Lowercase letters represent the differences between days of parasitism, while asterisks indicate differences between *Spodoptera frugiperda* egg masses with one and two egg layers.



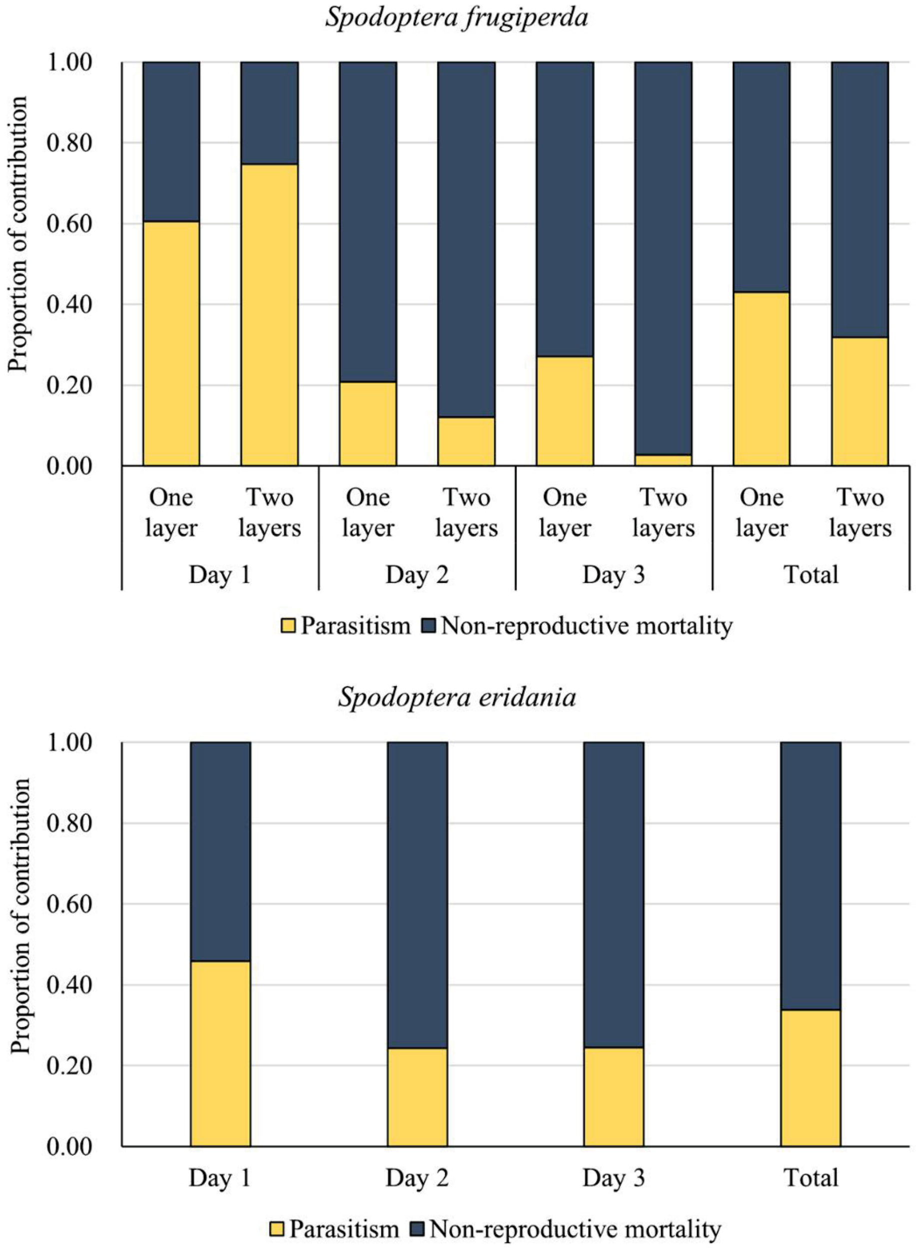
3.2 NON-REPRODUCTIVE MORTALITY

Trichogramma foersteri showed a remarkable ability to render the eggs of the examined hosts unviable through non-reproductive causes. The incidence of this type of mortality in *S. frugiperda* eggs exhibited significant variation in relation to the day of parasitism

($\chi^2 = 7.8$; df = 2; $p < 0.05$), number of layers ($\chi^2 = 17.3$; df = 1; $p < 0.05$), and the interaction between these two factors ($\chi^2 = 23.6$; df = 2; $p < 0.05$). In one-layer egg masses, the observed number of unviable eggs by non-reproductive causes was higher on the first two days (69.9 ± 9.0 eggs on the first and 71.2 ± 6.8 eggs on the second day) compared to the third day of parasitism (48.9 ± 7.9 eggs) (Figure 1c). A different pattern was observed when two-layer egg masses were offered to parasitism. In this case, the number of eggs killed by non-reproductive causes was higher on the second (53.4 ± 5.2 eggs) and third day (49.3 ± 6.7 eggs) in comparison to the first day (15.8 ± 4.3 eggs) (Figure 1c). Similar to the observed parasitism trend, the non-reproductive mortality was higher in one-layer compared to two-layer egg masses. In total, *T. foersteri* rendered an average of 190.1 ± 4.7 eggs unviable in one-layer egg masses of *S. frugiperda* over the three-day evaluation period, and 118.6 ± 3.8 eggs in egg masses with two layers. On the second and third days, non-reproductive mortality was higher than parasitism when *T. foersteri* was exposed to one- and two-layer egg masses (Figure 2).

Conversely, the day of parasitism did not have a significant influence on the mortality through non-reproductive causes in eggs of *S. eridania* ($\chi^2 = 1.7$; df = 2; $p = 0.4$, Figure 1d). The number of eggs rendered unviable due to non-reproductive causes tended to be higher on all evaluation days, with average values of 106.2 ± 13.7, 103.3 ± 9.3, and 85.1 ± 13.2 eggs on the first, second, and third days, respectively. For *S. eridania*, the total number of eggs rendered unviable due to non-reproductive causes during the three-day evaluation was 294.7 ± 7.0. Non-reproductive mortality accounted for most of the observed mortality for *S. eridania* on all evaluated days (Figure 2).

FIGURE 2 - Decomposition of the total mortality caused by *Trichogramma foersteri* on eggs of *Spodoptera frugiperda* and *Spodoptera eridania*.



3.3 TOTAL NUMBER OF EGGS KILLED BY *Trichogramma foersteri*

The total number of *S. frugiperda* eggs controlled by *T. foersteri*, which includes eggs killed by parasitism and non-reproductive causes, was significantly influenced by the evaluation day ($\chi^2 = 60.8$; df = 2; $P < 0.05$), number of egg layers ($\chi^2 = 68.0$; df = 1; $p < 0.05$), and the interaction between these two factors ($\chi^2 = 18.4$; gl = 2; $p < 0.05$). One-layer egg masses had a higher number of eggs controlled by *T. foersteri* compared to two-layer egg masses. In total, the parasitoid controlled an average of 334.2 ± 7.6 eggs of *S. frugiperda* in one-layer egg masses and 174.0 ± 4.0 eggs in two-layers egg masses. The total number of killed eggs decreased with the day of parasitism, starting from 177.0 ± 8.6 eggs on the first day to 66.7 ± 7.8 on the third day in one-layer egg masses (Figure 1e). Conversely, there were no significant differences in the total number of eggs controlled by *T. foersteri* in two-layers egg masses between the first and second days of parasitism. However, on the third day, a significant decrease was observed, with only 50.7 ± 6.8 eggs being controlled (Figure 1e).

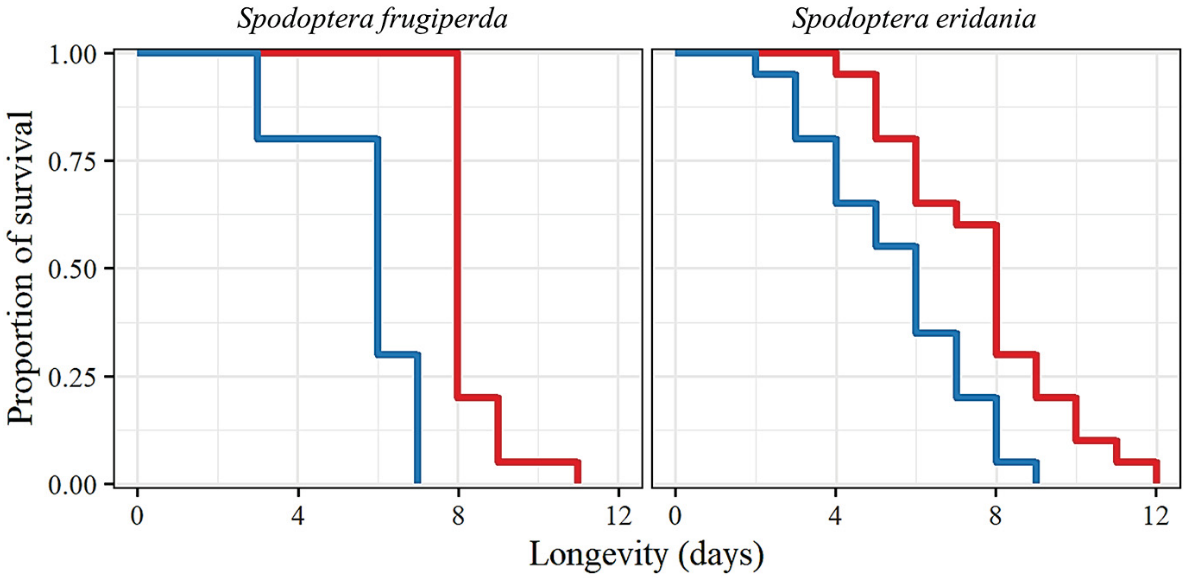
Significant differences between evaluation days were also recorded for *S. eridania* ($\chi^2 = 33.8$; df = 2; $p < 0.05$). The highest number of eggs killed by *T. foersteri* in this host occurred on the first day (196.0 ± 10.0 eggs), while the lowest number was recorded on the third day (112.7 ± 10.9 eggs) (Figure 1f). For *S. eridania*, a total of 445.4 ± 7.2 eggs were killed by *T. foersteri* during the three evaluation days.

3.4 EMERGENCE AND SURVIVAL OF *Trichogramma foersteri*

The emergence rate was significantly influenced by the host species ($\chi^2 = 51.7$; df = 1; $p < 0.05$) and evaluation days ($\chi^2 = 115.0$; df = 2; $p < 0.05$). However, there was no significant interaction between these two factors ($\chi^2 = 0.17$; df = 2; $p = 0.9$). A higher emergence rate of 80.5% was observed on the first evaluation day when *T. foersteri* developed on *S. frugiperda* eggs. There was a sharp decline in parasitism in the second and third evaluation days, with only a few parasitoids emerging from the parasitized eggs. The recorded emergence rates for these days were 26.0% and 2.8%, respectively. Lower emergence rates were observed for parasitoids developed on eggs of *S. eridania*. In this host, an emergence rate of 23.0% was observed on the first day of parasitism, and only 2.2% of the parasitoids emerged on the second day. No emergence of parasitoids was observed from egg masses offered for parasitism on the third day.

Parasitoid longevity from egg masses of *S. frugiperda* was influenced by the sex of parasitoids ($\chi^2 = 50.9$; df = 1; $p < 0.05$). The average longevity of female *S. frugiperda* (8.3 days ± 0.1) was higher than males (5.7 days ± 0.3). A similar pattern was observed for *S. eridania* ($\chi^2 = 10.1$; df = 1; $p < 0.05$), with females (7.6 ± 0.4) living longer than males (5.5 days ± 0.4). These findings were further supported by the survival analysis (Figure 3), which demonstrated significant differences in survival between female and male parasitoids for both *S. frugiperda* ($\chi^2 = 41$; df = 1; $p < 0.05$) and *S. eridania* ($\chi^2 = 8.9$; df = 1; $p < 0.05$).

FIGURE 3 - Survival curves constructed according to the Kaplan-Meier method for adults of *Trichogramma foersteri* reared on eggs of *Spodoptera frugiperda* and *Spodoptera eridania*. The blue and red lines indicate the survival curves of males and females, respectively.



4 DISCUSSION

In this study, the parasitism of a newly discovered species of *Trichogramma* was evaluated on eggs of *S. frugiperda* and *S. eridania*, two relevant agricultural pests of growing importance. This is the first investigation examining the efficacy of *T. foersteri* in controlling these two *Spodoptera* species. The results of our study revealed that *T. foersteri* successfully parasitizes eggs of both *S. frugiperda* and *S. eridania* and shows a substantial capacity to induce host mortality through non-reproductive means. These findings provide valuable and fundamental insights, suggesting that *T. foersteri* has the potential to be incorporated into integrated pest management programs for the effective control of *S. frugiperda* and *S. eridania*.

Most of the parasitism occurred on the first day of evaluation; however, subsequent days exhibited effective control through parasitism. The number *S. frugiperda* and *S. eridania* eggs parasitized by *T. foersteri* within the first 24 hours is remarkably high compared to other *Trichogramma* species evaluated at the same temperature and photoperiod conditions as in our study. For instance, Yang et al. (2022) reported that *Trichogramma chilonis* Ishii, *T. dendrolimi* Matsumura, and *T. pretiosum* Riley parasitized approximately 16, 30, and 26 eggs of *S. frugiperda*, respectively, within the same time frame. In another study, Bueno et al. (2010) found that *T. pretiosum* parasitized only 6.5 eggs of *S. frugiperda* within the same period, while Tian et al. (2020) reported that *T. dendrolimi* and *T. bilingensis* He and Pang parasitized 20 and 9.6 eggs of *S. frugiperda*, respectively. Similar disparities in parasitism between *T. foersteri* and other *Trichogramma* species were also observed on eggs of *S. eridania*. Carvalho et al. (2012) reported that *T. pretiosum* parasitized 9.9 eggs of *S. eridania* within 24 hours, *T. pratissolii* Querino and Zucchi parasitized 9.2, *T. galloi* Zucchi parasitized 7.3 eggs, *T. exiguum* Pinto and Platner parasitized 5.0 eggs, and *T. atopovirilia* Oatman and Platner parasitized only 3.8 eggs. When compared to other *Trichogramma* species, these findings clearly demonstrate the high parasitization capacity of *T. foersteri* on eggs of both *S. frugiperda* and *S. eridania*.

Certain species of *Spodoptera*, such as *S. frugiperda*, often lay egg masses with multiple layers. This characteristic is recognized as an important factor that limits parasitism by *Trichogramma* species (Beserra et al., 2005). Tyipically, female *S. frugiperda* lay egg masses with one to four layers, with the majority of egg masses containing two layers (Hou et al., 2022). However, many studies did not provide information about the number of layers in the egg masses used for parasitism tests. Moreover, some studies performed parasitism tests using single-layer egg masses without scales, claiming comparability with other host species (e.g. Jin et al., 2021). In this study, we examined the parasitism of *T. foersteri* on *S. frugiperda* egg masses containing one and two layers, without the removal of scales. Surprisingly, *T. foersteri* exhibited a high level of parasitism even in two-layer egg masses.

Although the parasitism in two-layer egg masses of *S. frugiperda* was lower compared to one-layer egg masses, it was relatively higher than the ones reported for other *Trichogramma* species (e.g. Bueno et al., 2010; Tian et al., 2020; Jin et al., 2021; Yang et al., 2022). For instance, Jin et al. (2021) evaluated the parasitism of seven *Trichogramma* species in one-layer egg masses of *S. frugiperda* with the scales artificially removed. Even under those conditions, the parasitism by the seven species was significantly lower than the observed for *T. foersteri* in this study. When exposed to one- and two-layer egg masses, the parasitism by *T. foersteri* was 11 to 27, and 4.5 to 12 times higher, respectively, than the parasitism reported by Jin et al. (2021). As a recently discovered species, *T. foersteri* requires further studies to fully understand its bioecology. The observed high reproductive capacity of *T. foersteri* may be attributed to its larger size compared to other species commonly found in Brazil. Laboratory observations confirmed that *T. foersteri* is larger in size than both *T. atopovirilia* and *T. pretiosum*, which exhibited lower parasitism rates on *S. frugiperda* eggs. Previous studies have demonstrated a positive correlation between parasitoid size and fecundity (Boivin, 2010), as well as the existence of physiological and behavioral advantages over their smaller counterparts (Godfray, 1994). Furthermore, our results also indicate that *T. foersteri* females tend to rapidly lay most of their eggs upon contact with a potential host. Therefore, investigating *T. foersteri* parasitism throughout the female lifespan may shed light on whether the observed differences are associated to this behavior or to other factors, such as parasitoid size.

Interestingly, the visual observations of the egg masses after 24h of parasitism indicated that *T. foersteri* primarily parasitized the outer eggs while failing to reach the inner eggs of the clutch. While this finding might suggest that the number of egg layers, rather than the presence of scales, played a more significant role in limiting the parasitism by *T. foersteri*, further investigation considering that previous studies have reported that the thickness of scales on egg masses had a significant impact on the parasitism success of different *Trichogramma* species (Dong et al., 2021; Hou et al., 2022). Therefore, it is crucial to delve deeper into this matter to gain a comprehensive understanding of the parasitism behavior of *T. foersteri* when parasitizing egg masses with multiple layers.

While it is widely recognized that parasitoids of the genus *Trichogramma* primarily induce host mortality through parasitism (Abram et al., 2019), it has been documented that these parasitoids can also cause host death through non-reproductive means (Asgari and Rivers, 2011; Marchioro et al., 2015; Abram et al., 2016; Cebolla et al., 2017; Kaser et al., 2018). Throughout the three-day evaluation period, *T. foersteri* consistently induced mortality in eggs of both *S. frugiperda* and *S. eridania* through non-reproductive factors. Notably, the mortality caused by non-reproductive factors surpassed that caused by parasitism for both host species. This outcome aligns with previous findings that demonstrate the potential for non-reproductive mortality to exceed reproductive mortality in certain instances (Kaser et al., 2018). Throughout the three days of parasitism examined in this study, *T. foersteri* played a significant role in the high number of eggs affected by non-reproductive mortality in both single and double-layer egg masses of *S. frugiperda*. Notably, the impact of this effect on the total mortality of *S. eridania* was even more pronounced, reaching almost 300 eggs. Parasitoids can induce non-reproductive mortality through various mechanisms, including (1) mechanical damage to the host egg, (2) death of the embryo during egg perforation, (3) larval feeding before parasitoids' eventual death, (4) release of chemical compounds or teratocytes without subsequent parasitoid development, or (5) adult feeding (Asgari and Rivers, 2011; Abram et al., 2013; Strand, 2014; Huang et al., 2017; Abram et al., 2019). This diverse range of mechanisms leading to host mortality through non-reproductive means helps to explain the efficacy of biological control programs, even when low rates of parasitism are observed (Kaser et al., 2018). It remains to be determined the cause of the high non-reproductive mortality levels of *T. foersteri* on eggs of both hosts.

The findings of our study carry significant implications, as the non-reproductive effects induced by egg parasitoids are often under-evaluated and overlooked in research assessing the impacts of *Trichogramma* species on their hosts (Abram et al., 2016; Cebolla et al., 2017; Kaser et al., 2018). Neglecting this aspect not only underestimates the true potential of biological control agents but can also influence the accuracy of insect population models in understanding the host-parasitoid dynamics (Cebolla et al., 2017). Currently, detailed information regarding the mechanisms employed by *T. foersteri* in causing this type of mortality is lacking. Conducting behavioral studies and biochemical analyses of the contents of eggs rendered unviable by non-reproductive causes could offer more definitive insight into this process.

The genus *Trichogramma* exhibits a complex relationship with respect to the nutritional quality of host eggs (Goulart et al., 2010; Zang et al., 2021), and larval survival serves as a crucial indicator when assessing the nutritional suitability of hosts. For instance, eggs of *Chilo suppressalis* Walker (Lepidoptera: Crambidae) were found to be suitable for *T. dendrolimi*, *T. japonicum* and *T. chilonis*, but not for *T. ostriniae*, which exhibited low rates of parasitism and progeny survival in this particular host (Zhang et al., 2013). Similarly, our results indicate significant variations in emergence rates depending on the host species, with *S. eridania* eggs displaying a lower emergence rate compared to *S. frugiperda*. Based on this biological parameter, one could infer that *S. eridania* eggs are less nutritious for *T. foersteri* than *S. frugiperda* eggs. This assumption is further supported by the shorter lifespan of *T. foersteri* when parasitizing *S. eridania* compared to *S. frugiperda*. One factor that might explain these findings is the potential presence of a more robust immune response of *S. eridania* against the parasitoid, as in certain situations the host's immune system can eliminate the parasitoid eggs (Abram et al., 2016). The extended lifespan of female parasitoids is a desirable trait as it promotes the search for food sources, mates, and hosts (Foerster et al., 2014; Tian et al., 2016). Females of both species exhibited long lifespans, particularly in the case of *S. frugiperda*, where all evaluated females survived for at least eight days.

Together, parasitism and non-reproductive mortality exerted control over a significant proportion of the eggs available to female *T. foersteri*. Understanding the mechanisms through which the parasitoid eliminates its host is crucial in determining the most effective strategy for biological control programs (Huang et al., 2017). For instance, during the three-day evaluation period, we observed that *T. foersteri* successfully controlled a total of 334 and 174 eggs in one- and two-layer egg masses of *S. frugiperda*, respectively, along with 445 eggs of *S. eridania*. These results indicate that *T. foersteri* is a potential candidate to be employed in inundative biological control which involves the mass production and release of parasitoids to rapidly control the pest through the action of the released parasitoids rather than their offspring (Abram et al., 2016). The non-reproductive mortality plays a pivotal role in reducing the pest population and, in certain instances, constitutes the primary cause of host mortality (Abram et al., 2019), as observed for *T. foersteri*. On the other hand, if the goal is to use the inoculative biological control, entailing the periodic release of parasitoids to restore an imbalanced system that has not been naturally maintained, preference should be given to parasitoids displaying high parasitization capacity and emergence rates.

In conclusion, this study represents the first assessment of the potential of the newly discovered egg parasitoid *T. foersteri* for controlling species of *Spodoptera*. Our laboratory experiments demonstrate that *T. foersteri* has the potential to control *S. frugiperda* and *S. eridania*, both through parasitism and non-reproductive mechanisms. In fact, a significant portion of the mortality inflicted on the hosts by *T. foersteri* was due to non-reproductive factors, highlighting the importance of considering this aspect when evaluating the impact of *Trichogramma* species on insect pests. Moreover, *T. foersteri* exhibited a notably higher capacity for parasitization compared to other *Trichogramma* species. Given the global demand for sustainable agricultural production, the prospection of new parasitoids like *T. foersteri* can serve as a vital tool in the development of Integrated Pest Management Programs. This information holds even greater significance for pests such as *S. frugiperda* and *S. eridania*, which continue to expand their distribution range and cause economic losses in various regions worldwide. While the number of eggs killed by *T. foersteri* demonstrates its potential as a biocontrol agent against these pests, further studies should be conducted to evaluate the feasibility of its mass rearing using factitious hosts, as well as its ability to parasitize hosts under field conditions.

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Consent for publication: All authors consent to publication.

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3 CAPÍTULO II - CAN *Telenomus remus* AND *Trichogramma foersteri* BE USED IN COMBINATION AGAINST THE FALL ARMYWO*RM, Spodoptera frugiperda*?[2]

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ABSTRACT

The fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae), poses a global threat to agriculture, causing significant economic losses in numerous cash crops. Various control methods, including chemical insecticides, have proven insufficient against *S. frugiperda*, leading to a demand for alternative strategies, such as biological control. In this context, laboratory experiments were conducted to evaluate the parasitism of *Trichogramma foersteri* (Hymenoptera: Trichogrammatidae) and *Telenomus remus* (Hymenoptera: Scelionidae) on egg masses of *S. frugiperda* with one and two layers. Additionally, the potential synergistic use of both species against the fall armyworm were assessed. Although both species parasitized single and double layered egg masses of *S. frugiperda*, *Te. remus* showed higher parasitism compared to *T. foersteri*. The parasitism of *Te. remus* was not affected by the competition with *T. foersteri*. Conversely, an increase in parasitism of *T. foersteri* was observed due to competition with *Te. remus*, especially when both species had simultaneous access to *S. frugiperda* egg masses. The total number of parasitized eggs was significantly higher when *Te. remus* was allowed to parasitize first, and when both parasitoids had simultaneous access to the egg masses. These results are crucial for the development of biological control programs using *T. foersteri* and *Te. remus*, as they indicate that both parasitoids could be used, either individually or in combination, against *S. frugiperda*.

Keywords: Agricultural pest, biological control, Hymenoptera, Lepidoptera, Scelionidae, Trichogrammatidae.

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1 INTRODUCTION

The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is recognized as one of the most important insect pests worldwide (Abbas et al., 2022). It causes significant economic losses to various cultivated crops, with maize being particularly susceptible (Parra et al., 2021). Originally native to the Neotropical region (Sparks, 1979), this species has recently spread to numerous countries in sub-Saharan Africa (Goergen, Kumar, Sankung, Togola & Tamò, 2016), Asia, Europe, and Oceania (EPPO, 2023). *Spodoptera frugiperda* is now considered a highly destructive pest in several countries within its invaded region (Wu et al., 2019; Wan et al., 2021; Anyanda et al., 2022, EPPO, 2023), demanding global efforts to promote its control, especially in countries recently invaded (Feldmann, Rieckmann & Winter, 2019; FAO, 2023).

The fall armyworm exhibits several traits commonly associated with the success of biological invaders. For example, it uses a wide range of preferred host plants, feeding on more than 274 plant species, including numerous cultivated ones (Montezano et al., 2018). This species also demonstrates strong flight capabilities, enabling it to cover long distances and undertake migration to various regions (Chen et al., 2022), and high reproductive potential, with females laying an average of more than 1,800 eggs depending on the plant used as host during the larval stage (Barros, Torres & Bueno, 2010). In addition, *S. frugiperda* is naturally tolerant to genetically modified plants expressing toxic proteins from the bacterium *Bacillus thuringiensis* Berliner (Eubacteriales: Bacillaceae) (Wu et al., 2019; Jurat-Fuentes, Heckel & Ferré, 2021; Wan et al., 2021), making difficult its control and facilitating its establishment in the invaded areas.

The exclusive use of chemical insecticides has proven insufficient in managing *S. frugiperda* after it successfully established in invaded regions (Dong et al., 2021), prompting the need for alternative and sustainable strategies for its control. In this context, biological control approaches are emerging as a promising alternative. Egg parasitoids are widely used in biological control programs to control insect pests affecting numerous crops worldwide (Parra & Coelho 2019; Zang, Wang, Zhang & Desneux, 2021; Colmenarez, Babendreier, Ferrer Wurst, Vásquez-Freytez & Bueno, 2022). In particular, parasitoids belonging to the genus *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) and *Telenomus* Haliday (Hymenoptera: Scelionidae) have demonstrated their ability to parasitize the eggs of several pests, including *S. frugiperda* (Abbas et al., 2022; Colmenarez et al., 2022; Jin et al., 2021; Yang et al., 2022; Koffi et al., 2023; Li et al., 2023a).

The effectiveness of these natural enemies can be influenced by specific characteristics of the egg masses laid by *S. frugiperda* females (Dong et al., 2021), such as the number of egg layers (Beserra & Parra, 2005; Beserra, Dias & Parra, 2005) and the presence of scales deposited during oviposition (Dong et al., 2021). *Trichogramma* species are particularly affected by these factors (Beserra & Parra, 2005; Beserra et al., 2005; Jin et al., 2021; Yang et al., 2022; Li et al., 2023b). In contrast, *Telenomus* species show comparatively better performance when parasitizing two-layered egg masses covered with scales (Carneiro & Fernandes 2012; Kenis et al., 2019; Colmenarez et al., 2022).

Given the rich diversity of natural enemies found in tropical agroecosystems, there is the potential for interactions among species competing for the same hosts. Interspecific competition, for example, can significantly impact the distribution, abundance, and evolutionary trajectory of species (Boivin & Brodeur, 2006; Boivin, 2010; Carneiro & Fernandes 2012; Silva, Vieira, Loiácomo & Parra, 2015; Ode, Vyas & Harvey, 2022). Among the natural enemies commonly found in agroecosystems, parasitoid wasps use various strategies to locate and exploit hosts, thereby influencing the intensity and outcome of competition among females (Boivin & Brodeur 2006; Ode et al., 2022). Therefore, comprehending how interspecific competition among egg parasitoids ultimately impacts the number of parasitized eggs elucidates parasitoid abundance in the field and holds practical applications for implementing biological control programs using multiple species simultaneously.

The egg parasitoid *Trichogramma foersteri* Takahashi was recently discovered parasitizing eggs of the velvetbean caterpillar *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae) in southern Brazil (Takahashi, Nishimura, Querino & Foerster, 2021). At the same time, *Telenomus remus* Nixon was also documented in the same region, marking the first recorded case of natural parasitism of this species on eggs of *Spodoptera* spp. in Brazil (Wengrat et al., 2021). While *Te.* remus is a well-studied species (Colmenarez et al., 2022), our understanding of the biology of *T. foersteri* remains comparatively limited. Laboratory experiments have shed light on the parasitic behavior of T. foersteri, revealing its ability to parasitize eggs of at least 10 economically important noctuids, including S. frugiperda (Takahashi et al., 2021). In particular, *T. foersteri* has a remarkable reproductive capacity when compared to other Trichogramma species (Takahashi et al., 2021; Villalba et al., 2023).

Although *T. foersteri* and *Te. remus* both coexist and target the eggs of *S. frugiperda*, there is currently a lack of knowledge regarding their reproductive behavior when females of both parasitoid species have access to the same egg masses. To fill this gap, laboratory experiments were specifically designed to investigate the dynamics of parasitism by *T. foersteri* after exposure to *S. frugiperda* egg masses that had already been parasitized by *Te. remus*, and *vice versa*. This information is important because it helps understand whether competition among parasitoids influences their efficacy in controlling the target pest. Consequently, these findings could have practical implications for the development of pest management strategies using these parasitoids.

2 MATERIAL AND METHODS

2.1 ORIGIN AND REARING OF *T. foersteri* AND *Te. remus* AND THEIR HOSTS

Individuals of *T. foersteri* were collected on eggs of *A. gemmatalis* during the season 2017/2018 in commercial soybean crops in the municipality of São José dos Pinhais Pinhas (25° 36' 49.0" S, 49° 08' 01" W), state of Paraná, southern Brazil. This species was reared on *A. gemmatalis* eggs according to the methodology adapted from Parra (2001). Its host was reared according to the approaches described by Hoffman-Campo, Oliveira & Moscardi (1985). Before proceeding with the experiments, *T. foersteri* was reared on *S. frugiperda* eggs for two consecutive generations to ensure consistency and to avoid potential variation due to the use of different host species.

Parasitoids of *Te. remus* were obtained from parasitized eggs of *Spodoptera cosmioides* Walker (Lepidoptera: Noctuidae) collected from the same soybean crop where *T. foersteri* was found. The rearing of *Te. remus* was performed on eggs of *S. frugiperda* because this host was previously maintained in the laboratory. The methodology for rearing *S. frugiperda* was based on Parra (2001), using the artificial diet developed by Marchioro & Foerster (2012). The rearing of the insects and all experiments were carried out in controlled climatic chambers set at 25 ± 1 ºC, with a relative humidity of 70 ± 10% RH, and a 14:10 h photophase.

2.2 PARASITISM IN ONE- AND TWO-LAYER EGG MASSES

Single and double-layered egg masses of *S. frugiperda*, each containing approximately 250 eggs and less than 24h old, were provided early in the morning for parasitism by females of *T. foersteri* and *Te. remus*, with 20 replicates for each treatment. For each species, 20 copulated females, with no oviposition experience and less than 24 hours old were individually placed in glass tubes measuring 7.5 cm in length and 1.0 cm in diameter. These tubes were sealed with a cotton ball and contained droplets of pure honey as a food source. The tubes were placed in chambers at 25 ± 1 ºC, 70 ± 10% RH, and a photoperiod of 14:10 hours. The females had access to single or double-layered egg masses for 24 hours. After this period, the females were removed from the glass tubes, and the parasitized egg masses were observed daily until adult emergence. Egg masses containing one and two layers laid by female *S. frugiperda* were used to minimize manipulation and preserve the scales. These scales were visually categorized based on the classification proposed by Hou et al. (2022), ranging from 0 to 71.6 µm. The number of eggs parasitized in single- and double-layer egg masses was the biological parameter used to assess the influence of the number of egg layers on the parasitism of *T. foersteri* and *Te. remus.* Larvae of *S. frugiperda* hatched from non-parasitized eggs were counted and removed from the egg masses twice daily to prevent egg cannibalism.

2.3 INTERSPECIFIC COMPETITION BETWEEN *T. foersteri* AND *Te. remus*

In this experiment, interspecific competition between *T. foersteri* and *Te. remus* was assessed under three different conditions: (i) egg masses were initially parasitized by *T. foersteri* for 24 hours and subsequently exposed to *Te. remus* for an additional 24 hours; (ii) *Te. remus* had access to the egg masses for 24 hours, followed by *T. foersteri* for another 24 hours, and (iii) females of both parasitoids had simultaneous access to the egg masses for 24 hours. Each association was replicated twenty times. A replication consisted of two-layered egg masses containing approximately 250 *S. frugiperda* eggs (< 24 hours old), which were provided early in the morning for parasitism by copulated females, with no oviposition experience and less than 24 hours old, of each parasitoid species. The egg masses employed in this experiment followed the same protocol adopted in the previous experiment, although only two-layer egg masses were used in this instance. The egg masses were exposed to parasitism in glass tubes measuring 7.5 cm in length and 1.0 cm in diameter, with droplets of pure honey deposited on the walls of the glass tube. The tubes were sealed with a cotton ball and placed in chambers at 25 ± 1 ºC, 70 ± 10% RH, and a photoperiod of 14:10 hours. Larvae of *S. frugiperda* hatched from non-parasitized eggs were counted and removed from the egg masses twice daily to prevent egg cannibalism.

After parasitism, the females were removed from the glass tubes, and the parasitized egg masses were observed daily until adult emergence. The emerged parasitoids were then separated by species and counted. The biological parameters evaluated in this experiment were the number of emerged parasitoids of each species. Laboratory tests indicated that both parasitoid species tended to oviposit a single egg per *S. frugiperda* egg. Therefore, the number of emerged parasitoids for each species can also be interpreted as the number of parasitized eggs from which emergence was obtained.

2.4 STATISTICAL ANALYSIS

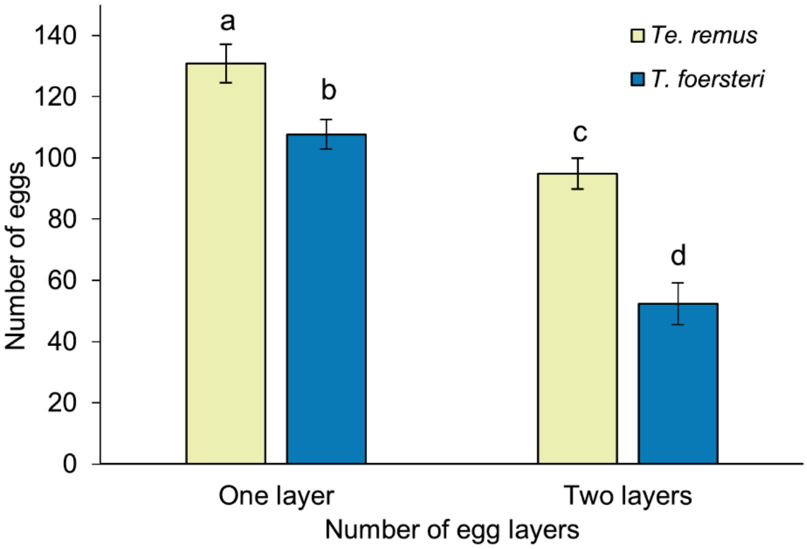
Generalized linear models were used to analyze the data on the parasitism in one- and two-layer egg masses, and on the interspecific competition between *T. foersteri* and *Te. remus*. The number of parasitized eggs and emerged parasitoids were analyzed with a quasi-Poisson distribution using a log link function. When statistical differences were observed, the means were compared with Tukey's post-hoc test at 5% probability. The relative contribution of each species to the total parasitism was compared with the chi-square test. All analyses were performed in the R statistical environment (R Core Team, 2022) using the packages *emmeans* (Lenth, 2023), *multcomp* (Hothorn, Bretz & Westfall, 2008), and *agricolae* (de Mendiburu, 2021).

3 RESULTS

3.1 PARASITISM IN ONE- AND TWO-LAYER EGG MASSES

The parasitism of *T. foersteri* and *Te. remus* was significantly influenced by the parasitoid species ($\chi^2 = 52.80$; df = 1; $p < 0.001$), the number of egg layers ($\chi^2 = 27.25$; df = 1; $p < 0.001$), and by the interaction between these two factors ($\chi^2 = 8.35$; df = 1; $p < 0.01$). Regardless of the number of egg layers, *Te. remus* parasitized more eggs of *S. frugiperda* than *T. foersteri*. Parasitism was higher when parasitoids were exposed to one-layer egg masses, with *Te. remus* parasitizing, on average, 130.95 ± 6.28 eggs, and *T. foersteri* parasitizing 107.65 ± 4.82 eggs. These differences were more pronounced when the parasitoids were exposed to egg masses containing two layers. In this case, *Te. remus* parasitized an average of 94.85 ± 5.02 eggs, while *T. foersteri* parasitized 52.30 ± 6.77 eggs (Figure 1).

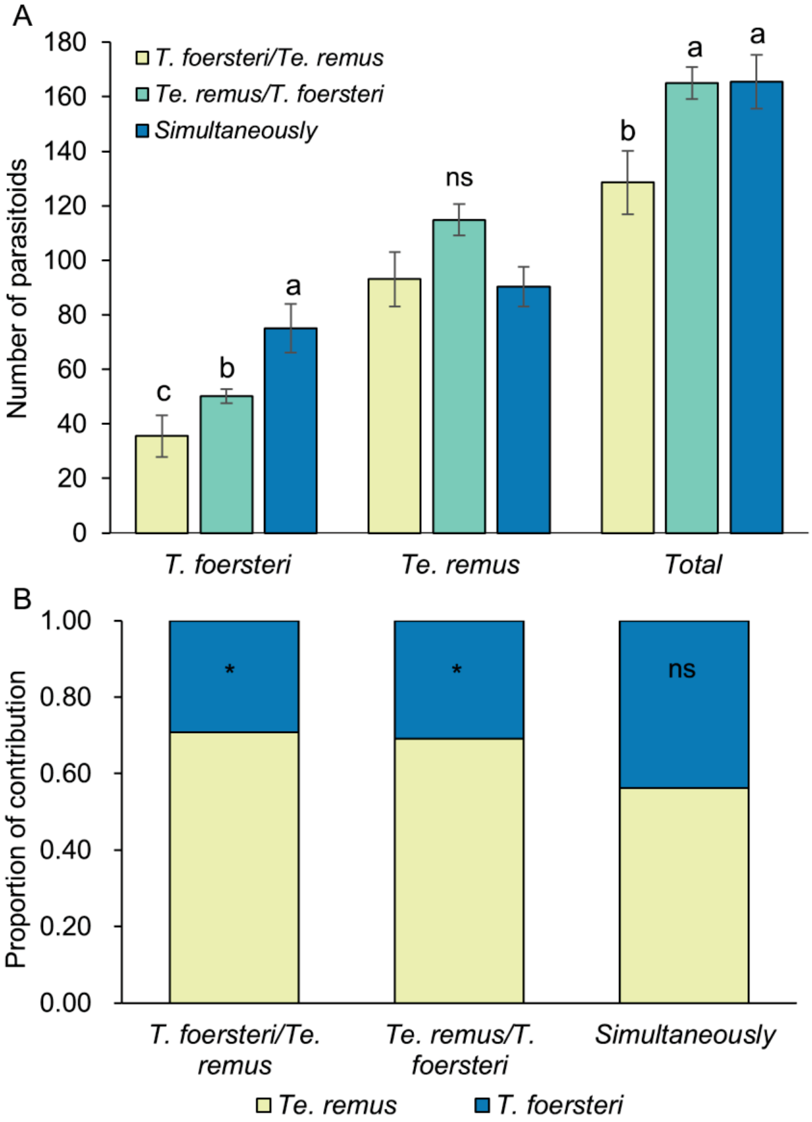
FIGURE 1 - Parasitism (mean ± standard error) of *Telenomus remus* and *Trichogramma foersteri* on single and double layered *Spodoptera frugiperda* egg masses. Different lowercase letters indicate statistical differences in the performance of both species due to the interaction between parasitoid species and number of egg layers, according to generalized linear models with Poisson distribution and Tukey's post-hoc test (P < 0.05).



3.2 INTERSPECIFIC COMPETITION BETWEEN PARASITOIDS

The number of emerged parasitoids by *T. foersteri* varied depending on the order of access to the egg masses ($\chi^2 = 15.90$; df = 2; P < 0.001). Interestingly, the emerged parasitoids of *T. foersteri* was higher when the parasitoid competed with *Te. remus* (Figure 2a). For example, *T. foersteri* exhibited increased emerged parasitoids when allowed to parasitize *S. frugiperda* eggs simultaneously with *Te. remus* (Figure 2a, b). On the other hand, the emerged parasitoids of *Te. remus* was not affected by competition with *T. foersteri* ($\chi^2 = 5.40$; df = 2; P = 0.06). In all tested associations, emerged parasitoids by *Te. remus* was consistently higher than that by *T. foersteri.* The highest number registered by *Te. remus* was achieved when the parasitoid first had access to the egg masses (114.85 ± 5.81 parasitized eggs).

FIGURE 2 - **a)** Number (mean ± standard error) of adults of *Telenomus remus* and *Trichogramma foersteri* emerged from double layered *Spodoptera frugiperda* egg masses under different competitive conditions. Different lowercase letters indicate statistical differences in the performance of each parasitoid species according to the order of access to the egg masses and based on generalized linear models with Poisson distribution and Tukey's post-hoc test ($P < 0.05$). **b)** Contribution of *Telenomus remus* and *Trichogramma foersteri* to the total number of parasitoids emerged under different competitive conditions. Asterisks indicate statistical differences in the contribution of species according to the order of access to the egg masses, and according to the chi-square test ($p < 0.05$). In both graphs, "ns" means non-significant.



The total number of emerged parasitoids by *T. foersteri* and *Te. remus* was influenced by the order of parasitoid access to host eggs ($\chi^2 = 9.81$; df = 2; $P < 0.01$). The number of emerged parasitoids was significantly higher when *Te. remus* was allowed to parasitize first, and when both parasitoids had simultaneous access to *S. frugiperda* egg masses (Figure 2a). Notably, the difference in the contribution of each species to the total number of emerged parasitoids was significantly similar when both species had simultaneous access to the egg masses ($\chi^2 = 1.40$; df = 1; p = 0.2374) (Figure 2b). When *T. foersteri* ($\chi^2 = 25.76$; df = 1; p <

0.001) and *Te. remus* ($\chi^2 = 25.32$; df = 1; $p < 0.001$) parasitized *S. frugiperda* eggs first, *Te. remus* contributed more to the total number of emerged parasitoids than *T. foersteri* (Figure 2b).

4 DISCUSSION

In this study, we investigated the parasitism of *T. foersteri* and *Te. remus* on *S. frugiperda* egg masses with one and two layers. In addition, we evaluated their parasitism when both parasitoids had access to *S. frugiperda* egg masses to elucidate whether they could be used together in a potential biological control program. Our results show that both species can parasitize *S. frugiperda* eggs, but the effectiveness of the parasitoids varies between species, the number of egg layers, and the order of access by the parasitoids to the host eggs.

The parasitism of *Te. remus* and *T. foersteri* on *S. frugiperda* eggs was reduced in egg masses with two layers compared to those with one layer. Similar findings have been observed in other parasitoid species (Beserra & Parra, 2005, Beserra et al., 2005, Mohamed, El-Heneidy & Dahi, 2023). For example, the number of eggs parasitized by *T. atopovirilia* Oatman & Platner and *T. pretiosum* Riley was higher in single-layer egg masses than in those with two layers. Likewise, a decrease in the parasitism of *Trichogrammatoidea bactrae* Nagaraja was observed when the parasitoid had access to egg masses containing more than 250 eggs arranged in two or three layers (Mohamed, El-Heneidy & Dahi, 2023). Generally, variations in parasitism in egg masses with multiple layers are attributed to the inability of certain parasitoids to parasitize the eggs in the lower layers, except for those located at the edge of the egg masses, which can be easily reached from the side (Carneiro & Fernandes 2012).

Telenomus remus parasitized more eggs than *T. foersteri* in egg masses containing one and two layers. Similar results were reported in other studies comparing the parasitism of *Te. remus* with other *Trichogramma* species (Goulart, Bueno, Bueno & Vieira, 2011; Carneiro & Fernandes 2012; Dong et al., 2021). The fall armyworm produces egg masses with one to four layers covered with scales, and the majority of egg masses laid during the female's lifespan consist of two layers (Hou et al., 2022). This behavior adversely impacts parasitism by *Trichogramma* species, which parasitize the outer layers but exhibit low efficiency in parasitizing eggs in the lower layers (Dong et al., 2021; Jin et al., 2021). In contrast, females of *Te. remus* are larger than *Trichogramma* (Carneiro & Fernandes 2012), facilitating access to eggs in the lower layers. For example, female *Te. remus* displace eggs from the outer layer to reach and parasitize the lower egg layer (Carneiro & Fernandes 2012; Dong et al., 2021).

Moreover, this species can move more easily over the egg masses by removing the scales that typically stick to the parasitoid's body (Carneiro & Fernandes 2012).

Although the number of parasitized eggs was lower compared to *Te. remus*, *T. foersteri* exhibited higher parasitism on *S. frugiperda* eggs than other *Trichogramma* species, as evidenced by a comparison with parasitism data from the literature. For example, in a study conducted by Jin et al., (2021), the number of eggs parasitized by seven *Trichogramma* species on single-layered egg masses of *S. frugiperda* ranged from 39.8 eggs for *T. dendrolimi* Matsumura to 101.5 eggs for *T. chilonis* Ishii. Similarly, *T. dendrolimi*, *T. bilingensis* He and Pang (Tian et al., 2020), *T. chilonis*, *T. dendrolimi*, and *T. pretiosum* (Yang et al., 2022) parasitized, on average, only 20.0, 9.6, 16.0, 30.0, and 26.0 *S. frugiperda* eggs, respectively. These parasitism rates were lower than the average of 107.6 eggs parasitized by *T. foersteri* in our study.

Numerous studies have highlighted the effective performance of *T. foersteri* in controlling various agricultural pests. For example, in comparison to *T. pretiosum*, *T. foersteri* demonstrated a significantly higher parasitization rate of eggs belonging to *Palpita forficifera* Munroe (Lepidoptera: Crambidae), the primary pest affecting olive crops (Villalba et al., 2023). In laboratory settings, *T. foersteri* could parasitize eggs of thirteen pest species spanning four lepidopteran families, including *S. frugiperda* (Takahashi et al., 2021). Both studies concluded that *T. foersteri* holds promising potential for integration into pest management programs. Moreover, prior research has indicated that this parasitoid can be successfully reared on eggs of the alternative host *Anagasta kuehniella* Zeller (Lepidoptera: Pyralidae) (Takahashi et al., 2021). This characteristic facilitates its mass rearing and commercial production, reinforcing its viability for practical implementation in pest control strategies.

Under field conditions, host eggs tend to remain available for parasitism and predation by different species, leading to direct and indirect competition (Boivin & Brodeur, 2006; Ode et al., 2022). The experiments carried out to evaluate the parasitism of *T. foersteri* and *Te. remus*, when both parasitoids had access to the host eggs, show that they can parasitize eggs previously foraged by the other species. In cases of multiparasitism, as observed in our study, when parasitoid females from different species attack the same host, the relationship between the parasitoid species tends to be asymmetrical due to interspecific competition (Boivin & Brodeur, 2006). Indeed, this asymmetry was observed in our study, with number of emerged parasitoids by *Te. remus* consistently higher than the observed for *T. foersteri* in all experiments. This finding is consistent with previous studies demonstrating a better performance of *Te. remus* when compared to *Trichogramma* species (Carneiro & Fernandes

2012; Dong et al., 2021). Similar results were observed under field conditions when 52 egg masses of *S. frugiperda* were exposed to parasitism (Silva et al., 2015). Adults of *Te. remus* emerged from all egg masses, while *T. atopovirilia* and *T. pretiosum* emerged only from three and 19 egg masses, respectively. Interestingly, these authors observed the emergence of all three parasitoid species from a single egg mass (Silva et al., 2015).

The observed competitive dominance of *Te. remus* over *Trichogramma* is likely due to its superior performance in parasitizing the lower layers of multilayered egg masses laid by female *Spodoptera* species (Silva et al., 2015; Li et al., 2023b). Under competitive conditions, physical and/or chemical alterations made by the parasitoids on hosts can inhibit or promote parasitism by another species (Aguirre et al., 2021). In the case of direct competition, these changes are even more pronounced (Boivin & Brodeur, 2006), as demonstrated in this study. While our findings indicate that parasitism by *Te. remus* is not affected by indirect and direct interactions with *T. foersteri*, the parasitism of the latter species increased as a result of interaction with *Te. remus*. This increase in parasitism was significantly higher when *T. foersteri* had access to the egg masses at the same time as *Te. remus*, resulting in improved performance of *T. foersteri* under competitive conditions. However, other *Trichogramma* species showed divergent behavior under competitive conditions. For example, contrary to our results, *T. pretiosum* did not parasitize *S. frugiperda* eggs previously foraged by *Te. remus* (Carneiro & Fernandes 2012). These results highlight the behavioral plasticity of *Trichogramma* and emphasize the importance of assessing how each species behaves in response to the competition with other parasitoids.

Although it is more difficult for a parasitoid to recognize the mark of another species (Boivin & Brodeur, 2006), it is possible that changes in the host eggs caused by *Te. remus*, or even its presence, promoted the parasitism behavior of *T. foersteri*. For example, the larger size of *Te. remus* females (Silva et al., 2015) may have favored the opening of pathways on the scales covering the eggs, thus facilitating the locomotion and parasitism of *T. foersteri* (Carneiro e Fernandes 2012; Dong et al., 2021). Regardless of the factors underlying the increase in parasitism by *T. foersteri*, our results show that competition between the two species may be beneficial for the control of *S. frugiperda*. This result is particularly important considering that competition between parasitoid species should be considered when selecting natural enemies (Boivin & Brodeur, 2006). Furthermore, both species were collected in the same region and at the same time, suggesting that the species not only coexist in the same area but may also share the same hosts.

In summary, the results show that parasitism is influenced by the parasitoid species and the number of egg layers. Regardless of the number of layers in the egg mass, *Te. remus* exhibited higher parasitism than *T. foersteri.* Furthermore, although the interaction with *T. foersteri* did not affect the parasitism by *Te. remus,* the number of parasitized eggs by *T. foersteri* increased significantly due to competition with *Te. remus.* The findings of our study are crucial for the development of biological control programs, as they indicate the potential use of both parasitoids, either individually or in combination, for controlling *S. frugiperda*.

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Author contributions: FS, CAM, and LAF contributed to the study conception and design. FS conducted experiments and statical analyses. FS, CAM, and LAF wrote the manuscript. All authors read and approved the manuscript.

Data availability statement

The data that support the findings of this study are openly available in Mendeley Data at http://doi.org/10.17632/wfr2nmfgzp.1.

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4 CAPÍTULO III - MODELING PARASITOID DEVELOPMENT: CLIMATE CHANGE IMPACTS ON *Telenomus remus* (NIXON) AND *Trichogramma foersteri* (TAKAHASHI) IN SOUTHERN BRAZIL[3]

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ABSTRACT

BACKGROUND

The egg parasitoids *Telenomus remus* (Nixon) and *Trichogramma foersteri* (Takahashi) were recently collected in southern Brazil, expanding their potential use in biological control. Understanding how these species respond to temperature is essential to the effective implementation of biological control programs, especially in the context of worldwide global warming. In this study, phenological models were employed to assess the effects of temperature and climate change on their development.

RESULTS

Temperature had a significant impact on the development of *Te. remus*, with development times ranging from 52.7 days at 15 °C to 8.1 days at 35 °C. Parasitism peaked at 35 °C (124.15 eggs) and lowest at 15 °C (38.5 eggs). Emergence rates declined under extreme temperatures, especially at 15 °C. The Brière-2 and Shi models were identified as the most appropriate for

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Te. remus and *T. foersteri*, respectively. Under the SSP2-4.5/2080 scenario, an increase in the number of generations was projected. In contrast, in the SSP5-8.5 scenario, higher temperatures may exceed the thermal thresholds of these species, potentially reducing voltinism in warmer regions while promoting it in colder areas.

CONCLUSION

Telenomus remus and *T. foersteri* exhibit broad thermal tolerance; however, extreme temperatures, including those predicted under climate change scenarios, can restrict their development. This study offers valuable insights for laboratory rearing programs, mass production, and field release programs while enhancing the understanding of thermal interactions in Hymenopteran parasitoids.

Keywords: Biological control, egg parasitoids, voltinism, development rate, global warming.

1 INTRODUCTION

The use of egg parasitoids for the biological control of insect pests is becoming increasingly common in modern agriculture (van Lenteren et al., 2018; Parra and Coelho Junior, 2019; Zang et al., 2021). In recent years, biological control has gained particular relevance in the management of *Spodoptera* spp., especially in *Bt* crops expressing *Bacillus thuringiensis* (Berliner) toxins, where insecticide applications are less frequent (Wengrat et al., 2021). Parasitoid wasps of the order Hymenoptera rank among the most important natural enemies of insect pests (Fei, Gols, and Harvey, 2023). Several species of micro-wasps from the genera *Trichogramma* (Westwood) and *Telenomus* (Haliday) are employed to control a broad range of pests in various regions worldwide (Parra and Coelho Junior, 2019; Zang et al., 2021; Colmenarez et al., 2022; Fortes et al., 2023).

The genus *Trichogramma* is notable for its widespread use, primarily because these parasitoids can be easily mass-reared in factitious hosts, facilitating large-scale production for field release (Parra and Coelho Junior, 2019). Conversely, some *Telenomus* species still lack well-established mass-rearing techniques (Colmenarez et al., 2022). Despite this, numerous reports document the natural occurrence of *Telenomus* species in field crops, where they contribute to pest control in these ecosystems (Kenis et al., 2019; Liao et al., 2019; Wengrat et al., 2021).

Trichogramma foersteri (Takahashi) was recently identified in southern Brazil (Takahashi et al., 2021), and recent studies have highlighted its high parasitism potential against various agricultural pests (Takahashi et al., 2021; Villalba et al., 2023; Sampaio et al., 2024; Sampaio, Marchioro, and Foerster, 2024). In contrast to *T. foersteri*, *Telenomus remus* (Nixon) is a well-known and extensively studied species worldwide (Kenis et al., 2019; Colmenarez et al., 2022). More than 35 years after the introduction of *Te. remus* in Brazil, the species was recently collected from *Spodoptera cosmioides* (Walker) eggs in the same region where *T. foersteri* was found (Wengrat et al., 2021). This discovery supports the hypothesis that the parasitoid can parasitize multiple species and sustain its population in the field.

The field capture of *T. foersteri* and *T. remus* opens new possibilities for the use of these parasitoids in Brazil. These findings provide valuable opportunities for further bioecological research on both species, which are crucial for biological control programs (Parra and Coelho Jr., 2022). Notably, the collection area for these species experiences well-defined seasons, characterized by warm summers and harsh winters (Alvares et al., 2013; Mintegui et al., 2019). Such climatic conditions may challenge the parasitoid’s survival, motivating an investigation into the influence of temperature on their development, as temperature is considered the primary abiotic factor affecting insect growth (Bale et al., 2002; Rebaudo and Rabhi, 2018). While a previous study evaluated the effects of temperature on the biology of *T. foersteri* (Nishimura et al., 2020), no information is currently available regarding the lineage of *Te. remus* collected in Southern Brazil.

Phenological models are increasingly used to understand how temperature affects insect development (Quinn, 2017; Rebaudo and Rabhi, 2018; Sampaio, Krechemer, and Marchioro, 2021a), although research on egg parasitoids remains scarce. Fitting multiple functions to developmental data across different temperatures is crucial for understanding parasitoid biology (Quinn, 2017), particularly in the case of *T. foersteri*, for which fundamental biological studies are still lacking. This knowledge has practical applications for developing laboratory rearing protocols aimed at mass production for commercial purposes, as well as for assessing the potential effects of climate change on parasitoids (Rebaudo and Rabhi, 2018; Jacques et al., 2019; Sampaio, Krechemer, and Marchioro, 2021b).

Climate change poses a significant challenge for parasitoids, as it is expected to substantially impact trophic interactions depending on species-specific biology and ecology (Hance et al., 2007; Furlong and Zalucki, 2017). In this context, determining the thermal thresholds that parasitoids can tolerate is essential for understanding how global warming may influence their survival and development. This knowledge is critical for guiding the management of these species in the coming years, considering that climate change could alter their capacity to persist in current habitats (Castex et al., 2018; Harvey et al., 2023). Despite its importance, there is a notable lack of studies evaluating the impacts of climate change on parasitoids, as most research focuses on insect pests. While projections generally indicate an increase in pest outbreaks and new invasions, few models account for the impact of climate change on natural enemies (Hance et al., 2007). In this context, climate change could not only reshape existing biological control programs but also create new opportunities for using parasitoids, depending on the species and region involved. Therefore, this study aimed to evaluate the effect of temperature on the development of *Te. remus*. Various phenological models were fitted to the data generated for *Te. remus* and to data from the literature for *T. foersteri* (Nishimura et al., 2020), with the most appropriate models for each parasitoid selected. These models were subsequently used to estimate the number of generations and the days when temperatures exceed the thermal thresholds tolerated by each species, both under current conditions and across different climate change scenarios.

2 MATERIAL AND METHODS

2.1 OBTAINING AND REARING *Te. remus* AND ITS HOST

Specimens of *Te. remus* were collected from parasitized eggs of *Spodoptera cosmioides* (Walker) in soybean crops (*Glycine max* L. Merril) during the 2018/19 growing season in São José dos Pinhais (25°36'49.0"S, 49°08'01"W), Paraná, Brazil (Wengrat et al., 2021). The fall armyworm (*Spodoptera frugiperda* (J. E. Smith)), the natural host of *Te. remus*, was collected from a maize crop during the same growing season at the Canguiri Experimental Farm in Pinhais (25°24'01"S, 49°07'01"W), Paraná, Brazil. The collected insects were maintained in the laboratory under controlled conditions of temperature, humidity, and photoperiod (25 ± 1 °C, 70 ± 10% RH, and a 14:10 light/dark cycle). Rearing of *Te. remus* followed the methodology described in the literature with adaptations (Parra, 2001). The parasitoid was reared on *S. frugiperda* eggs, which were kept in the laboratory according to established protocols (Parra, 2001) and fed with an artificial diet (Marchioro and Foerster, 2012).

2.2 EFFECT OF TEMPERATURE ON THE DEVELOPMENT AND SURVIVAL OF *Te. remus*

The experiments were conducted using a completely randomized design, with five treatments (constant temperatures) and 20 replicates per treatment. The effect of temperature on the development time and survival of *Te. remus* was evaluated in climate-controlled chambers set to constant temperatures of 15, 20, 25, 30, and 35 ± 1 °C, 70 ± 10% RH, and a 14:10 light/dark cycle. At each temperature, 20 glass tubes (7.5 cm in length and 1.0 cm in diameter) were used, each containing egg masses of approximately 200 *S. frugiperda* eggs (less than 24 hours old), fixed onto cardstock rectangles (6 cm x 1.5 cm). A single mated female *Te. remus*, less than 24 hours old and with no prior exposure to eggs, was placed in each tube to parasitize the egg mass for 24 hours. Drops of honey were provided as food inside the tubes, which were sealed with cotton. After the parasitism period, the females were removed. Emerging *S. frugiperda* larvae from non-parasitized eggs were counted and removed twice daily to prevent cannibalism. Following parasitoid emergence, the egg masses were examined under a stereoscopic microscope to determine the number of parasitized eggs, the emergence rate, and the sex of the emerged parasitoids.

At each temperature, the following parameters were evaluated: development time (from egg to adult), number of parasitized eggs (total number of darkened eggs), emergence rate (number of eggs with exit holes/total number of darkened eggs x 100), sex ratio (number of females/total number of adults), adult longevity, and survival of *Te. remus* adults. Adult longevity was assessed using 20 newly emerged pairs at 25 °C, each pair individually placed in glass tubes (7.5 cm in height x 1.0 cm in diameter) containing pure honey as food, without exposure to eggs. The tubes were maintained at the specified temperatures, with daily observations to record the death date of both males and females.

2.2.1 STATISTICAL ANALYSIS

Parameters related to the development of *Te. remus* at different temperatures were analyzed in the R computational environment (R Core Team, 2022), using generalized linear models with the *multcomp* package (Hothorn, Bretz, and Westfall, 2008). Development time was modeled with a Gamma distribution (inverse link function). A quasi-Poisson distribution (log link function) was applied for the number of parasitized eggs and adult longevity, while logistic regressions with a quasi-binomial distribution (logit link function) were used for the emergence rate and sex ratio. The chi-square test of independence was used to assess the effects of temperature on the evaluated parameters. When significant differences were detected between treatments, post-hoc comparisons were conducted using Tukey's test (Tukey, 1949), at a 5% significance level with the *emmeans* package (Lenth, 2024). Survival curves for each temperature were generated from the longevity data of females and males and compared using the non-parametric Kaplan-Meier method (Kaplan and Meier, 1958) with the *survival* package (Therneau, 2024).

2.3 SELECTION OF MATHEMATICAL MODELS

The development time data for *Te. remus* and *T. foersteri* at each temperature were used to estimate the thermal development thresholds of both species. Data on the development of *T. foersteri* at the constant temperatures of 15, 20, 25, 28 and 30 ºC were obtained from the literature (Nishimura et al., 2020). Twelve commonly used models from the literature were evaluated, including one linear and eleven non-linear models, which were fitted to the observed development rate (1/development time) across the tested temperatures for each parasitoid. The models and their estimated parameters are presented in Table 1.

TABLE 1 – Mathematical models employed to describe the temperature-dependent development rate of *Telenomus remus* and *Trichogramma foersteri.*

Model	**Function**†	**Reference**
Linear	$D(T) = a + b * T$	Campbell et al. (1974)
Beta-16	$D(T) = c_m \left[\left(\frac{T_H - T}{T_H - T_{opt}}\right)\left(\frac{T - T_L}{T_{opt} - T_L}\right)^{(T_{opt} - T_b)/(T_H - T_{opt})}\right]^{\delta}$	Shi et al. (2016)
β type	$D(T) = \rho \cdot \left(a \frac{T}{10}\right) \cdot \left(\frac{T}{10}\right)^{\beta}$	Damos et al. (2008)
Brière-1	$D(T) = aT(T - T_L)(T_H - T)^{1/2}$	Brière et al. (1999)
Brière-2	$D(T) = aT(T - T_L)(T_H - T)^{1/m}$	Brière et al. (1999)
Kontodimas	$D(T) = \alpha(T - T_L)^2(T_H - T)$	Kontodimas (2004)
Lactin-1	$D(T) = e^{(\rho T)} - e^{\left(\rho T_H - \frac{T_H - T}{\Delta T}\right)}$	Lactin et al. (1995)
Lactin-2	$D(T) = e^{(\rho T)} - e^{\left(\rho T_H - \frac{T_H - T}{\Delta T}\right)} + \lambda$	Lactin et al. (1995)
Logan-6	$D(T) = \varphi\left[e^{(\rho T)} - e^{(\rho T_H - \frac{(T_H - T)}{\Delta T}}\right]$	Logan et al. (1976)
Performance-2	$D(T) = c(T - T_L)(1 - e(K(T - T_H)))$	Shi et al. (2011)
Shi	$D(T) = c(1 - e[-K1(T - T_L)])(1 - e[K2(T - T_H)])$	Shi et al. (2011)
Taylor	$D(T) = Rm\, e\left\{-0{,}5\left[\frac{(T - T_{opt})}{T_L}\right]^2\right\}$	Taylor (1981)

† T_L. T_H, T_{opt} are, respectively, the lower temperature threshold, upper temperature threshold and the optimum temperature for development (ºC), *K* is the thermal constant. The remaining parameters are fitted coefficients.

The linear model estimates the lower thermal threshold (T_L) and the thermal constant (*K*) using the x-axis intersection method ($T_L = -a/b$) and the reciprocal slope calculation ($K = 1/b$), respectively (Campbell et al., 1974). Non-linear models are more complex due to their larger number of parameters and, in addition to describing the relationship between temperature and development rate, some can also explain the physiological and biochemical mechanisms underlying insect responses to temperature (Brière et al., 1999). Therefore, besides being evaluated using statistical criteria to assess the quality of fit to the observed data, the models were also assessed for their ability to accurately estimate biologically meaningful parameters (Brière et al., 1999; Roy, Brodeur, and Cloutier, 2002; Kontodimas et al., 2004; Marchioro, Krechemer, and Foerster, 2017). All model parameters were estimated using the *devRate* package (Rebaudo, Struelens, and Dangles, 2018) in the R computational environment (R Core Team, 2022), employing the Levenberg-Marquardt algorithm.

2.3.1 EVALUATION OF MATHEMATICAL MODELS

2.3.1.1 STATISTICAL CRITERIA

The quality of the models was evaluated using regression error (S), Akaike Information Criterion (AIC) (Akaike, 1974), and Bayesian Information Criterion (BIC) (Schwarz, 1978). AIC is widely used to assess the relative quality of models by balancing complexity (number of parameters) with goodness of fit. BIC, another statistical tool used for model selection, is partially based on the likelihood function and closely related to AIC. Models with lower S, AIC, and BIC values were considered more suitable (Sandhu et al., 2010; Negahban et al., 2021). Additionally, ΔAIC and ΔBIC (differences between the lowest AIC and BIC values for each model) were calculated, with values ≤ 2 indicating comparable model performance (Burnham, Anderson, and Huyvaert, 2011). These criteria were calculated using the *devRate* package (Rebaudo, Struelens and Dangles, 2018) in R (R Core Team, 2022).

2.3.1.2 BIOLOGICALLY MEANINGFUL PARAMETERS

The lower (T_L) and upper (T_H) thermal thresholds, optimal temperature (T_{opt}), and thermal constant (K) were considered biologically meaningful parameters (Brière et al., 1999; Kontodimas et al., 2004; Marchioro, Krechemer, and Foerster, 2017). Observed laboratory data for both species were compared with values predicted by each model. For *Te. remus*, thermal thresholds outside the ranges between 6.0 and 15.0 °C for T_L, 32.0 and 34.0 °C for T_{opt}, and 34.0 and 36.0 °C for T_H were regarded as unrealistic. For *T. foersteri*, thresholds outside 8.0 and 15.0 °C for T_L, 30.0 and 32.0 °C for T_{opt}, and 32.0 and 33.0 °C for T_H: were considered unrealistic. Preference was given to models that estimated thermal thresholds within these expected ranges.

2.4 ANNUAL GENERATION ESTIMATES

2.4.1 STUDY AREA

The number of annual generations of *Te. remus* and *T. foersteri* were estimated under current climatic conditions and future projections for southern Brazil. This region, covering approximately 576,774 km², encompasses humid subtropical and oceanic climates, according to the Köppen classification (Alvares et al., 2013). Mountainous areas in Santa Catarina and Rio Grande do Sul are characterized by colder climates, mild summers, and harsh winters, with frequent frosts and occasional snowfall (Mintegui et al., 2019). Conversely, warmer regions, such as northwestern Paraná and parts of Rio Grande do Sul, experience hot summers and mild winters.

2.4.2 CLIMATE DATA

Daily climatic data representative of current conditions for simulate parasitoid voltinism, were obtained from the literature (https://utexas.box.com/Xavier-etal-IJOC-DATA), covering 908 grids of 0.25° each across the entire study area. These data include 20 years (1994–2013) of minimum and maximum daily air temperatures, interpolated from meteorological stations (Xavier, King, and Scanlon, 2016). For each grid and day of the year, a 20-year average of the climatic data was calculated prior to its use in voltinism estimations.

To assess future warming in the study area, data were obtained from the Sixth Assessment Report of the IPCC on climate change (2021) (IPCC, 2023) and sourced from the WorldClim version 2.1 database (https://worldclim.org). These data included current climate conditions (1970–2000) (Fick and Hijmans, 2017) as well as projections for 2040 and 2080 based on SSP2-4.5 and SSP5-8.5 climate change scenarios. SSP2-4.5 predicts an intermediate emissions scenario, with projected global warming of 2.0 °C for 2041–2060 and 2.7 °C for 2081–2100. SSP5-8.5 is a high emissions scenario, with projected global warming of 2.4 °C for 2041–2060 and 4.4 °C for 2081–2100 (IPCC, 2023).

Projections were based on four GCMs: BCC - Beijing Climate Center; MIROC6 - Model for Interdisciplinary Research on Climate; CMCC-ESM2 - Euro-Mediterranean Center on Climate Change - Earth System Model; and CNRM-CM6-1 - Centre National de Recherches Météorologiques - Climate Model 6.1. To reduce uncertainties, the average of the four GCMs was calculated, generating consensus maps for each year and climate scenario. The magnitude of warming in the area was estimated by calculating the differences between future and current mean annual temperatures (in °C) using the R statistical environment (R Core Team, 2022). These differences, specific to each grid cell and climate scenario, were then added to the minimum and maximum daily air temperatures (Xavier, King, and Scanlon, 2016) to account for regional variations in global warming (Xia et al., 2014).

2.4.3 VOLTINISM OF *Te. remus* AND *T. foersteri*

The best models selected in the previous step were used to calculate voltinism. In this approach, the daily development rate was calculated as the average of the values obtained from the minimum and maximum air temperatures. These daily development rates were then accumulated over consecutive days, with a complete generation considered when the total development reached a value of 1. The voltinism of *Te. remus* and *T. foersteri* was estimated using current and future climatic data, with the egg-to-adult lifecycle considered as a complete generation. All calculations were performed in the R computational environment (R Core Team, 2022).

2.4.4 DAYS EXCEEDING THERMAL THRESHOLDS OF *Te. remus* AND *T. foersteri*

To simulate the impact of current and future climatic conditions on parasitoid development, the thermal threshold estimates (lower threshold, T_L, and upper threshold, T_H) from the selected models were applied. The number of days per year with daily minimum temperatures below T_L and daily maximum temperatures above T_H was analyzed using daily climatic data for the study area. This analysis provides insights into potential climatic constraints and favorable periods for the development of *Te. remus* and *T. foersteri*, offering projections of how shifts in temperature regimes might influence their biological cycles.

3 RESULTS

3.1 EFFECT OF TEMPERATURE ON THE DEVELOPMENT AND SURVIVAL OF *Te. remus*

Temperature significantly influenced the development time of *Te. remus* ($\chi^2 = 110203$; df = 4; $P < 0.001$), with an inverse relationship between temperature and development duration. The development time ranged from 52.70 ± 0.41 days at 15 ºC to 8.10 ± 0.03 days at 35 ºC (Table 2).

TABLE 2 – Development (mean ± standard error) of *Telenomus remus* at different constant temperature regimes parasitizing *Spodoptera frugiperda* eggs.

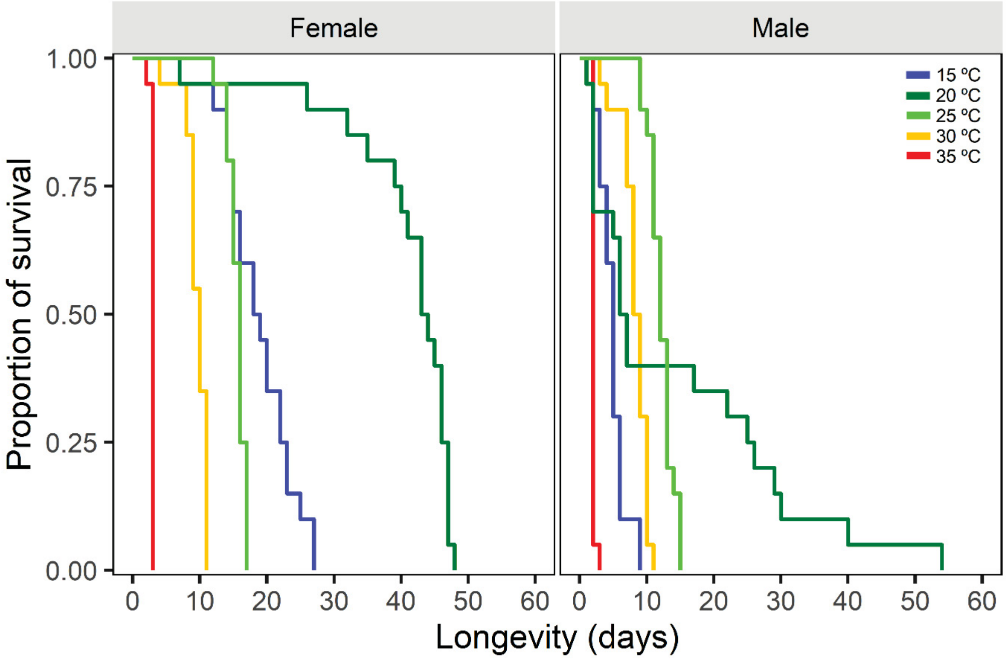
T (°C)	Egg-adult duration (days)†	Eggs parasitized†	Emergence rate (%)†	Sexual ratio (%)†
15	52.70 ± 0.41 a	38.50 ± 2.91 e	15.29 ± 2.95 b	0.94 ± 0.03 a
20	21.09 ± 0.11 b	106.45 ± 3.51 d	93.73 ± 0.90 a	0.87 ± 0.01 ab
25	12.49 ± 0.03 c	110.95 ± 6.14 c	95.15 ± 0.95 a	0.83 ± 0.01 b
30	8.26 ± 0.02 d	120.60 ± 7.35 b	96.28 ± 1.43 a	0.82 ± 0.02 b
35	8.10 ± 0.03 e	124.15 ± 5.02 a	78.16 ± 2.05 a	0.86 ± 0.01 b

† Means followed by the same letters within the columns are not statistically different according to Tukey test at a significance level of 5%.

The number of eggs parasitized by *Te. remus* varied significantly with temperature ($\chi^2 = 227.46$; df = 4; $P < 0.001$). Parasitism peaked at 35 ºC (124.15 eggs) and was lowest at 15 ºC (38.50 eggs). Similarly, temperature significantly influenced the emergence rate of *Te. remus* ($\chi^2 = 671.64$; df = 4; $P < 0.001$), with lower rates observed at extreme temperatures, particularly at 15 ºC, where only 15.29% of parasitized eggs emerged. However, no significant differences in emergence rates were detected among the other temperatures tested (Table 2). Additionally, development was monitored for 100 days at 12 ºC, but no parasitoid emergence occurred, indicating that this temperature is below the threshold required for *Te. remus* development under constant exposure.

The sex ratio of *Te. remus* was significantly influenced by temperature ($\chi^2 = 18.09$; df = 4; $P < 0.01$). At low temperatures (15 and 20 ºC), a higher proportion of females emerged compared to males, with the highest ratio observed at 15 ºC (0.94 ± 0.03) (Table 2). Longevity was significantly influenced by temperature ($\chi^2 = 491.45$; df = 4; $P < 0.001$), sex ($\chi^2 = 149.39$; df = 1; $P < 0.001$), and the interaction between these factors ($\chi^2 = 53.49$; df = 4; $P < 0.001$). Females exhibited greater longevity than males at all evaluated temperatures (Fig. 1). The longest lifespan for both sexes occurred at 20 ºC, with females living an average of 40.60 ± 2.17 days and males 14.55 ± 4.07 days. Conversely, at 35 ºC, females and males survived only 2.95 ± 0.05 and 2.05 ± 0.11 days, respectively. Survival analysis confirmed these findings, showing significant differences in longevity for females ($\chi^2 = 178$; df = 4; $P < 0.001$) and males ($\chi^2 = 94.5$; df = 4; $P < 0.001$) across the temperature range tested (Fig. 1).

FIGURE 1 - Longevity of *Telenomus remus* adults reared at different constant temperatures regimes. Survival curves were generated with the non-parametric analysis of Kaplan-Meier.



3.2 SELECTION AND PERFORMANCE OF MATHEMATICAL MODELS

The fit and performance of the twelve evaluated models evaluated varied according to the development data of each species (Figs. 2 and 3). Based on S, ΔAIC, and ΔBIC values, the models with the best fit for *Te. remus* and *T. foersteri* were Brière-2 and Shi, respectively (Table 3). The estimated thermal thresholds differed depending on the mathematical model used. For example, for *Te. remus*, T_L ranged from -17.89 to 12.66 ºC, while T_H ranged from 35.04 to 46.79 ºC. For *T. foersteri*, T_L varied between 5.88 and 11.87 ºC, and T_H between 32.22 and 41.55 ºC. The linear model estimated T_L as 11.16 for *Te. remus* and 9.55 ºC for *T. foersteri*, with a thermal constant of 176.94 degree-days for *Te. remus* and 162.43 for *T. foersteri* to complete the egg-to-adult development (Table 4).

FIGURE 2 - Fitting of the mathematical models used to describe the temperature-dependent development rate of *Telenomus remus* for egg-adult life cycle.

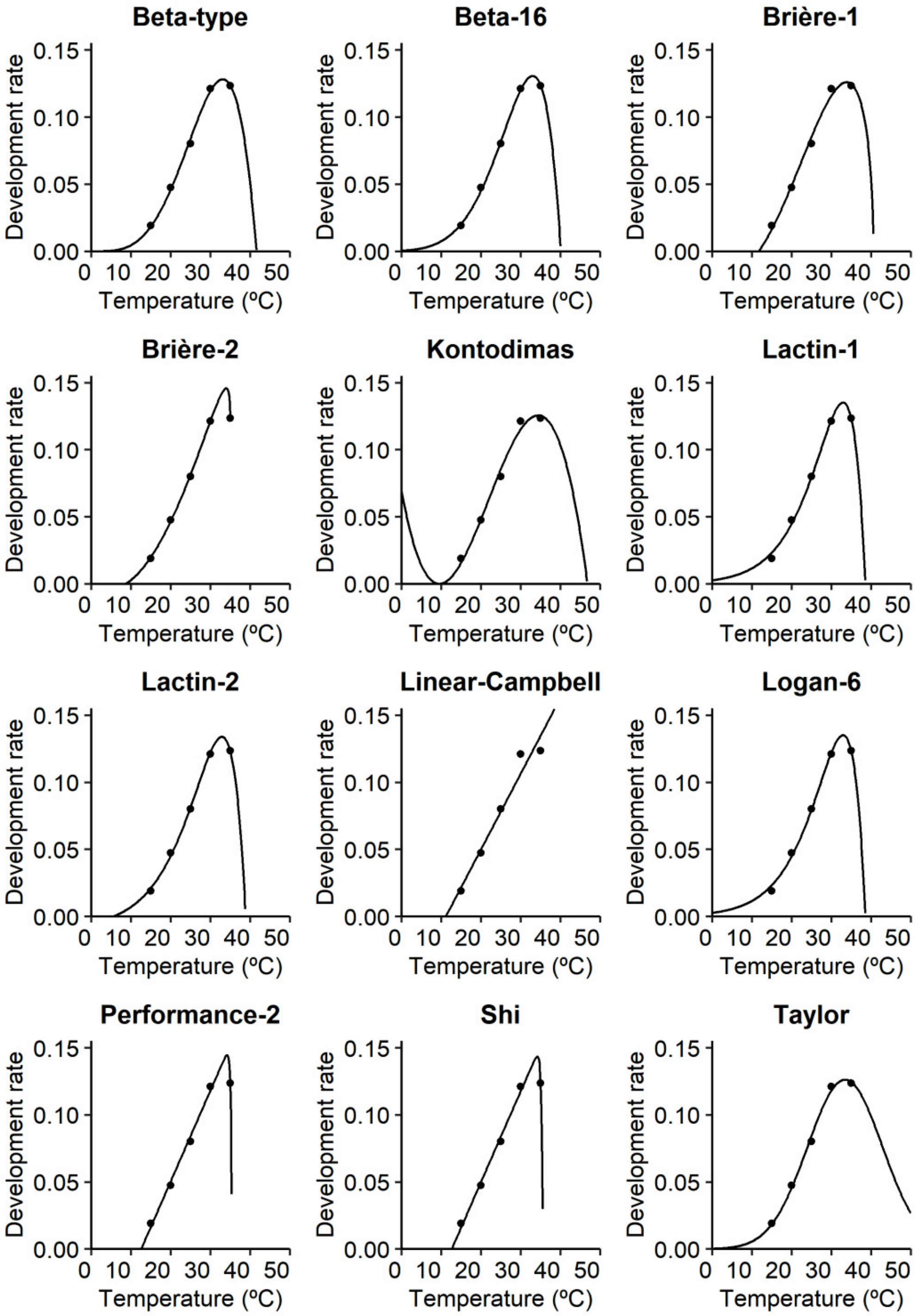


FIGURE 3 - Fitting of the mathematical models used to describe the temperature-dependent development rate of *Trichogramma foersteri* for egg-adult life cycle.

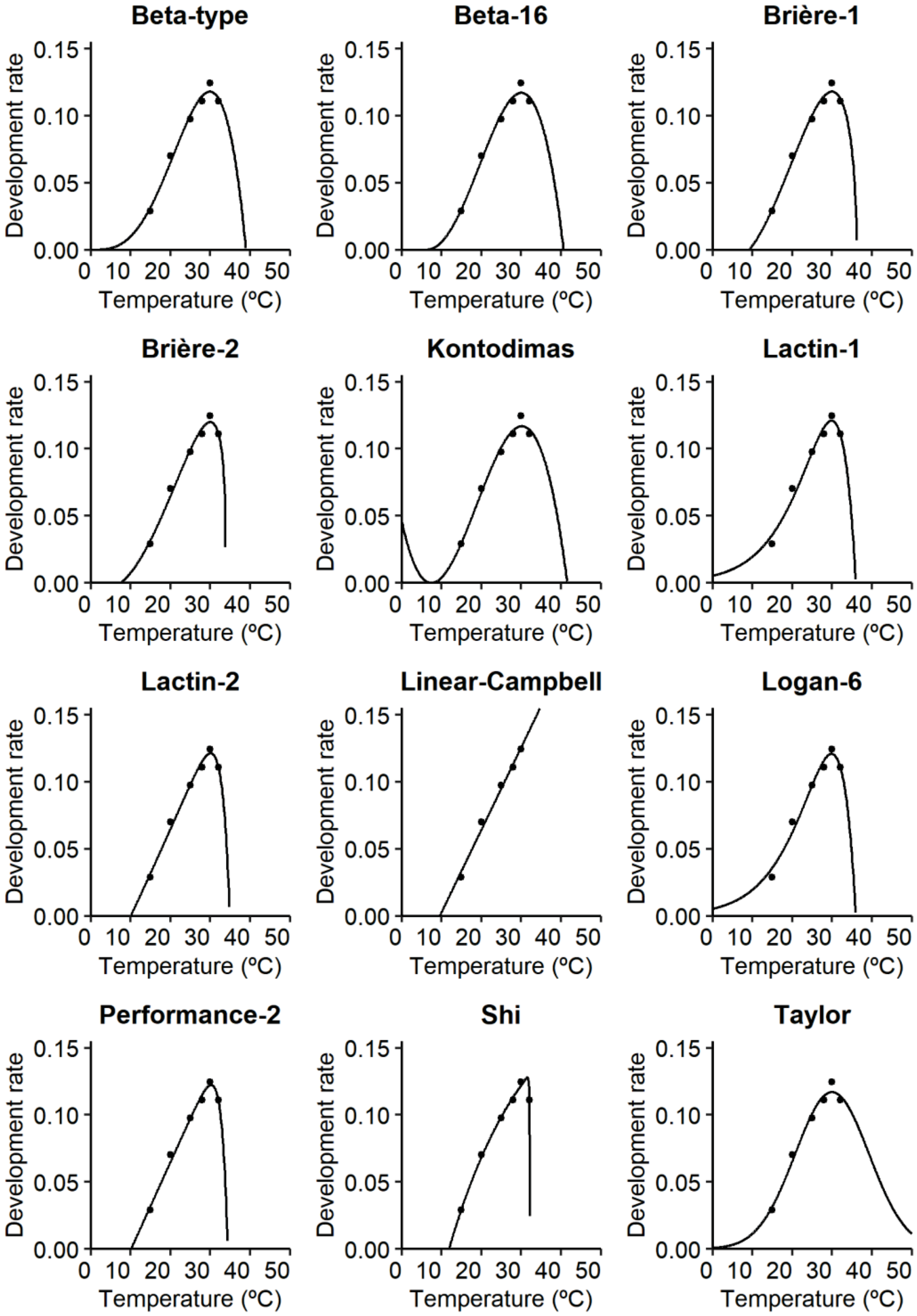


TABLE 3 – Performance of the twelve mathematical models used to describe the temperature-dependent development rate of *Telenomus remus* and *Trichogramma foersteri* based on the standard error of the regression (S), the Akaike Information Criterion (AIC), and Bayesian Information Criterion (BIC).

Model	Egg to adult					
	Telenomus remus			*Trichogramma foersteri*		
	S (10^{-3})	ΔAIC[†]	ΔBIC	S (10^{-3})	ΔAIC[†]	ΔBIC
Linear	10.94	20.25	21.03	4.80	-[‡]	-[‡]
Beta-16	3.22	7.99	8.38	5.80	7.49	7.91
β type	3.34	7.20	7.20	6.97	9.28	9.49
Brière-1	5.70	13.70	14.09	5.30	6.42	6.84
Brière-2	**1.67**	**0.00**	**0.00**	6.30	8.06	8.27
Kontodimas	6.25	14.63	15.02	5.71	7.32	7.74
Lactin-1	3.70	9.39	9.78	6.98	9.72	10.14
Lactin-2	3.49	7.33	7.33	5.71	6.88	7.09
Logan-6	5.24	11.40	11.40	8.55	11.73	11.94
Performance-2	6.35	13.32	13.32	5.45	6.32	6.53
Shi	6.35	15.52	15.13	**3.85**	**0.00**	**0.00**
Taylor	4.05	10.30	10.69	6.23	8.36	8.78

[†] Bold S, ΔAIC and ΔBIC values indicate models with better performance (ΔAIC ≤ 2).

[‡] Values not calculated because of the differences in the number of samples due to the exclusion of the data obtained at 35 ºC when fitting the linear model.

TABLE 4 – Parameter values (± SE) for each mathematical model used to describe the relationship between temperature and development rate of *Telenomus remus* and *Trichogramma foersteri*.

Model[†]	Egg-adult life cycle	
	Telenomus remus	*Trichogramma foersteri*
Linear		
a	-0.06 ± 0.01	-0.05 ± 0.01
b (10^{-3})	5.65 ± 0.69	6.15 ± 0.39
T_L	11.16	9.55
K	176.94	162.43
Beta-16		
c_m (10^{-1})	1.30 ± 0.04	1.17 ± 0.04
T_L	-17.89 ± 34.72	5.88 ± 12.11
T_H	40.07 ± 1.61	40.62 ± 6.11
T_{opt}	32.96 ± 0.35	30.06 ± 1.39
β type		
ρ (10^{-2})	0.14 ± 0.02	0.34 ± 0.07
a	4.16 ± 0.09	3.89 ± 0.18
β	3.90 ± 0.22	3.30 ± 0.35
T_H	41.60	38.90
T_{opt}	34.05	29.90
Brière-1		
a (10^{-5})	6.51 ± 1.00	7.65 ± 1.13
T_L	11.73 ± 1.32	9.20 ± 1.52
T_H	40.53 ± 1.46	36.11 ± 0.99
T_{opt}	33.80	30.00
Brière-2		
a (10^{-4})	1.69 ± 0.09	1.20 ± 0.56
m	14.22 ± 10.32	3.98 ± 3.14
T_L	8.72 ± 1.14	7.5 ± 4.26

T_H	35.04 ± 11.33	33.81 ± 2.82
T_{opt}	34.00	30.00
Kontodimas		
α (10^{-5})	1.63 ± 0.54	1.99 ± 0.59
T_L	9.50 ± 1.45	7.47 ± 1.37
T_{opt}	34.40	30.20
T_H	46.79 ± 3.04	41.55 ± 2.15
Lactin-1		
ρ	0.17 ± 0.008	0.16 ± 0.01
Δ	5.60 ± 0.28	6.07 ± 0.60
T_H	38.53 ± 0.45	35.94 ± 0.99
T_{opt}	32.90	29.90
Lactin-2		
ρ	0.16 ± 0.02	0.59 ± 0.07 (10^{-2})
Δ	6.11 ± 0.90	1.88 ± 1.45
λ	-0.008 ± 0.01	-1.06 ± 0.01
T_L	5.70	10.10
T_H	39.03 ± 1.02	38.66 ± 4.70
T_{opt}	32.90	30.10
Logan-6		
ρ	0.17 ± 1.70	0.16 ± 2.78
Δ	5.46 ± 56.04	5.91 ± 106.25
φ (10^{-1})	0.08 ± 29.07	0.19 ± 1101.93
T_H	38.53 ± 1.83	35.94 ± 2.89
T_{opt}	32.90	29.90
Performance-2		
cc (10^{-3})	6.78 ± 0.89	6.56 ± 0.76
K	3.43 ± 3.14	0.63 ± 0.54
T_L	12.63 ± 1.11	10.10 ± 1.24
T_H	35.49 ± 4.47	34.36 ± 1.81
T_{opt}	34.20	30.2
Shi		
c	9.22 ± 1.26	0.20 ± 0.08
$K1$(10^{-2})	0.07 ± 7.53	4.97 ± 3.14
$K2$	2.95 ± 0.04	8.57 ± 5.33
T_L	12.66 ± 11.31	11.87 ± 1.00
T_H	35.57 ± 0.09	32.22 ± 1.39
T_{opt}	34.20	31.60
Taylor		
Rm (10^{-1})	1.26 ± 0.03	1.16 ± 0.03
T_L	9.39 ± 0.66	9.28 ± 1.07
T_{opt}	33.44 ± 0.79	29.99 ± 1.17

† T_L. T_H, T_{opt} are, respectively, the lower temperature threshold, upper temperature threshold and the optimum temperature for development (ºC), K is the thermal constant. The remaining parameters are fitted coefficients.

Some models produced unrealistic thermal thresholds. For *Te. remus*, the Beta-16 model estimated a T_L of -17.89 ºC, and the Kontodimas model estimated a T_H of 46.79 ºC. Despite these discrepancies, certain models provided acceptable thresholds, consistent with the experimental conditions and the region where the parasitoids were collected, such as the linear and Performance-2 models for both species. The number and accuracy of the estimated thermal thresholds, along with the quality of fit (Table 5), confirmed that the Brière-2 and Shi models were the most suitable for *Te. remus* and *T. foersteri*, respectively. Therefore, these models were employed to estimate the voltinism of *Te. remus* and *T. foersteri*.

TABLE 5 – Thermal thresholds estimated by each model used to describe the temperature-dependent development rate of *Telenomus remus* and *Trichogramma foersteri*, and their accuracy inferred based on the observed development times and on the known species distribution range.

Models	Number of estimated thermal thresholds	Accuracy†					
		Telenomus remus			*Trichogramma foersteri*		
		T_L	T_{opt}	T_H	T_L	T_{opt}	T_H
Linear	1	+	•	•	+	•	•
Beta-16	3	-	+	-	+	+	-
β type	2	•	+	-	•	+	-
Brière-1	3	+	+	-	+	+	-
Brière-2	**3**	+	+	+	+	+	+
Kontodimas	3	+	+	-	+	+	-
Lactin-1	2	•	+	-	•	+	-
Lactin-2	3	-	+	-	+	+	-
Logan-6	2	•	+	-	•	+	-
Performance-2	3	+	+	+	+	+	+
Shi	**3**	+	+	+	+	+	+
Taylor	2	+	+	•	+	+	•

T_L and T_H are, respectively, the lower and upper thermal thresholds, and T_{opt} is the optimum temperature.
† +, yes; -, no; •, parameter not estimated by the model.

3.3 VOLTINISM UNDER CURRENT AND PROJECTED CLIMATE CONDITIONS

The lower and upper thermal thresholds estimated by the Brière-2 model for *Te. remus* were 8.72 °C and 35.04 °C, respectively. Using the Shi equation, the T_L estimated for *T. foersteri* was 11.87 ºC, while the T_H was 32.22 ºC. Under current climatic conditions, the number of generations in the study area varied from 8.27 to 13.89 for *Te. remus* and from 12.94 to 30.87 for *T. foersteri* (Fig. 4 and 5). Both species exhibited a similar geographic pattern in voltinism, with fewer generations in high-altitude areas of southern Santa Catarina and northern Rio Grande do Sul, and more generations in warmer regions, such as western, northwestern, and coastal Paraná, as well as northeastern Santa Catarina.

FIGURE 4 – Voltinism of *Telenomus remus* in southern Brazil estimated by the model Brière-2. Future climate projections for 2040 and 2080 derived from the climate change scenarios SSP-4.5 and SSP-8.5.

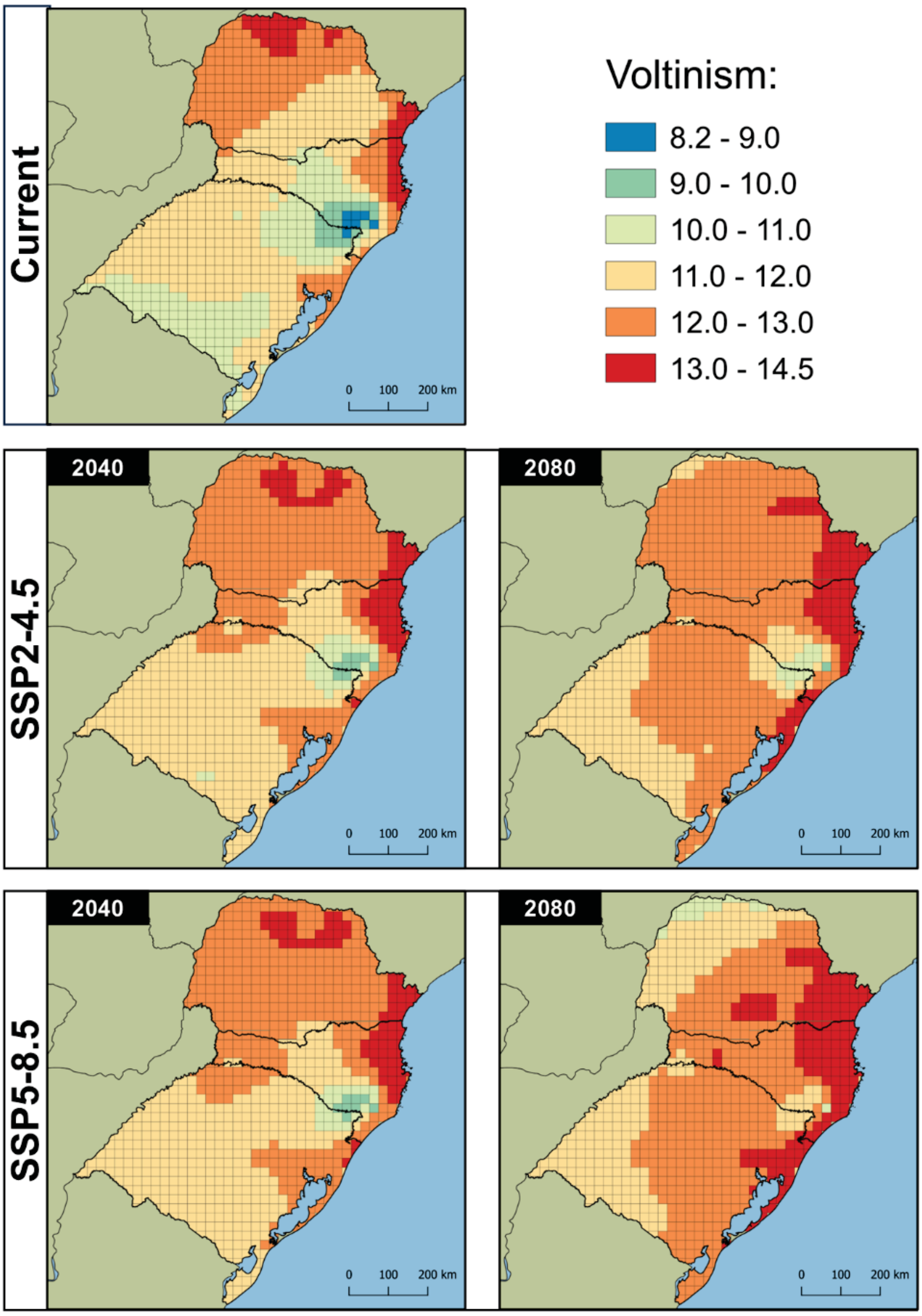
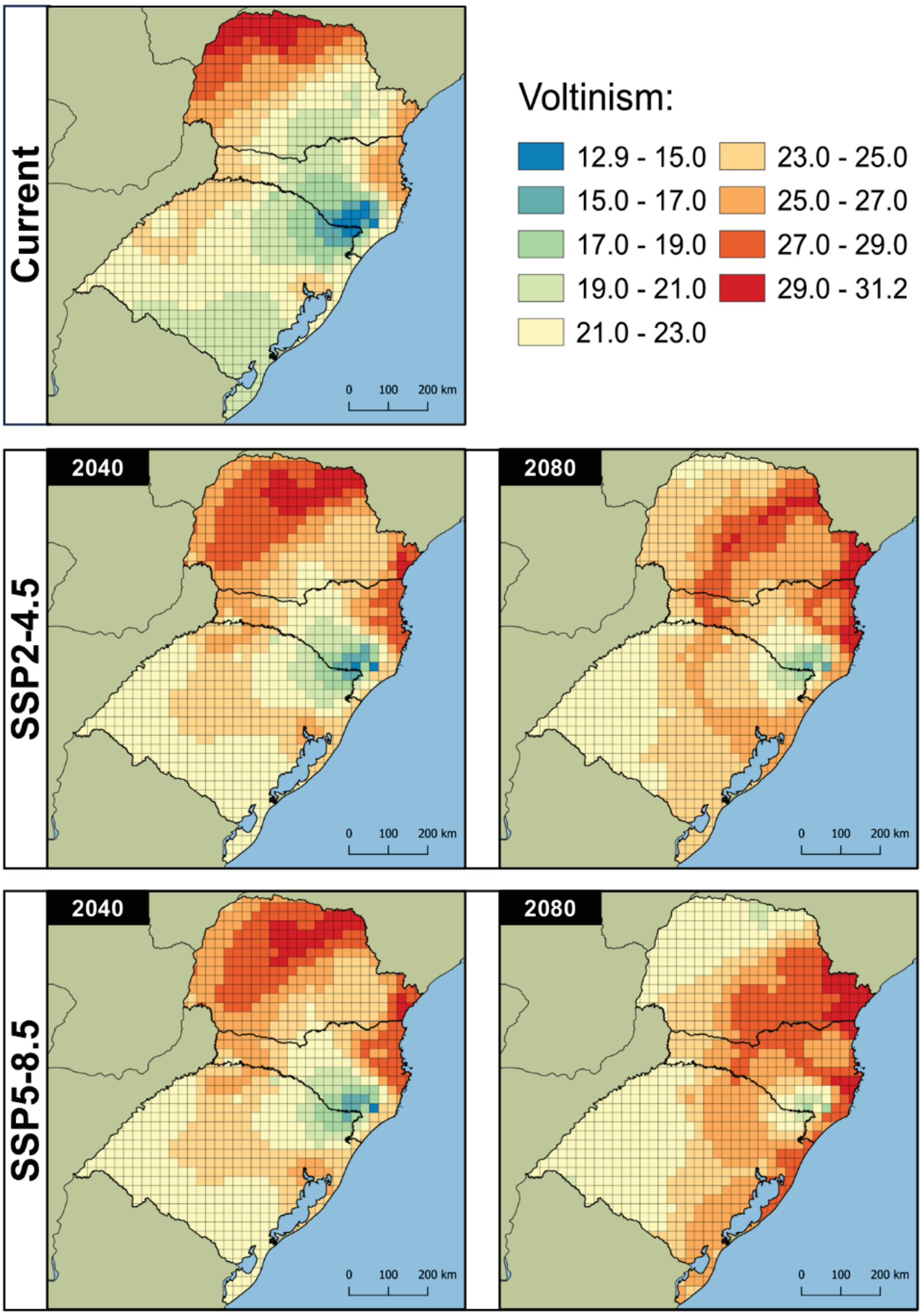


FIGURE 5 – Voltinism of *Trichogramma foersteri* in southern Brazil estimated by the model Shi. Future climate projections for 2040 and 2080 derived from the climate change scenarios SSP-4.5 and SSP-8.5.



When the parasitoids' voltinism was estimated under different climate change scenarios and periods, an increase in the number of generations was observed for both species. In the SSP2-4.5/2040 scenario, the number of generations ranged from 9.11 to 14.32 for *Te. remus* and from 14.67 to 30.38 for *T. foersteri*. By 2080, under the same scenario, the estimated number of generations increased to 9.98 - 14.52 for *Te. remus* and 16.52 - 31.20 for *T. foersteri*. Under the SSP5-8.5/2040 scenario, the range was 9.23 - 14.37 for *Te. remus* and 14.92 - 30.41

for *T. foersteri*. Finally, in the SSP5-8.5/2080 scenario, the number of generations rose to 10.53 - 14.47 for *Te. remus* and 18.61 - 30.89 for *T. foersteri*.

The SSP2-4.5/2080 scenario showed the greatest increase in the number of generations. Notably, the higher voltinism observed under SSP2-4.5 for both species suggests that the temperature rise projected in the SSP5-8.5 scenario (2.4 ºC for 2041-2060 and 4.4 ºC for 2081-2100) may exceed the upper thermal thresholds tolerated by the parasitoids, thereby reducing voltinism in warmer regions (Fig. 4 and 5). Conversely, in colder regions of the study area, this temperature increase may promote the development of both species, leading to a higher number of annual generations.

Regardless of the species evaluated, the models projected an increase in the number of generations in colder regions under future climate scenarios. The SSP5-8.5/2080 scenario showed the highest increases, with up to 33.98% (2.81 generations) for *Te. remus* and 47.07% (6.22 generations) for *T. foersteri*. In the same scenario, the largest decrease in the number of generations was observed in warmer regions, with potential reductions of up to 19.01% (-2.51 generations) for *Te. remus* and 31.22% (-9.59 generations) for *T. foersteri* (Fig. 6 and 7).

FIGURE 6 – Differences (%) between current and future projections of voltinism for *Telenomus remus* in southern Brazil estimated with the model Brière-2. Future climate projections for 2040 and 2080 derived from the climate change scenarios SSP-4.5 and SSP-8.5. Negative and positive values refer to decrease and increase in the number of generations, respectively.

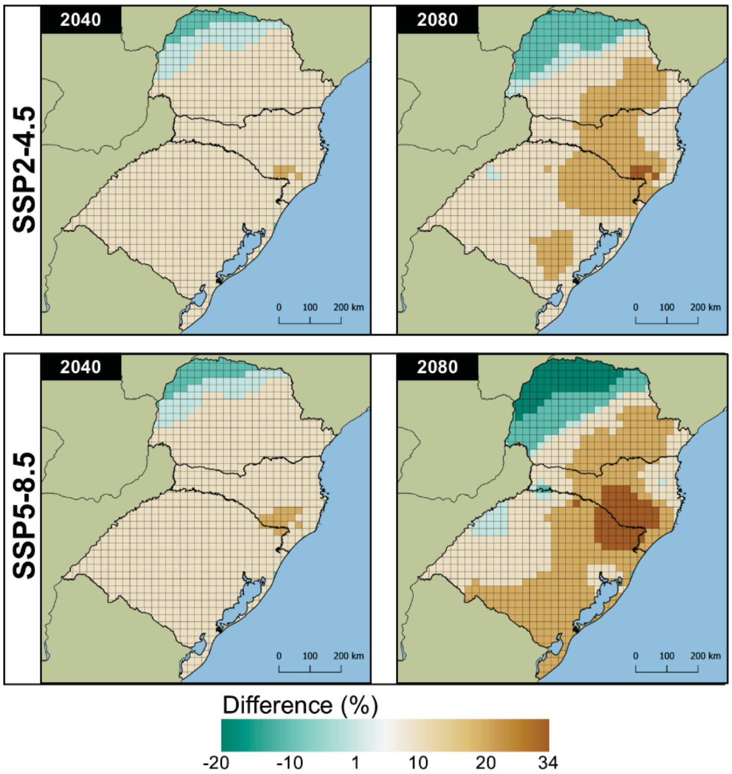
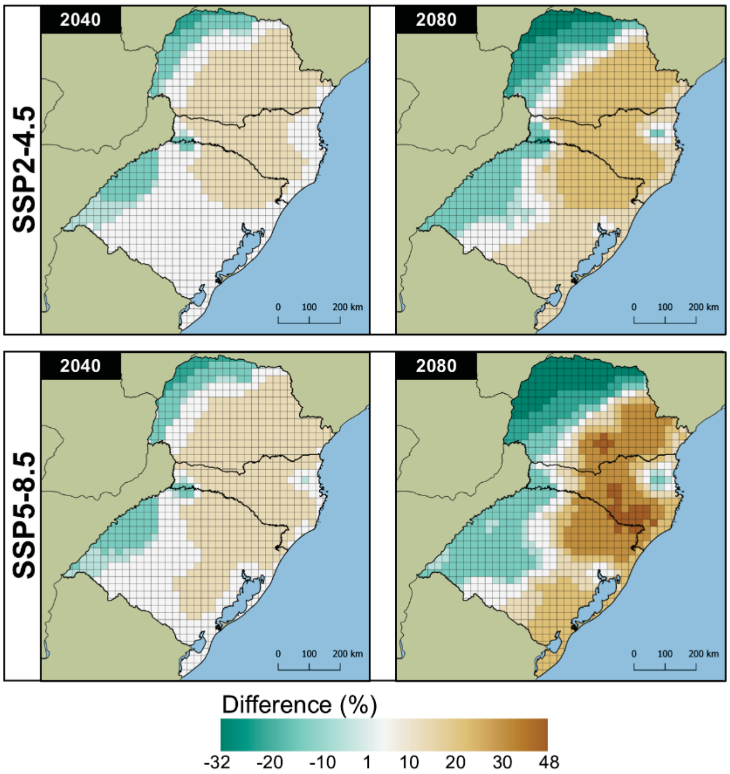


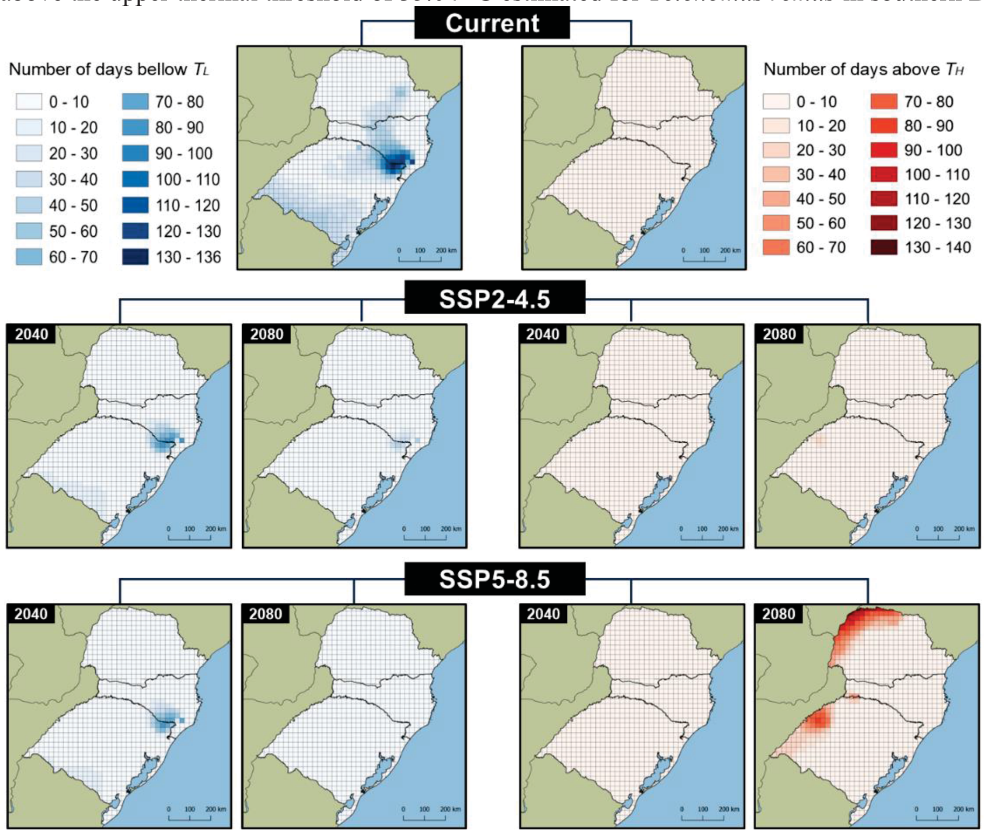
FIGURE 7 – Differences (%) between current and future projections of voltinism for *Trichogramma foersteri* in southern Brazil estimated with the model Shi. Future climate projections for 2040 and 2080 derived from the climate change scenarios SSP-4.5 and SSP-8.5. Negative and positive values refer to decrease and increase in the number of generations, respectively.



3.4 NUMBER OF DAYS EXCEEDING THE THERMAL THRESHOLDS

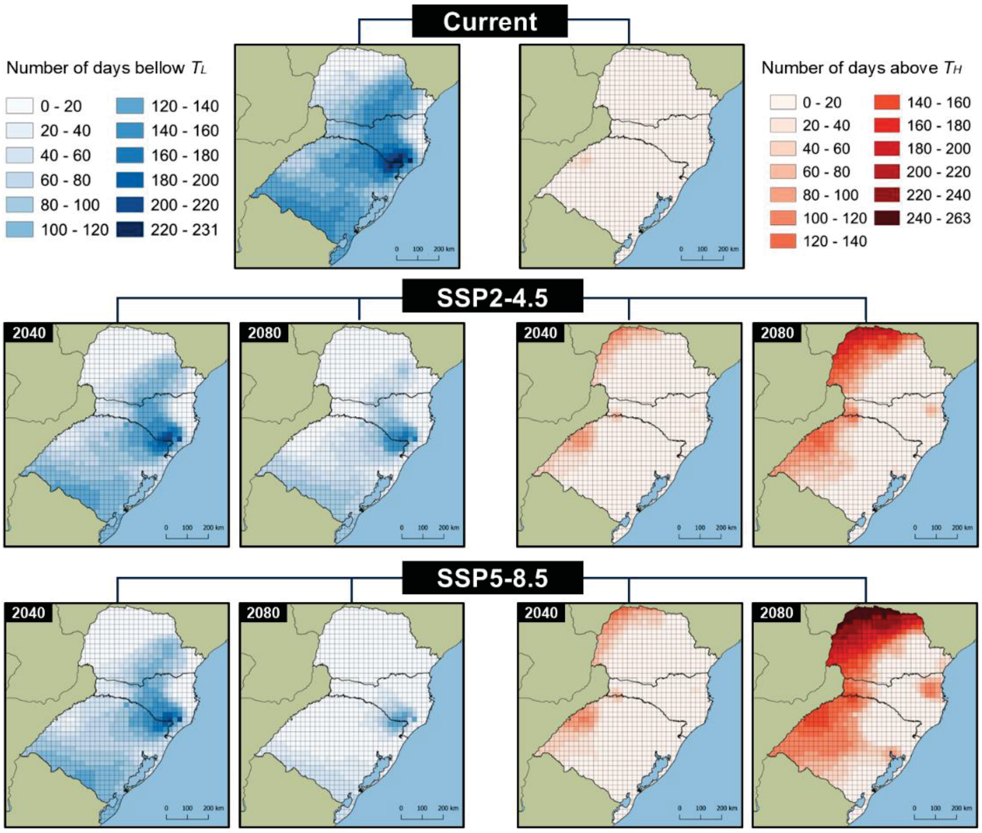
Under current climatic conditions, the daily minimum temperature falls below the estimated T_L for *Te. remus* for up to 136 days per year. This is projected to decrease to as few as 10 days in future climate scenarios, such as SSP5-8.5/2080 (Fig. 8). The greatest variations in relation to T_L were observed in the colder regions of southern Brazil. Currently, no temperatures equal to or exceeding the estimated T_H for *Te. remus* (35.04 ºC) have been recorded. However, under future climate change scenarios, up to 140 annual days are expected to reach or exceed this threshold (Fig. 8), particularly in the northwestern regions of Paraná and Rio Grande do Sul.

FIGURE 8 – Number of days in the year in which the daily temperature was below the lower thermal threshold of 8.72 °C, and above the upper thermal threshold of 35.04 °C estimated for *Telenomus remus* in southern Brazil.



For *T. foersteri*, the T_L is reached for up to 231 days per year under current climatic conditions (Fig. 9). Even in future projections, the number of days remains high, with the highest value recorded in the SSP5-4.5/2040 scenario (207 days). Currently, the T_H for *T. foersteri* is reached on 50 days per year, and projections suggest this could increase to as many as 263 days in warmer areas under future climatic conditions.

FIGURE 9 – Number of days in the year in which the daily temperature was below the lower thermal threshold of 11.87 °C, and above the upper thermal threshold of 32.22 °C estimated for *Trichogramma foersteri* in southern Brazil.



4 DISCUSSION

Numerous studies have shown that temperature significantly influences insect development and survival. However, because the response to temperature varies among species, research on its effects on insect biology remains essential (Sampaio et al., 2020). This study examined the direct impact of temperature on the development of a lineage of *Te. remus* adapted to the climatic conditions of southern Brazil. Additionally, phenological models were fitted to explore the relationship between temperature and development in *Te. remus* and *T. foersteri*. Finally, potential climate change impacts on the voltinism of these parasitoids were assessed.

Temperature influenced the developmental time and survival of *Te. remus*, as evidenced by the parasitoid's response to extreme temperatures. A notable difference of 44.6 days in the average development time was observed between individuals reared at 15 ºC and 35 °C. Lower temperatures reduced parasitism, while higher temperatures (e.g., 35 °C) stimulated parasitic activity. However, at these temperature extremes, the lowest emergence rates were recorded, particularly at 15 °C. These findings indicate that the population growth of *Te. remus* may be constrained in regions where temperatures frequently approach or fall below 15 °C.

Cold conditions can cause metabolic damage, such as osmotic stress, and reduce the degree days accumulated for complete development, leading to slower growth rates (Hance et al., 2007). Additionally, low temperatures may prematurely interrupt feeding (Denlinger and Lee, 1998), increasing larval mortality and reducing emergence rates, as observed in this study. Conversely*,* *Te. remus* appears to thrive at higher temperatures (up to 35 °C), particularly regarding development time and parasitism. At elevated temperatures, developmental changes likely result from metabolic adjustments in the insect (Hernández and Diáz, 1996; Ma, Ma, and Pincebourde, 2021; Negahban et al., 2021). Notably, the development time decreases as temperature rises, up to a threshold beyond which survival becomes compromised (Bueno et al., 2008).

Although an emergence rate of 78.16% was observed in this study at 35 ºC, previous research reported no emergence at this temperature (Bueno et al., 2008) or only 5.83% at 34 °C (Pomari et al., 2012). Additionally, a higher proportion of females was observed at 15 °C, whereas another study under similar conditions reported a predominance of males (Bueno et al., 2008). When a different field-collected lineage of *Te. remus* from Piracicaba, SP, Brazil, was analyzed, considerably lower parasitism rates were recorded compared to this study (Melo et al., 2024). Despite the close timing of collection for both lineages, the regions are approximately 500 km apart (Wengrant et al., 2021) and experience significantly different climatic conditions. Although the lineages likely share a common origin (Wengrant et al., 2021), distinct performance among them may result from adaptation to local environmental conditions (Castex et al., 2018), which could explain the differences observed in our study.

The most effective approach for conducting studies with phenological models to explain insect development involves testing multiple models and using a multi-criteria process to select the most suitable equation (Quinn, 2017; Rebaudo and Rabhi, 2018; Sampaio, Krechemer, and Marchioro, 2021a). In addition to evaluating the quality of the model fit, we also considered the precision of the estimated thermal thresholds. Among the twelve models tested, Brière-2 and Shi were the ones that best described the relationship between temperature and developmental rate for *Te. remus* and *T. foersteri*, respectively. Although the same equations were fitted for both parasitoids, each species responded differently to the functions, leading to the selection of distinct models. This variation among models highlights that model selection and evaluation should be carried out on a case-by-case basis (Sampaio, Krechemer, and Marchioro, 2021a). Few models met the pre-established criteria, indicating that fitting and selecting the most appropriate model can be particularly challenging when working with egg parasitoids. These difficulties may help explain why, despite the importance of such studies, relatively less research has focused on model selection for egg parasitoid Hymenoptera. This emphasizes the need for a thorough evaluation of equations for this insect group.

Overall, our findings indicate that the selected models provided parameter estimates consistent with empirical observations. For *Te. remus*, the upper thermal threshold and optimal temperature estimated by the Brière-2 model aligned well with laboratory data. A similar pattern was observed for *T. foersteri* using the Shi model. However, some discrepancies were noted in the estimation of the lower thermal threshold for *Te. remus*. Although no parasitoid emergence was recorded under laboratory conditions at a constant temperature of 12 °C over 100 days, the Brière-2 model estimated a lower threshold of 8.72 °C for this species. For *T. foersteri*, the Shi model estimated a lower threshold of 11.87 °C, consistent with another study in which no development of *T. foersteri* was observed at 10 °C when reared on olive moth eggs, *Palpita forficifera* (Munroe) (Lepidoptera: Pyralidae)) (Villalba et al., 2023). Despite these discrepancies, the Brière-2 model appears to provide a more realistic estimate of the lower threshold. The thermal thresholds estimated by the model for *Te. remus* (8.72 °C) and *T. foersteri* (7.5 °C) represent temperatures occasionally experienced by the parasitoids in the field. This supports the notion that, despite our findings and those of other studies, *Te. remus* and *T. foersteri* are capable of surviving at 12 ºC and 10 °C, respectively, under natural field conditions. Moreover, they likely withstand even lower temperatures, as the collection region frequently experiences low winter temperatures (Alvares et al., 2013), with frosts being common during this season (Mintegui et al., 2019).

The estimated lower thresholds may help explain the difficulty in collecting larger numbers of both parasitoid species in the field, despite repeated sampling efforts. When temporarily exposed to temperatures below their lower thermal threshold, development is halted but resumes once temperatures rise above the lower threshold (Hance et al., 2007). However, even if the parasitoids are capable of tolerating lower temperatures, such as those experienced in southern Brazil, prolonged exposure may negatively impact their development, survival, and reproduction (Hance et al., 2007). Therefore, although parasitoids can endure such conditions, severe winters may disrupt their populations, leading to a significant decline in abundance in the region.

Non-linear models are widely regarded as more appropriate for generating reliable estimates in studies assessing the impacts of climate change on insect development (Quinn, 2017; Rebaudo and Rabhi, 2018; Sampaio, Krechemer, and Marchioro, 2021b), because they incorporate an upper thermal threshold into their function. Upper thermal thresholds in insects tend to show less variation than lower thresholds (Furlong and Zalucki, 2017), as they are confined to a narrow range that usually follows the optimal temperature. In our study, we observed a 3 °C difference between the upper thermal thresholds estimated for the two parasitoid species, consistent with laboratory data. This difference proved sufficient to illustrate how identical global warming scenarios could have distinct impacts on each species. For example, *T. foersteri* may experience developmental constraints in certain regions of the study area, as higher temperatures could surpass its upper threshold (32.22 °C) for up to 263 days per year.

In colder regions, rising temperatures could increase the number of generations of both parasitoids, as their estimated lower thresholds would be exceeded more frequently. Currently, these areas, which include mountainous regions in southern Santa Catarina, northern Rio Grande do Sul, and southern Paraná, are subject to frequent frosts and occasional snowfalls during winter (Mintegui et al., 2019), resulting in fewer generations for *Te. remus* and *T. foersteri* under these climatic conditions. Consequently, the region's climatic heterogeneity contributed to the variability in the predicted number of generations for both parasitoids across different climate scenarios. In several studies, geographical location consistently emerges as the primary factor explaining variation in the predicted voltinism of insects (Ziter et al., 2012; Jaques et al., 2019; Srinivasa Rao et al., 2020; Sampaio, Krechemer, and Marchioro, 2021b).

The thermal ecology of most economically important species in agriculture, horticulture, and forestry remains largely unexplored (Ma, Ma, and Pincebourde, 2021). This study is the first to assess the potential impact of climate change on the development of *Te. remus* and *T. foersteri* in Brazil. Beyond temperature, other environmental factors can significantly influence the number of generations of these species, highlighting the importance of validating models under natural conditions as a critical step in the modeling process. Nevertheless, we recognize the challenges of obtaining such field data, which likely contributes to the limited number of studies addressing the effects of fluctuating temperatures on parasitoids (Furlong and Zalucki, 2017).

Our data demonstrated that temperature plays a significant role in the development and survival of the *Te. remus* lineage collected in southern Brazil. Among the twelve models tested, Brière-2 and Shi are recommended for describing the temperature-dependent development rates of *Te. remus* and *T. foersteri*, respectively. Based on the thermal thresholds estimated by these models, both parasitoids can develop across a broad temperature range. These findings are essential for optimizing parasitoid rearing laboratory settings, improving mass production in biofactories, and planning future field releases. Climate change is expected to affect parasitoid development in southern Brazil, with impacts varying according to the region’s climatic conditions. While some areas may become more conducive to parasitoid development, others could see a reduction in voltinism. This study provides valuable insights into the temperature-driven dynamics of Hymenopteran egg parasitoids and highlights the potential consequences of climate change on these biological control agents. The methods applied, grounded in the biology of *Te. remus* and *T. foersteri*, offer critical perspectives to improve forecasting of the ecological consequences of global warming on parasitoids.

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5 CONSIDERAÇÕES FINAIS

A realização desta tese foi motivada diante da ocorrência natural de *T. foersteri* e *Te. remus* registrada em campo pela equipe do Laboratório de Controle Integrado de Insetos (LCII). Os resultados obtidos enfatizam a importância de investigar o potencial de parasitismo de novas espécies de parasitoides de ovos, ampliando o entendimento sobre suas capacidades como agentes de controle biológico. Além disso, os dados destacam a necessidade de avaliar o desempenho desses organismos quando em associação, considerando as interações entre espécies e os impactos potenciais no equilíbrio ecológico. Particularmente relevante foi a análise da influência de fatores abióticos, como a temperatura, que afeta diretamente o desenvolvimento, a sobrevivência e a eficiência dos parasitoides. Esse entendimento foi aprofundado por meio da aplicação de modelos fenológicos robustos, que forneceram *insights* detalhados sobre as respostas térmicas das espécies estudadas. Por fim, este trabalho oferece uma perspectiva abrangente sobre os impactos das mudanças climáticas nesses agentes biológicos, evidenciando como as alterações ambientais poderão influenciar principalmente seu desenvolvimento, e como consequência sua distribuição e dinâmica populacional.

No primeiro capítulo verificamos que *Trichogramma foersteri* Takahashi, 2021 (Hymenoptera: Trichogrammatidae) é capaz de parasitar eficientemente ovos de *Spodoptera frugiperda* J.E. Smith, 1797 e *Spodoptera eridania* Stoll, 1782 (Lepidoptera: Noctuidae), além de induzir uma mortalidade significativa por meio de mecanismos não reprodutivos, o que amplia sua eficácia como agente de controle biológico destas pragas. Esse comportamento ressalta a necessidade de incluir os efeitos não reprodutivos nas avaliações de eficiência de parasitoides, uma vez que tais mecanismos podem ser decisivos na redução das populações de pragas. Além disso, a capacidade de parasitismo de *T. foersteri* superou a de outras espécies do gênero avaliadas em condições semelhantes, evidenciando seu elevado potencial. Ambos os insetos-pragas aqui estudados, têm se tornado problemas cada vez mais sérios em diversas regiões do mundo e, diante das dificuldades envolvidas no seu manejo, novos agentes de controle biológico representam uma ferramenta importante no contexto de programas de Manejo Integrado de Pragas (MIP).

O segundo capítulo deste estudo oferece informações relevantes sobre as dinâmicas de parasitismo de *Telenomus remus* Nixon, 1937 (Hymenoptera: Platygastridae) e *T. foersteri* e em massas de ovos de *S. frugiperda*, abordando fatores que influenciam sua eficácia como agentes de controle biológico. Os resultados destacam o impacto da estrutura das massas de ovos (camada única versus dupla) e das interações interespecíficas nas taxas de parasitismo de ambas as espécies. O estudo revelou que *T. remus* apresenta uma eficiência de parasitismo superior em comparação com *T. foersteri*, especialmente em massas de ovos com múltiplas camadas, devido ao seu maior tamanho e à capacidade de manipular as camadas de ovos. No entanto, *T. foersteri* demonstra uma capacidade notável de parasitismo, superando muitas espécies de *Trichogramma*, o que sugere seu potencial utilitário em programas de manejo de pragas. Uma contribuição significativa deste capítulo reside na exploração da competição interespecífica. Os resultados mostram que *T. foersteri* se beneficia das condições competitivas com *Te. remus*, já que as características físicas e comportamentais deste último aprimoram o acesso aos ovos para o primeiro. Essa interação sinérgica inesperada sugere que o uso combinado desses parasitoides pode otimizar o controle biológico de *S. frugiperda*. Por fim, nota-se que considerar diferentes parasitoides, como *T. foersteri* e *Te. remus*, como agentes complementares no controle de *S. frugiperda*, pode ser um método importante no planejamento do manejo de pragas.

O último capítulo deste estudo, abordou a influência da temperatura no desenvolvimento e sobrevivência dos parasitoides *Te. remus* e *T. foersteri*, destacando também as potenciais implicações das mudanças climáticas sobre essas espécies. Por meio da aplicação de modelos fenológicos, foi possível compreender a relação entre temperatura e desenvolvimento dos parasitoides, além de estimar os impactos de cenários de aquecimento global. Os resultados mostram que a temperatura exerce uma influência significativa no tempo de desenvolvimento, parasitismo e taxas de emergência de *Te. remus*. Temperaturas extremas representam limitações no crescimento populacional devido a estresses metabólicos e interrupções no desenvolvimento. Por outro lado, temperaturas mais altas estimularam a atividade parasítica, destacando o potencial do parasitoide em regiões mais quentes.

A seleção dos modelos Brière-2 e Shi, que melhor representaram o desenvolvimento térmico de *Te. remus* e *T. foersteri*, respectivamente, reforça a necessidade de uma abordagem criteriosa na escolha de modelos fenológicos. Esses modelos forneceram estimativas realistas das faixas de temperatura adequadas para o desenvolvimento das espécies, indicando que ambas podem tolerar uma ampla variação térmica. O capítulo traz contribuições importantes para o manejo integrado de pragas, especialmente no contexto das mudanças climáticas. Regiões mais frias podem tornar-se favoráveis ao aumento do número de gerações dos parasitoides, enquanto áreas com temperaturas extremas poderão limitar o desenvolvimento, como evidenciado para *T. foersteri*. Este estudo pioneiro no Brasil apresenta dados importantes para a criação em laboratório e a liberação em campo desses agentes de controle biológico, oferecendo subsídios para adaptar estratégias frente ao aquecimento global. Neste capítulo evidenciou-se a relevância de estudos sobre a ecologia térmica de parasitoides e sua aplicação prática no manejo sustentável de pragas agrícolas. Os achados reforçam o impacto direto da temperatura e a importância de modelos adequados para prever os efeitos das mudanças climáticas sobre agentes de controle biológico. O importante papel dos parasitoides na agricultura, seja por meio do controle natural de pragas ou de programas de controle biológico, pode ser afetado com as mudanças climáticas.

Esses resultados não apenas ampliam o conhecimento sobre a biologia e o comportamento de *T. foersteri* e *Te. remus*, mas também fornecem subsídios essenciais para otimizar estratégias de manejo integrado de pragas. Futuramente, é possível que os parasitoides aqui estudados possam colaborar para manter populações de insetos-pragas, como as do gênero *Spodoptera* spp, abaixo do nível de dano econômico. Além de colaborarem na redução de emprego de inseticidas e na prolongação da vida útil de cultivares transgênicas. Ao explorar o potencial dessas espécies, tanto individualmente quanto em interação, e ao considerar os efeitos das mudanças climáticas, esta tese destaca a relevância de abordagens integrativas e adaptativas para o controle biológico sustentável. Nossos dados permitem alertar sobre a importância do setor agrícola em se adaptar a essas alterações, que em alguns casos já são irreversíveis. No entanto, a necessidade de validações em condições de campo e estudos adicionais sobre os impactos de fatores ambientais destaca os desafios futuros no uso prático desses agentes de controle.

5.1 RECOMENDAÇÕES PARA TRABALHOS FUTUROS

Os resultados desta tese fornecem uma base sólida para futuras investigações e avanços no uso de *T. foersteri* e *Te. remus* em programas de controle biológico. Contudo, há ainda importantes lacunas que precisam ser abordadas para maximizar o potencial desses parasitoides como agentes de manejo de pragas.

Recomenda-se a realização de estudos em condições de campo para validar os resultados laboratoriais, especialmente quanto à eficácia de *T. foersteri* e *Te. remus* no controle de *S. frugiperda* e *S. eridania*. Esses experimentos devem considerar fatores ambientais como disponibilidade de hospedeiros e competição natural. Adicionalmente, uma investigação mais profunda sobre os mecanismos bioquímicos e comportamentais responsáveis pela mortalidade não reprodutiva induzida por *T. foersteri* pode ampliar a compreensão de seu papel no controle de pragas, além de identificar compostos químicos e estratégias comportamentais associadas.

Outro aspecto relevante é o estudo das interações competitivas e sinérgicas entre *T. foersteri* e *Te. remus*, considerando a possibilidade de uso combinado para otimizar o manejo de pragas. Além disso, devem-se explorar a resiliência e a capacidade de adaptação dessas espécies às mudanças climáticas, investigando como eventos extremos, como ondas de calor e geadas, podem impactar sua sobrevivência e eficácia. Pesquisas sobre aclimatação e enriquecimento de ambientes agrícolas poderão desempenhar um papel crucial na adaptação desses organismos nos ecossistemas agrícolas.

Para fomentar o uso prático desses parasitoides, é essencial o aprimoramento de protocolos de criação massal, com especial atenção ao uso de hospedeiros alternativos, como *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae), visando reduzir custos e aumentar a eficiência da produção comercial. Paralelamente, recomenda-se expandir o uso de modelos fenológicos para prever a dinâmica populacional dos parasitoides em função de variáveis ambientais, como temperatura, umidade e fotoperíodo, integrando dados de campo e laboratório para a construção de modelos mais robustos e aplicáveis.

Por fim, estudos econômicos e sociais devem ser conduzidos para avaliar a viabilidade financeira do uso de *T. foersteri* e *Te. remus* em comparação com outras estratégias de manejo integrado de pragas. A análise de benefícios ambientais e a aceitação dos agricultores também são aspectos fundamentais para promover a sustentabilidade do controle biológico com esses parasitoides. Essas recomendações oferecem caminhos promissores para ampliar o conhecimento gerado nesta tese e contribuir para estratégias agrícolas mais eficazes, sustentáveis e adaptadas aos desafios impostos pelas mudanças climáticas.