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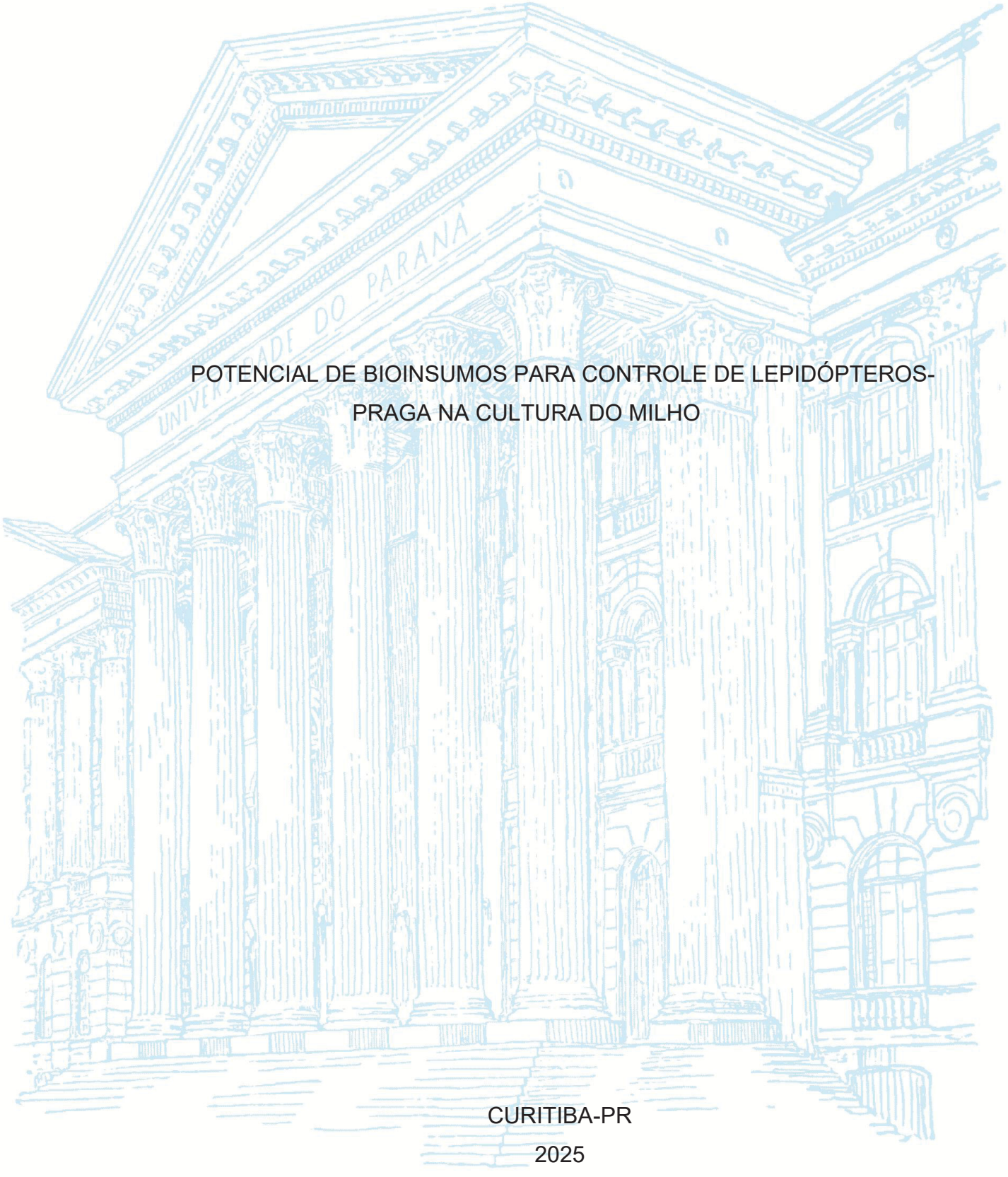

POTENCIAL DE BIOINSUMOS PARA CONTROLE DE LEPIDÓPTEROS-PRAGA NA CULTURA DO MILHO

CURITIBA-PR

2025

WEIDSON PLAUTER SUTIL

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Tese apresentada ao curso de Pós-Graduação em Entomologia, Setor de Ciências Biológicas, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutor em Ciências Biológicas (Entomologia).

Orientador: Prof. Dr. Adeney de Freitas Bueno

Coorientadora: Dra. Yelitza Coromoto Colmenarez

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação CIÊNCIAS BIOLÓGICAS (ENTOMOLOGIA) da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **WEIDSON PLAUTER SUTIL**, intitulada: **POTENCIAL DE BIOINSUMOS PARA CONTROLE DE LEPIDÓPTEROS-PRAGA NA CULTURA DO MILHO**, sob orientação do Prof. Dr. ADENEY DE FREITAS BUENO, 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, 29 de Agosto de 2025.

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RESUMO

A agricultura global busca intensificar a produção de forma sustentável, o que tem incentivado a pesquisa por novas estratégias de manejo de pragas com menor impacto ambiental, especialmente contra lepidópteros-praga de elevado reconhecimento agrícola como espécies do complexo *Spodoptera* e *Helicoverpa spp.*, responsáveis por severos danos em milho, soja, algodão e diversas outras culturas. Neste contexto, o uso de parasitoides de ovos como agentes de controle biológico e o desenvolvimento de produtos derivados de plantas surgem como soluções promissoras para o manejo dessas espécies. Desta forma, o objetivo geral deste trabalho foi prospectar e otimizar abordagens de manejo para pragas da cultura do milho, com foco em três frentes principais: (1) avaliar a dinâmica competitiva entre os parasitoides de ovos *Telenomus remus* e *Trichogramma pretiosum* no manejo de *Spodoptera frugiperda*; (2) otimizar a tecnologia de liberação de *T. remus* com uma dieta sólida e tempos de armazenamento; e (3) investigar o potencial inseticida de uma nanoemulsão de óleo essencial de orégano contra *Spodoptera littoralis*, utilizada para ensaios de bioatividade. Nossos resultados demonstraram que *T. remus* superou consistentemente *T. pretiosum* em todas as condições testadas, do laboratório ao campo, com este último apresentando contribuição mínima no parasitismo em liberações conjuntas. O uso da dieta sólida permitiu o armazenamento de *T. remus* por até quatro dias em cápsulas de liberação sem perda de eficácia. Em campo, as densidades testadas não foram suficientes para um controle efetivo, sugerindo a necessidade de densidades mais elevadas. Por fim, a nanoemulsão de óleo essencial de orégano mostrou alta eficácia, causando mortalidade em ovos e larvas de *S. littoralis* e reduzindo significativamente sua atividade alimentar. Portanto, conclui-se sobre a utilização combinada dos parasitoides não é vantajosa para o manejo de *S. frugiperda*, e que a densidade para uso de T. remus isolado deve ser aumentada, por meio da utilização de adultos já alimentados e copulados os quais podem ser armazenados por até quatro dias dentro das capsulas avaliadas. Por fim, a nanoemulsão do óleo essencial de orégano apresenta grande potencial como um novo bioinsumo inseticida e deve ser avaliado também contra *S. frugiperda*. Os resultados obtidos fornecem informações importantes para o aprimoramento de estratégias sustentáveis de manejo integrado de pragas, com implicações práticas não apenas para o controle de *Spodoptera* spp., mas também de outras pragas-chave como *Helicoverpa spp.* em sistemas agrícolas.

Palavras-chave: *Spodoptera* spp.; parasitoides de ovos; óleos essenciais; manejo integrado de pragas.

ABSTRACT

Global agriculture seeks to intensify production in a sustainable manner, which has encouraged the search for new pest management strategies with lower environmental impact, especially against lepidopteran pests of major agricultural importance such as species of the *Spodoptera* complex and *Helicoverpa* spp., responsible for severe damage in maize, soybean, cotton, and several other crops. In this context, the use of egg parasitoids as biological control agents and the development of plant-derived products emerge as promising solutions for the management of these species. Thus, the general objective of this study was to prospect and optimize management approaches for maize pests, focusing on three main fronts: (1) to evaluate the competitive dynamics between the egg parasitoids *Telenomus remus* and *Trichogramma pretiosum* in the management of *Spodoptera frugiperda*; (2) to optimize the release technology of *T. remus* using a solid diet and different storage periods; and (3) to investigate the insecticidal potential of an oregano essential oil nanoemulsion against *Spodoptera littoralis*, used as a model species for bioactivity assays. Our results demonstrated that *T. remus* consistently outperformed *T. pretiosum* under all tested conditions, from laboratory to field, with the latter contributing minimally to parasitism in combined releases. The use of the solid diet enabled *T. remus* storage for up to four days in release capsules without loss of efficacy. In the field, the tested release densities were not sufficient for effective control, suggesting the need for higher densities. Finally, the oregano essential oil nanoemulsion showed high efficacy, causing mortality in eggs and larvae of *S. littoralis* and significantly reducing its feeding activity. Therefore, we conclude that the combined use of parasitoids is not advantageous for the management of *S. frugiperda*, and that the release density of *T. remus* alone should be increased, using pre-fed and mated adults, which can be stored for up to four days in the tested capsules. Moreover, the oregano essential oil nanoemulsion shows great potential as a new insecticidal bioinput and should also be evaluated against *S. frugiperda*. The results obtained provide important insights for improving sustainable strategies of integrated pest management, with practical implications not only for the control of *Spodoptera* spp. but also for other key pests such as *Helicoverpa* spp. in agricultural systems.

Keywords: *Spodoptera* spp.; egg parasitoids; essential oils; integrated pest management.

SUMÁRIO

1 INTRODUÇÃO

O gênero *Spodoptera* reúne algumas das pragas mais impactantes para a agricultura global, caracterizando-se por seu elevado potencial destrutivo, alta capacidade de dispersão, polifagia acentuada e seleção recorrente de populações resistentes aos inseticidas utilizados (CABI, 2019; FAO, 2020). Essa combinação de atributos confere a suas espécies uma grande relevância econômica em diversos sistemas de produção.

Dentre as espécies de maior importância agrícola, destaca-se a lagarta-do-cartucho, *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae), também conhecida como fall armyworm, por sua natureza polífaga e voraz. Sua capacidade de se alimentar de estruturas vegetativas e reprodutivas de mais de 350 espécies de plantas, distribuídas em pelo menos 76 famílias botânicas (Montezano *et al*., 2018; CABI, 2019; Overton *et al*., 2021; Kenis *et al*., 2022), é um fator chave para seu sucesso. Originalmente restrita ao continente americano, *S. frugiperda* demonstrou uma notável plasticidade ecológica, resultando em sua rápida expansão para a África, Ásia e Europa nas últimas décadas (Goergen *et al*., 2016; EPPO, 2025; Kartasis *et al*., 2025; Togola *et al*., 2025). Essa invasão global é amplificada pela ausência de inimigos naturais eficientes nas novas áreas invadidas e pelo aumento do comércio internacional, que facilita sua dispersão acidental (Faulkner *et al*., 2017; Early *et al*., 2018; Kenis *et al*., 2022).

Além de *S. frugiperda*, outras espécies, como a lagarta-do-mediterrâneo, *Spodoptera littoralis* (Boisduval, 1833) (Lepidoptera: Noctuidae) e a lagarta-da-espiga, *Helicoverpa armigera* (Hübner, 1809) (Lepidoptera: Noctuidae), também representam ameaças significativas à agricultura, compartilhando características como ampla distribuição geográfica, elevada capacidade de adaptação e comportamento alimentar generalista. Todas essas lagartas causam prejuízos consideráveis em diversas culturas de importância econômica, incluindo algodão, milho, hortaliças e plantas ornamentais (CABI, 2022; ElShahed *et al*., 2023).

A ação conjunta dessas pragas tem impulsionado o uso intensivo e recorrente de inseticidas químicos em várias regiões agrícolas. No entanto, a dependência excessiva desses produtos tem gerado uma série de consequências negativas, como os riscos à saúde humana e à biodiversidade (Jacquet *et al*., 2022), a seleção de populações resistentes (Carvalho *et al*., 2013), a supressão de inimigos naturais (Torres; Bueno, 2018) e o ressurgimento de pragas primárias ou surtos de pragas secundárias (Bueno *et al*., 2021). Como resultado, a redução do uso de pesticidas químicos tem se tornado uma

prioridade em políticas públicas voltadas ao desenvolvimento agrícola sustentável (Lee *et al*., 2019), impulsionando a busca por alternativas de manejo sustentáveis.

Nesse contexto, alternativas ecologicamente corretas têm sido impulsionadas por políticas globais para reduzir a dependência dos químicos sintéticos, especialmente o controle biológico aumentativo (CBA), que envolve a liberação massal de inimigos naturais, como os parasitoides de ovos *Trichogramma pretiosum* (Riley, 1879) (Hymenoptera: Trichogrammatidae) e *Telenomus remus* Nixon, 1937 (Hymenoptera: Scelionidae) que se destacam pelo elevado potencial de controle de ovos de *Spodoptera* spp., com ação mais específica e impacto ambiental reduzido (Parra, 2019; Wengrat *et al*., 2021; Bueno *et al*., 2024a). Outra importante estratégia sustentável para o manejo de pragas é o uso de produtos de origem botânica, como óleos essenciais, que oferecem ação inseticida, repelente e de inibição da oviposição de insetos, geralmente com baixa toxicidade para organismos não alvo e baixo impacto ambiental quando comparados aos inseticidas químicos tradicionais (Campolo *et al*., 2020; Modafferi *et al*., 2024).

Apesar dos avanços recentes no uso de bioinsumos, ainda persistem lacunas relevantes para a consolidação de programas de controle biológico aumentativo com parasitoides de ovos, especialmente no que se refere à definição de densidade, frequência e momento das liberações, bem como ao uso combinado, tanto em laboratório quanto em campo de diferentes espécies de parasitoides e sua compatibilidade com outros produtos biológicos como os inseticidas botânicos, por exemplo. A compreensão das interações entre inimigos naturais, sobretudo quando compartilham o mesmo hospedeiro, como no caso do multiparasitismo, é fundamental para avaliar possíveis efeitos sinérgicos, antagônicos ou de exclusão competitiva que podem comprometer a eficácia do controle. Adicionalmente, é necessário investigar o efeito letal e subletais dos óleos essenciais sobre esses inimigos naturais e outros insetos benéficos, considerando seu potencial tóxico e impactos não intencionais (Modafferi *et al*., 2024). O aprofundamento desses aspectos permitirá o desenvolvimento de estratégias de manejo mais robustas, eficientes e adaptadas às necessidades práticas dos agricultores no manejo de *Spodoptera* spp.

Dessa forma, o objetivo desta tese de doutoramento é avaliar estratégias de manejo de *S. frugiperda* por meio do uso isolado e combinado dos parasitoides *T. remus* e *T. pretiosum*, considerando diferentes contextos experimentais (laboratório e campo), com foco na compreensão das interações ecológicas entre as espécies, como competição e complementaridade. Além disso, busca-se investigar o potencial do óleo essencial de orégano no controle de *S. littoralis*, analisando seus efeitos letal e subletais sobre a praga,

com vistas à ampliação de alternativas sustentáveis no manejo integrado de pragas (MIP) desses lepidópteros pragas.

2 REVISÃO DE LITERATURA

2.1 USO DE BIOINSUMOS E MANEJO INTEGRADO DE LEPIDÓPTEROS PRAGAS

A crescente demanda por práticas agrícolas mais sustentáveis, resilientes e ambientalmente seguras tem impulsionado o aprimoramento do MIP, uma abordagem que busca manter as populações de pragas abaixo do nível de dano econômico por meio da integração racional de diferentes táticas de controle (Bueno *et al*., 2022; Robinson, 2024; FAO, 2025a).

Nesse cenário, os bioinsumos têm ganhado destaque como ferramentas essenciais. Conforme estabelecido pelo Ministério da Agricultura, Pecuária e Abastecimento (MAPA, 2021). Bioinsumos são definidos como o produto, o processo ou a tecnologia de origem vegetal, animal ou microbiana, destinado ao uso na produção, no armazenamento e no beneficiamento de produtos agropecuários, nos sistemas de produção aquáticos ou de florestas plantadas, que interfiram positivamente no crescimento, no desenvolvimento e no mecanismo de resposta de animais, de plantas, de microrganismos e de substâncias derivadas e que interajam com os produtos e os processos físico-químicos e biológicos. Esse conceito abrange uma ampla gama de produtos, incluindo agentes de controle biológico, biofertilizantes, inoculantes, extratos vegetais, óleos essenciais e outros compostos naturais com atividade inseticida ou fungicida (Bettiol, 2022).

Dentro desse escopo dos bioinsumos, o controle biológico ocupa papel de destaque, sendo caracterizado pelo uso aplicado de inimigos naturais, como insetos benéficos (predadores e parasitoides) e microrganismos (fungos, vírus e bactérias), para o controle de pragas agrícolas e vetores de doenças (EMBRAPA, 2023). Paralelamente, compostos naturais derivados de plantas, como extratos e óleos essenciais, também são considerados bioinsumos, apresentando propriedades inseticidas, repelentes ou fungicidas, e podendo ser utilizados de forma complementar ou integrada ao controle biológico tradicional (CROPLIFE, 2025a).

O uso de bioinsumos contribui diretamente para a redução da dependência na produção agrícola de inseticidas químicos, atenuando impactos ambientais, diminuindo o risco de seleção de populações resistentes de pragas e favorecendo a conservação de inimigos naturais e polinizadores, elementos essenciais para a maior resiliência dos agroecossistemas (Bueno *et al*., 2022; Medina *et al*., 2024). As principais vantagens da

adoção de bioinsumos incluem a especificidade ao organismo-alvo com menor impacto sobre organismos não-alvo, sua compatibilidade com sistemas orgânicos e agroecológicos, a ausência de resíduos tóxicos em alimentos e no ambiente, e sua contribuição crucial para o manejo da resistência aos inseticidas convencionais (CROPLIFE, 2025).

Apesar das vantagens aqui destacadas, a adoção em larga escala de bioinsumos possui desafios a serem vencidos. A variabilidade de desempenho em campo, o tempo de prateleira reduzido em comparação com químicos sintéticos, a necessidade de condições específicas de armazenamento e aplicação, bem como a demanda por infraestrutura técnica e regulamentação adequada, persistem como os maiores desafios para o sucesso de sua adoção (Mazaro *et al*., 2022; Policarpo *et al*., 2025). Contudo, o cenário brasileiro demonstra um notável avanço, impulsionado por mudanças regulatórias, como a simplificação do sistema de registro de agentes de controle biológico, que permitiu o registro por alvo biológico e acelerou sua disponibilização (Bettiol e Medeiros, 2023; [1]Andreata *et al*., 2025). Um marco importante no avanço do desenvolvimento e adoção do controle biológico e inseticidas botânicos no Brasil foi a criação do Programa Nacional de Bioinsumos em 2020, com o objetivo de acelerar o desenvolvimento e a adoção de biopesticidas (MAPA, 2020).

Até o início da década de 2010, o setor de biopesticidas era ainda incipiente no Brasil, com registros pouco expressivos, não ultrapassando dois produtos registrados por ano, com baixa representatividade no mercado agrícola nacional (Bueno *et al*., 2023; AGROFIT, 2025). No entanto, a partir de 2011, observou-se um crescimento contínuo e acelerado no número de produtos registrados, passando de apenas sete registros naquele ano para 381 em 2021 (Bueno *et al*., 2023; AGROFIT, 2025). Esse aumento expressivo reflete não apenas a maior aceitação desses produtos por parte do setor produtivo, mas também avanços regulatórios e tecnológicos que favoreceram o desenvolvimento e a inserção de bioinsumos no contexto do MIP. A ocorrência da praga *H. armigera* em 2013, que provocou severos surtos em diversas culturas e expôs a fragilidade de sistemas agrícolas fortemente dependentes de inseticidas químicos, impulsionou a retomada de práticas de MIP e fomentou o interesse dos agricultores por soluções biológicas (Bueno; Sosa-Gómez, 2014). Esse cenário marcou um ponto de inflexão na adoção do controle biológico e na regulamentação de bioinsumos no Brasil.

No Brasil há registro de mais de 600 produtos biológicos, com predomínio de microrganismos (65%) seguidos em sequência pelos macroorganismos (15%), bioquímicos

[1]ANDREATA, F. L.; ALVES, S. M.; ANDRADE, G.; BUENO, A. F.; VENTURA, M. U.; ALMEIDA, J. E. M.; FONSECA, I. E. A.; MOSELA, M.; SIMIONATO, A. S.; ROBAINA, R. R.; GONÇALVES, L. E. A. *The current increase and future*. *Frontiers in Microbiology*, [s.l.], v. 16, **no prelo**. Aceito em: 7 jul. 2025.

(13%) e semioquímicos (7%) (CROPLIFE, 2023; MAPA, 2025). Entre os microrganismos mais utilizados como bioinsumos destacam-se os fungos entomopatogênicos, como *Beauveria bassiana* e *Metarhizium anisopliae*, amplamente empregados no controle de pragas agrícolas (Sosa-Gómez *et al*., 2022*)*. Bioinseticidas bacterianos, como *Bacillus thuringiensis* (*Bt*), também são largamente aplicados, tanto na forma de produtos comerciais quanto por meio de cultivos geneticamente modificados que expressam suas proteínas inseticidas (Arias *et al*., 2022). Além disso, biofungicidas e promotores de crescimento, como *Bacillus subtilis*, *Bacillus amyloliquefaciens* e *Pseudomonas fluorescens*, têm mostrado grande eficácia no manejo de doenças e no estímulo ao desenvolvimento vegetal (Bettiol *et al*., 2022). Por fim, rizobactérias promotoras de crescimento, como *Bradyrhizobium* spp., *Rhizobium* spp. e *Azospirillum brasiliense*, são amplamente empregadas na fixação biológica de nitrogênio e na promoção do crescimento de diversas culturas (Hungria; Nogueira, 2022).

Tamanha expansão na disponibilidade de bioinsumos resultou em um crescimento de 13% no uso de bioinsumos na safra brasileira de 2024/2025, expandindo a área cultivada com esses produtos de 22 milhões de hectares em 2018 (CROPLIFE, 2025b) para mais de 56 milhões de hectares em 2024 (Medeiros; Bettiol, 2023; van Lenteren *et al*., 2025). De acordo com dados da safra 2024/2025, a soja foi a principal cultura a utilizar bioinsumos no Brasil, concentrando 62% da aplicação total, um aumento de 7% em relação à safra anterior. Em contrapartida, observou-se uma redução no uso de bioinsumos em outras culturas, como no milho, cujo percentual caiu de 27% para 23%; na cana-de-açúcar, de 12% para 10% (CROPLIFE, 2025b).

Com relação ao faturamento do mercado de biopesticidas brasileiro, que era inferior a US$ 58 milhões em 2017, atingiu aproximadamente US$ 690 milhões na safra 2023/24 (Andreata *et al*., 2025). Os bioinseticidas representam hoje a maior fatia do mercado de biopesticidas, respondendo por cerca de 42,5% do total, seguidos pelos bionematicidas (30%) e biofungicidas (27,5%) (Andreata *et al*., 2025). As projeções indicam que o setor pode ultrapassar US$ 1,69 bilhão anuais até 2027 (Borsari; Vieira, 2022), impulsionado pelo avanço tecnológico, flexibilização regulatória, aumento da demanda por produtos com menor impacto ambiental e adoção crescente das tecnologias sustentáveis disponíveis como a liberação de parasitoides de ovos e a aplicação de inseticidas botânicos dentro do contexto do MIP.

2.2 LEPIDÓPTEROS DE IMPORTÂNCIA ECONÔMICA NO MILHO

Os sistemas agrícolas baseados em monocultivos intensivos favorecem a ocorrência simultânea de diversas espécies de pragas, especialmente em regiões tropicais como o Brasil (Kaur *et al*., 2024). Nesse contexto, o MIP tem enfrentado desafios cada vez maiores, sobretudo devido à pressão seletiva imposta pelo uso intensivo e contínuo de inseticidas químicos, prática erroneamente comum em grandes culturas (Bueno *et al*., 2021; Jacquet *et al*., 2022; Zhang *et al*., 2025). Entre os principais grupos de pragas que afetam esses sistemas, destacam-se os lepidópteros, responsáveis por perdas econômicas expressivas em várias culturas agrícolas, com destaque para o milho. Dentro desse grupo, espécies dos gêneros *Spodoptera* e *Helicoverpa* são especialmente preocupantes por sua elevada polifagia, alto potencial de dano e a perda de eficácia de diversos grupos de inseticidas utilizados em seu controle (Valicente, 2015; Montezzano *et al*., 2018; Dourado *et al*., 2021; Kenis *et al*., 2022).

A elevada capacidade adaptativa das espécies desses grupos é um fator crucial, especialmente porque sua ampla gama de hospedeiros vegetais pode levar à formação do que se conhece como 'ponte verde' (Leite *et al*., 2021). As 'pontes verdes' referem-se à presença contínua de plantas hospedeiras no ambiente agrícola, oferecendo alimento e abrigo constantes às pragas, fato que não só dificulta seu manejo, mas também favorece a ocorrência de surtos populacionais (Leite *et al*., 2021). Entre os principais fatores que contribuem para a formação dessas pontes estão a presença de plantas daninhas como o caruru, *Amaranthus* spp. que atuam como hospedeiros alternativos (Hill *et al*., 2025), a ocorrência de culturas voluntárias (rebrotas ou tigueras) e o uso de culturas em sucessão ou coberturas vegetais mal planejadas, que mantêm hospedeiros disponíveis no campo por longos períodos (Favetti *et al*., 2017; Leite *et al*., 2021; Adnan *et al*., 2024; Hill *et al*., 2025).

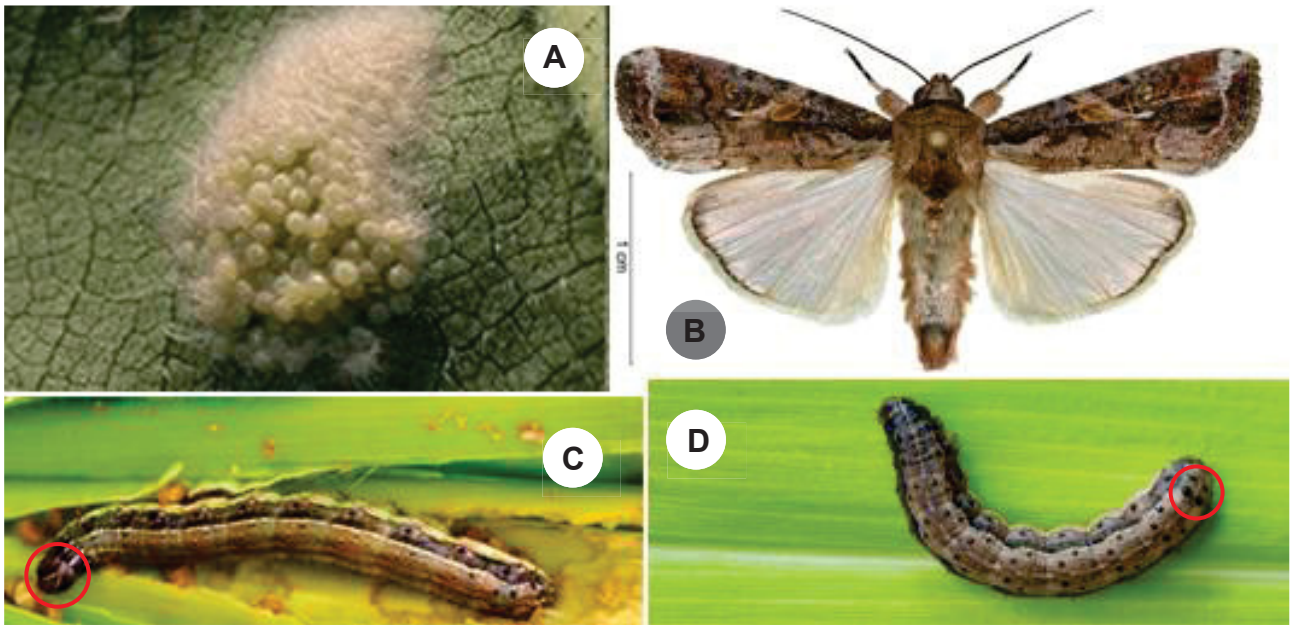
A constante presença de insetos-praga nos sistemas agrícolas impõe elevados custos à economia global, com perdas que podem atingir até 40% da produção agrícola mundial, além de prejuízos econômicos da ordem de bilhões de dólares anuais relacionados ao controle de pragas e doenças (FAO, 2023; FAO, 2025b). Apesar dos avanços e da eficácia comprovada de estratégias sustentáveis de proteção de plantas, como o uso de cultivares *Bt*, o emprego de inseticidas químicos ainda ocorre de forma indiscriminada por muitos produtores (Serrão *et al*., 2022; Ngegba *et al*., 2025). No entanto, a aplicação contínua e excessiva de defensivos sintéticos pode acarretar efeitos adversos a médio e longo prazo. Um exemplo emblemático é o do herbicida glifosato, cuja utilização recorrente tem contribuído para a seleção e a disseminação de biótipos resistentes, além do aumento da ocorrência de espécies tolerantes nas principais regiões produtoras de soja

do país (Lúcio *et al*., 2019). Esse cenário compromete a eficácia do controle químico, dificultando o manejo integrado das lavouras.

2.2.1 *Spodoptera frugiperda*

A lagarta-do-cartucho (Figura 1) é uma praga altamente destrutiva, que se alimenta em grandes quantidades das folhas, caules e partes reprodutivas, causando sérios prejuízos econômicos, especialmente em gramíneas cultivadas como milho, arroz, sorgo, cana-de-açúcar e trigo, além de outras culturas como hortaliças e algodão (Montezano *et al*., 2018; Overton *et al*., 2021; Kenis *et al*., 2022; Kartasis *et al*., 2025; Togola *et al*., 2025). Originária das Américas, onde está presente desde a Argentina até o Canadá, *S. frugiperda* tem se expandido rapidamente para outras regiões. Após sua primeira detecção na África em 2016 (Goergen *et al*., 2016), a praga foi registrada também na Ásia (EPPO, 2018) e na Europa (EPPO, 2021), gerando grande preocupação quanto ao seu impacto na agricultura em escala global (Kenis *et al*., 2022).

FIGURA 1 – Características morfológicas de *Spodoptera frugiperda* (Lepidoptera: Noctuidae): (A) massa de ovos em camadas, recoberta por escamas protetoras; (B) mariposa adulta (macho); (C) lagarta – vista dorso-lateral, evidenciando o padrão em “Y” invertido na cabeça; (D) detalhe dos quatro pontos escuros dispostos em quadrado na região posterior do corpo.



FONTE: Adaptado de CABI (2019).

No Brasil, a lagarta-do-cartucho está amplamente distribuída nas principais regiões produtoras de milho (EPPO, 2025). Essa ampla ocorrência é fortemente favorecida pela disponibilidade contínua de plantas hospedeiras ao longo do ano (“ponte verde”), resultado principalmente do cultivo sucessivo de culturas que servem como fontes de alimento para a espécie, como milho, soja e algodão, conforme discutido na seção anterior (Souza *et al*., 2017; Lian *et al*., 2021; Adnan *et al*., 2024). Além das culturas agrícolas, *S. frugiperda* pode se desenvolver em diversas plantas daninhas comuns nos agroecossistemas brasileiros. Dentre as espécies hospedeiras da família Poaceae, destacam-se o capim-pé-de-galinha, o sorgo-selvagem (*Sorghum verticilliflorum*) e o capim-amargoso (*Digitaria insularis*). Também são hospedeiras espécies de outras famílias botânicas, como a buva (*Conyza* sp., Asteraceae), o caruru-verde (*Amaranthus hybridus*, Amaranthaceae) e a trapoeraba (*Commelina benghalensis*, Commelinaceae) (Moraes *et al*., 2020; Leite *et al*., 2021).

O desenvolvimento de *S. frugiperda* é influenciado por fatores como temperatura e tipo de planta hospedeira (Silva *et al*., 2017). Quando associada ao milho, a praga pode completar seu ciclo de vida (larva-adulto) em torno de 21 dias, e necessita de 26 dias quando se alimenta de soja (Silva *et al*., 2017). As fêmeas adultas, após o acasalamento, ovipositam em massas compostas por até quatro camadas de ovos sobrepostas, geralmente posicionadas nas folhas da planta (CABI, 2019). Cada massa pode conter várias centenas de ovos, que são parcialmente recobertos por escamas das asas depositadas pela própria fêmea, formando uma barreira protetora contra inimigos naturais (Dong *et al*., 2021; Hou *et al*., 2022). A eclosão ocorre entre três e quatro dias após a oviposição, e as lagartas neonatas iniciam a alimentação diretamente do tecido foliar (CABI, 2019).

Estima-se que as perdas de rendimento de milho causadas por *S. frugiperda* atinjam até 73% globalmente, com danos severos em diferentes continentes (Rwomushana *et al*., 2018; Guo *et al*., 2018; Wu *et al*., 2021). Por exemplo, em regiões onde a FAW foi introduzida, levantamentos com agricultores estimaram perdas de rendimento de milho em Gana e Zâmbia entre US$ 284 e US$ 198 milhões, respectivamente. De modo geral, as perdas variaram entre um total no continente de US$ 2,5 e US$ 6,3 bilhões em 2017, logo após a invasão imediata ao continente africano (Day *et al*., 2017). Estimativas mais recentes das perdas anuais de rendimento causadas pela lagarta-do-cartucho reportam até US$ 9,4 bilhões no continente africano (Senay *et al*., 2022).

A iminente entrada da lagarta-do-cartucho no continente europeu tem gerado preocupações, dadas às grandes perdas já observadas em outras regiões, como África. Essa praga ainda é classificada como quarentenária na União Europeia (UE) e está listada

como uma praga quarentenária A1 pela EPPO, cujo status atual é transitório (EPPO, 2025). Sua presença já foi relatada em países como Espanha (Ilhas Canárias), Chipre, Grécia, Portugal, Malta e Romênia (Moreno; Gastón, 2020; EPPO, 2024; EU, 2019; Lytra *et al*., 2024; Seguna *et al*., 2024; Cean *et al*., 2024). Inicialmente restrita a pequenas ilhas, onde sua presença era considerada sob controle, a espécie passou a migrar para áreas continentais, inserindo-se efetivamente no contexto geográfico de países da União Europeia. Foi registrada também na Bulgária (Szanyi *et al*., 2025), evidenciando a rápida capacidade de dispersão já conhecida dessa espécie, cujos adultos podem migrar até 100 km em um único dia (FAO, 2020).

Estimativas preliminares indicam que os potenciais impactos econômicos no sul da Europa podem resultar em perdas anuais de até €546 por hectare na margem bruta do milho em grão, superando €900 milhões no pior cenário (Kartakis *et al*., 2025). É importante destacar que as táticas de controle atuais para o manejo da lagarta-do-cartucho são desafiadoras devido à sua alta fecundidade e mobilidade, elevado potencial para seleção de resistência a inseticidas e proteínas *Bt*, e à sua alta plasticidade fisiológica e comportamental (Paredes-Sánchez *et al*., 2021; Serrão *et al*., 2022). Além disso, o controle dessa praga ainda é baseado, em grande parte, no uso intensivo de inseticidas químicos (van Den Berg, du Plessis, 2022).

O uso excessivo e a aplicação inadequada de pesticidas tóxicos não apenas impactam negativamente a saúde humana e o meio ambiente (Jacquet *et al*., 2022), como também exercem pressão de seleção sobre populações de insetos-praga, contribuindo para a seleção de populações resistentes e comprometendo a eficácia do controle ao longo do tempo (Jacquet *et al*., 2022; Gong *et al*., 2023). Em escala global, a *S. frugiperda* tem sido selecionada para resistência à maioria das classes de inseticidas sintéticos, incluindo piretróides (como lambda-cialotrina, permetrina, cialotrina, tralometrina, bifentrina e fluvalinato) e organofosforados (como malation, clorpirifós, metil paration, diazinon e sulfofos) (Carvalho *et al*., 2013; Bolzan *et al*., 2019; Lira *et al*., 2020; Garlet *et al*., 2021; Nascimento *et al*., 2023**;** Roy *et al*., 2023; Guo *et al*., 2024; Kanno *et al*., 2024; Ngegba *et al*., 2025). De acordo com o Arthropod Pesticide Resistance Database (APRD), há pelo menos 272 casos reportados de resistência de *S. frugiperda* ao redor do mundo, distribuídos em pelo menos 11 grupos de modos de ação (considerando as classes distintas e relevantes com base no Insecticide Resistance Action Committee (IRAC), 34 ingredientes ativos químicos e sete proteínas *Bt* (Kenis *et al*., 2022; Mota-Sanchez; Wise, 2025; Ngegba *et al*., 2025).

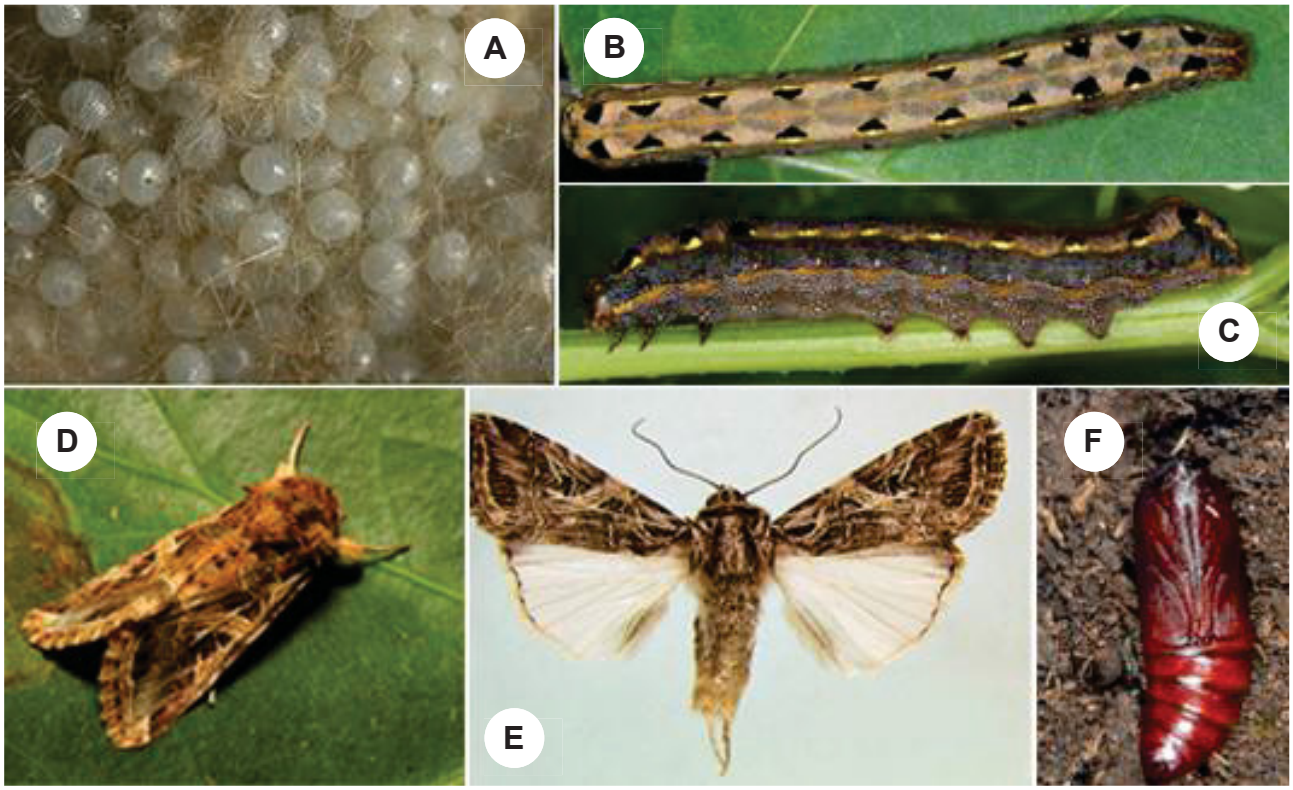
Um dos principais mecanismos associados à resistência em populações *de S. frugiperda* é o processo de detoxificação, ou seja, a capacidade dos insetos de utilizar enzimas específicas para quebrar ou modificar a molécula inseticida, tornando-a menos tóxica e facilitando sua excreção (Ismail *et al*., 2020; Hilliou *et al*., 2021; Chen *et al*., 2023). Enzimas, como citocromos P450 (P450s), as esterases (ESTs) e as glutationa S-transferases (GSTs), têm sido constantemente atribuídas a esse processo de detoxificação em *S*. *frugiperda* (Hilliou *et al*., 2021; Liu et al., 2022).

Além dos inseticidas químicos, populações de *S. frugiperda* também tem sido selecionadas para resistência às principais tecnologias de cultivos de plantas *Bt*. Populações resistentes foram selecionadas para diferentes proteínas inseticidas expressas no milho, incluindo Cry1Ab, Cry1Fa (Farias *et al*. 2016; Santos-Amaya *et al*. 2017; Tabashnik; Carrière, 2019), Cry1, Cry2, Cry1F e Vip3Aa (Huang *et al*., 2021; Banerjee *et al*., 2022; Yang *et al*., 2022; Liu *et al*., 2024).

2.2.2 *Spodoptera littoralis*

Outra espécie de grande relevância pertencente ao gênero *Spodoptera*, mas cuja ocorrência é ausente no Brasil, é *S. littoralis* (Figura 2), conhecida como lagarta-do-algodão africana. Trata-se também de uma espécie de lepidóptero da família Noctuidae, nativa da África e considerada uma das pragas mais destrutivas da agricultura (ElShahed *et al*., 2023). Extremamente polífaga, essa praga alimenta-se de mais de 130 espécies vegetais pertencentes a 56 famílias botânicas (Pogue, 2002; El-Sheikh *et al*., 2018). Sua notável capacidade de adaptação a diferentes ambientes e hospedeiros a torna uma ameaça significativa para a produção agrícola em diversas regiões.

FIGURA 2 – Características morfológicas de *Spodoptera littoralis*. (A) massa de ovos; (B) lagarta – vista dorsal; (C) lagarta – vista lateral; (D) mariposa adulta (fêmea); (E) mariposa adulta (macho) – vista dorsal; (F) pupa.



FONTE: Adaptado de Wagner (2025).

A lagarta-do-algodão passa por até seis estágios larvais. As larvas jovens apresentam coloração verde-clara com a cabeça acastanhada. À medida que se desenvolvem, a coloração pode variar de cinza a avermelhada ou amarelada, destacando-se por uma linha dorsal mediana ladeada por duas faixas laterais amarelo-avermelhadas ou acinzentadas, além de pequenos pontos amarelos distribuídos em cada segmento abdominal. Uma característica marcante que permite distinguir *S. littoralis* de outras espécies do mesmo gênero é a presença de quatro pontos pretos dispostos em forma de triângulo sobre o dorso. As fêmeas ovipositam de 20 a 500 ovos por postura, geralmente na face inferior das folhas, preferencialmente nas partes mais baixas da planta. Ao longo de sua vida, uma única fêmea pode depositar até 3.000 ovos. Assim como outras espécies do gênero *Spodoptera*, os ovos são recobertos por escamas amareladas ou castanho-amareladas provenientes do abdômen da fêmea, o que reduz sua visibilidade e contribui

para a proteção contra dessecação (EPPO BULLETIN, 2015; CABI, 2022; KOPPERT, 2025).

A distribuição geográfica de *S. littoralis* abrange principalmente a África, o Oriente Médio, o sul da Europa e algumas ilhas do Pacífico (ElShahed *et al*., 2023; Durand *et al*., 2025; EPPO, 2025). Embora não ocorra no Brasil, sua presença em outras regiões tropicais e subtropicais demonstra seu potencial invasivo e o risco de introdução em novas áreas (CABI, 2022). Além de atacar culturas como milho, algodão e soja, essa espécie também causa danos significativos em plantas ornamentais, frutíferas e olerícolas, como pepino, pimentão e alface, ampliando seu potencial de impacto econômico (Baskar *et al*., 2012; CABI, 2022; EPPO, 2025).

Os danos econômicos causados por *S. littoralis* resultam das injúrias ocasionadas pelas lagartas, que se alimentam vorazmente de folhas jovens, brotos, e frutos, comprometendo também as estruturas reprodutivas das plantas, reduzindo a qualidade e o rendimento das colheitas (CABI, 2022). O controle dessa praga, assim como de *S. frugiperda*, é particularmente desafiador devido à sua alta fecundidade, múltiplas gerações anuais e rápida evolução para resistência a inseticidas químicos (FAO, 2020; Amaral *et al*., 2022), características comuns ao gênero *Spodoptera* (Mota-Sanchez; Wise, 2025).

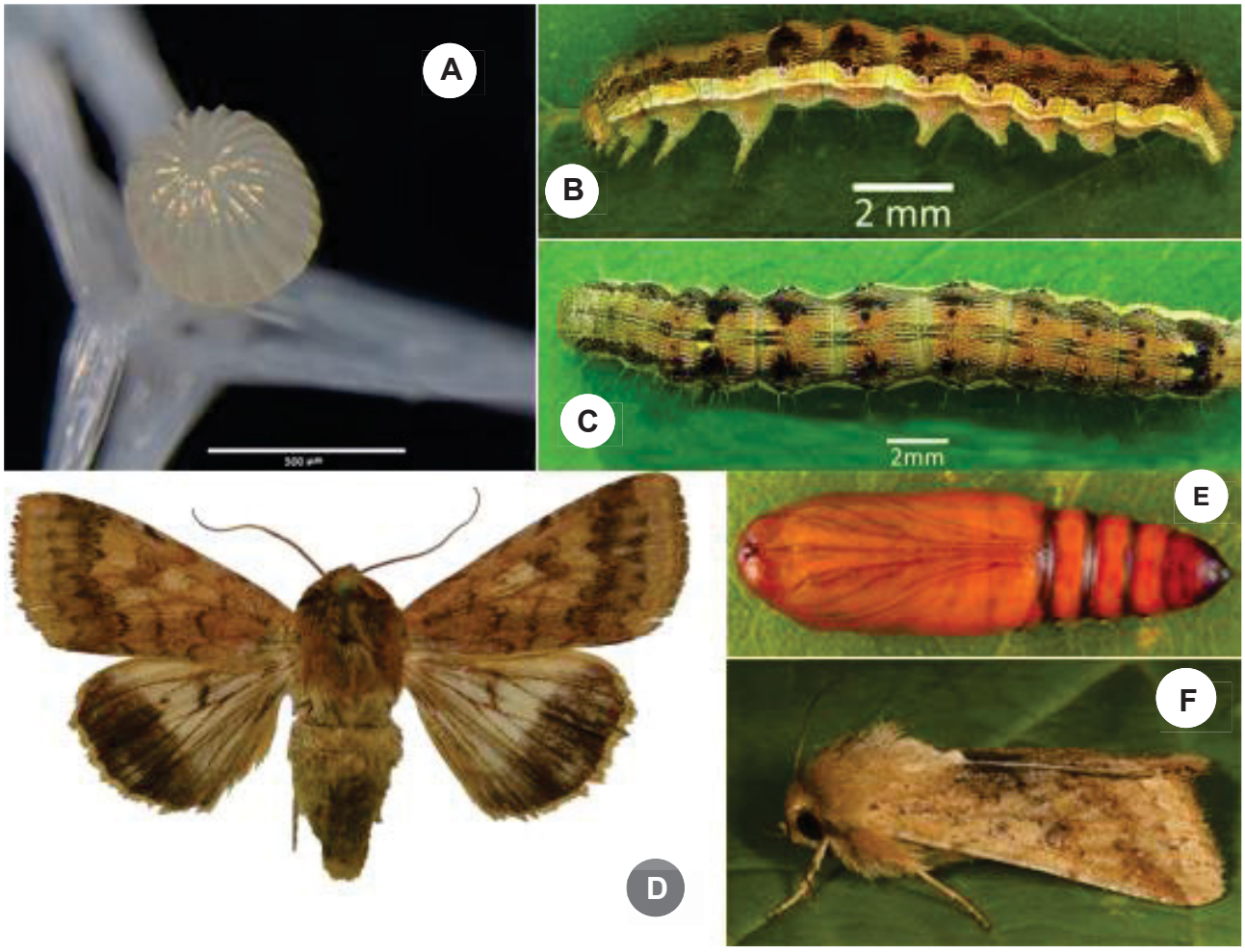
Já foram relatados ao menos 67 casos de resistência para *S. littoralis* (Mota-Sanchez; Wise, 2025), envolvendo diversos grupos químicos, incluindo organofosforados, carbamatos, piretroides e diamidas (Hilliou *et al*., 2021; Ismail *et al*., 2023; Mokbel *et al*., 2024; Mota-Sanchez; Wise, 2025). No caso de *S. littoralis*, a exemplo de *S*. *frugiperda*, citocromos P450 (P450s) e glutationa S-transferases (GSTs) estão entre as principais enzimas defensivas envolvidas nos mecanismos de resistência (Ismail *et al*., 2020; Hilliou *et al*., 2021; Yao *et al*., 2025). Esse cenário para ambas as espécies de *Spodoptera* evidencia não apenas o alto potencial de dano, mas também à crescente dificuldade de manejo imposta pela resistência a inseticidas. Tais desafios têm impulsionado a busca por alternativas mais sustentáveis e ambientalmente seguras, incluindo o desenvolvimento de bioinsumos e produtos de base natural, os chamados produtos “verdes”, que possam integrar-se a programas de MIP apresentando controles mais eficazes, duradouros e com menor impacto ambiental.

2.2.3 *Helicoverpa* spp.

As lagartas da subfamília Heliothinae (Lepidoptera: Noctuidae) também constituem um complexo de grande relevância nos sistemas produtivos. Essa subfamília inclui

espécies de importância agrícola como *Helicoverpa zea* (Boddie, 1850), conhecida como lagarta-da-espiga, *H. armigera* e *Chloridea virescens* (Fabricius, 1777) (anteriormente denominada *Heliothis virescens)*. No entanto, no presente estudo, iremos focar *Helicoverpa* spp. devidos sua importância econômica ao cultivo do milho, enquanto *C. virescens* não é considerada uma praga relevante nesse cultivo. A identificação entre *H. zea* e *H. armigera* (Figura 3) é frequentemente complexa devido à sua acentuada semelhança morfológica, genética e fisiológica, o que historicamente resultou em confusões taxonômicas, especialmente em regiões onde *H. armigera* foi recentemente introduzida (Tay *et al*., 2013; CABI, 2021). No entanto, *H. armigera* diferencia-se por sua maior agressividade e elevado grau de resistência a diversos grupos químicos de inseticidas, características que a tornam um desafio ainda maior para o MIP (Tay *et al*., 2013).

FIGURA 3 – Características morfológicas de *Helicoverpa armigera.* (A) ovo; (B) lagarta – vista lateral; (C) lagarta – vista dorsal; (D) mariposa adulta (fêmea) – vista dorsal; (E) pupa; (F) mariposa adulta (macho) – vista lateral.



FONTE: Adaptado de Santos et al. (2018).

Com ampla distribuição na África, sul da Europa e Oriente médio (EPPO, 2025), *H. armigera* representa uma séria ameaça para um vasto leque de culturas, incluindo milho, algodão, soja e tomate (Bueno; Sosa-Gómez, 2014; Pratissoli *et al*., 2015; Pomari-Fernandes *et al*., 2015). Sua polifagia, alta prolificidade e notável capacidade de resistência a múltiplos grupos químicos de inseticidas a qualificam como uma praga de difícil manejo e com significativo impacto econômico (Salvadori; Suzana, 2019; CABI, 2021). A espécie foi identificada morfológica e molecularmente nas Américas em 2013, após sua detecção em várias regiões agrícolas do Brasil (Specht *et al*., 2013; Bueno; Sosa-Gómez, 2014).

O estádio larval apresenta de cinco a seis ínstares e pode durar de duas a três semanas, dependendo das condições climáticas (CABI, 2021). Os danos causados pelas lagartas, especialmente *H. armigera*, na cultura do milho podem afetar as estruturas vegetativas; no entanto, os maiores prejuízos são geralmente observados durante a fase reprodutiva da planta (Valicente, 2015). A infestação da lavoura de milho inicia-se usualmente com a oviposição das fêmeas nos estilo-estigmas. Após a eclosão, as lagartas neonatas começam a se alimentar desses tecidos e, à medida que se desenvolvem, migram para o interior das espigas, onde consomem os grãos em formação, impactando diretamente a produtividade da cultura (Ávila *et al*., 2013; CABI, 2021).

Existem aproximadamente 892 relatos de resistência envolvendo *H. armigera* (Mota-Sanchez; Wise, 2025). Os casos registrados incluem resistência a inseticidas pertencentes a diferentes grupos químicos, como carbamatos, organofosforados, piretroides, espinosinas, oxadiazinas, diamidas e também a proteínas *Bt* (Liu *et al*., 2017; Ahmad *et al*., 2019; Bird *et al*., 2023; Mota-Sanchez; Wise, 2025).

No Brasil, um dos primeiros relatos de resistência em *H. armigera* foi registrado em 2017, relacionado à resistência a piretroides, associada a uma elevada frequência do gene CYP337B3 (Durigan *et al*., 2017). Posteriormente, uma linhagem resistente à flubendiamida foi selecionada em laboratório e, após 14 gerações sob pressão de seleção, apresentou uma razão de resistência superior a 50.000 vezes em relação à linhagem suscetível (Abbade-Neto *et al*., 2022). Apesar do elevado potencial para a seleção de resistência demonstrada por essa espécie, custos adaptativos estão associados, como redução da taxa intrínseca de crescimento populacional em linhagens resistentes à flubendiamida (Abbade-Neto, 2021; Abbade-Neto *et al*., 2022).

2.3 PARASITOIDES DE OVOS COMO AGENTES DE CONTROLE BIOLÓGICO

Dentre a diversidade de bioinsumos, os agentes de controle biológico desempenham um papel central no manejo de insetos-praga, com destaque para os parasitoides de ovos, que se mostram particularmente eficazes contra lepidópteros (Bueno *et al*., 2022). Os parasitoides de ovos são insetos de vida livre na fase adulta, conhecidos popularmente como “vespinhas”, que, durante a sua fase larval, vivem e se alimentam dentro de um ovo hospedeiro (geralmente um ovo de uma praga), provocando a morte e, portanto, o controle desse ovo (Parra *et al*., 2021; Bueno *et al*., 2024a).

Ao interromperem o ciclo da praga ainda na fase de ovo, impedem a eclosão das larvas e, consequentemente, os danos subsequentes às plantas. Tais agentes são reconhecidos por sua segurança, especificidade e potencial para produção em larga escala, o que viabiliza sua utilização em programas de liberação inoculativa ou aumentativa (Consoli *et al*., 2010; Bueno *et al*., 2024b). O avanço do controle biológico com parasitoides no Brasil tem sido acompanhado por progressos em tecnologias de produção massal, estratégias de liberação e na sua integração com outros componentes do MIP.

Esses avanços têm permitido o sucesso de programas de controle não só de lepidópteros, mas também de outros grupos de pragas, como os percevejos-pentatomídeos, incluindo *Euschistus heros* (Fabricius, 1798) (Hemiptera: Pentatomidae), além de apresentarem eficiência também contra espécies do gênero *Dichelops* spp., por meio da liberação de *Telenomus podisi* Ashmead, 1893 (Hymenoptera: Scelionidae), com registro comercial junto ao MAPA para o manejo de percevejos desde 2019 e adoção crescente por produtores de soja nos anos subsequentes.

2.3.1 *Trichogramma pretiosum*

Os parasitoides de ovos do gênero *Trichogramma spp*. (Hymenoptera: Trichogrammatidae) representam um dos grupos mais estudados e amplamente utilizados no controle biológico de pragas agrícolas, com particular relevância no Brasil (Parra; Coelho, 2019; Parra *et al*., 2021). Das 240 espécies identificadas globalmente neste gênero, 40 ocorrem no Brasil (Parra *et al*., 2015; Querino *et al*., 2025).

Dentre as diversas espécies que compõem o gênero, *T. pretiosum* (Figura 4) (destaca-se como a mais estudada e empregada globalmente para o manejo de lepidópteros-praga (Parra, 2019; Navik *et al*., 2023). Sua eficácia é evidenciada pela alta taxa de parasitismo natural em lavouras, sendo a espécie de parasitoide de ovos mais frequentemente encontrada em culturas como a soja, onde é responsável por mais de 90% do parasitismo natural observado (Foerster; Avanci, 1999). Adicionalmente, *T. pretiosum* é

também o parasitoide predominante no controle natural de *S. frugiperda* (Beserra *et al*., 2002).

FIGURA 4 – Adulto de *Trichogramma pretiosum* em ovos de *Corcyra cephalonica* (Stainton, 1866) (Lepidoptera: Pyralidae).

FONTE: Adeney de Freitas Bueno.

Apesar de seu tamanho diminuto, variando entre 0,2 mm e 1,5 mm de comprimento (Pinto, 1998), uma única fêmea de *T. pretiosum* pode parasitar mais de 50 ovos de pragas durante sua fase adulta no campo, demonstrando seu elevado potencial como agente de controle biológico aumentativo (Bueno *et al*., 2024a). O tempo de desenvolvimento de ovo a adulto de *T. pretiosum* a 25 °C em ovos de *Anticarsia gemmatalis* (Hübner, 1818) (Lepidoptera: Eribidae) e *Chrysodeixis includens* (Walker, 1858) (Lepidoptera: Noctuidae) foi de 10,3 e 10,0 dias, respectivamente (Bueno *et al*., 2009). Esse desenvolvimento é dividido em ovo, larva e pupa, que ocorrem obrigatoriamente dentro do ovo hospedeiro (endoparasitoide), enquanto a fase adulta é de vida livre (Bueno *et al*., 2009; Bueno *et al*., 2022). A coloração escurecida dos ovos, indicativa do parasitismo, pode ser observada entre quatro e cinco dias após a oviposição do parasitoide (Bueno *et al*., 2024a).

Trichogramma pretiosum está registrado para uso comercial no Brasil desde 2013 (MAPA, 2013) e apresenta como principais vantagens seu hábito generalista e possibilidade de criação em hospedeiros alternativos, tornando sua criação e consequente adoção de

baixo custo, com recomendações de uso no controle de diversas espécies de lepidópteros, incluindo *A. gemmatalis*, *C. includens*, *S. frugiperda* e *Helicoverpa* spp. (AGROFIT, 2025). Quando utilizado na cultura do milho, devem ser liberados segundo a especificação de referência para a cultura (recomendações da bioindústria produtora), na quantidade de 100.000 parasitoides por hectare, divididos em três liberações, em pelo menos 25 pontos por hectare, com intervalos de sete dias. A liberação pode ser realizada tanto com os parasitoides na fase de pupas quanto na fase adulta, com ou sem o uso de cápsulas de liberação (AGROFIT, 2025).

Entretanto, apesar do seu potencial, o manejo de *S. frugiperda* com *T. pretiosum* de forma isolada provavelmente não será suficiente para alcançar o sucesso de controle da praga, ou seja, para mantê-la a abaixo do nível de dano econômico (Bueno *et al*., 2023). Isso se deve, em parte, à eficiência de parasitismo de *T. pretiosum* em massas de ovos de espécies do gênero *Spodoptera*, que é consideravelmente limitada por características morfológicas dessas posturas. Dentre essas características, destacam-se a disposição em multicamadas e a presença de escamas protetoras (Dong *et al*., 2021). As escamas depositadas pelas fêmeas de *S. frugiperda* atuam como barreiras físicas que dificultam o acesso do parasitoide aos ovos das camadas inferiores, restringindo o parasitismo principalmente à camada superficial (Dong *et al*., 2021; Hou *et al*., 2022).

Estudos como o de Pinto e Fernandes (2020) evidenciaram que massas de ovos em camadas influenciam significativamente a taxa de parasitismo por *T. pretiosum*. Os autores observaram que, em posturas com apenas uma camada de ovos, a média de ovos parasitados foi de 38, enquanto em posturas com duas camadas esse número caiu para 19. Considerando que as fêmeas de *S. frugiperda* podem ovipositar massas com até quatro camadas de ovos, o parasitismo por *T. pretiosum* torna-se ainda mais desafiador nesse cenário (Kasige *et al*., 2022). Em contraste, o parasitismo não é afetado em ovos de *H. armigera*, já que esses são depositados de forma isolada em camada única, facilitando o acesso do parasitoide (Santos *et al*., 2018).

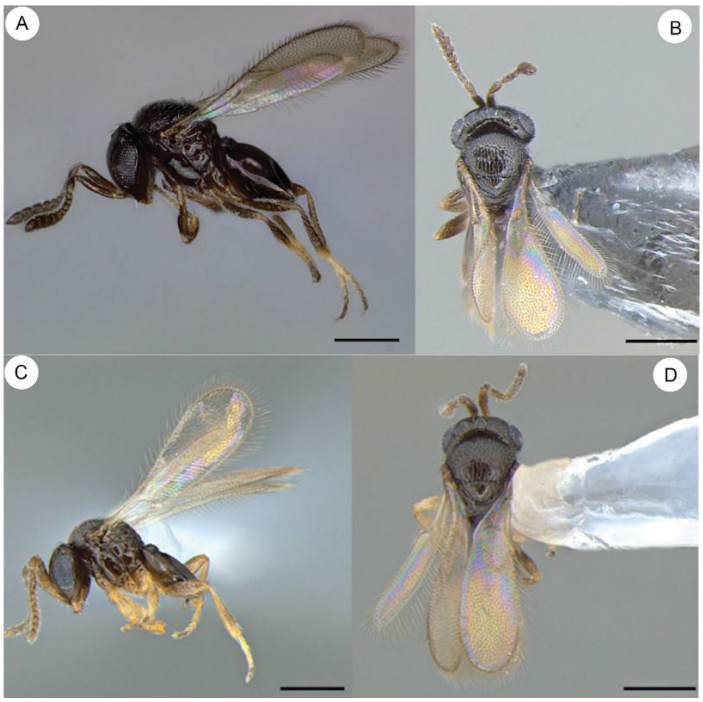
Além disso, a eficácia de *Trichogramma* spp. pode ser limitada em grandes áreas cultivadas, devido à sua reduzida capacidade de dispersão e quando sua liberação não é mecanizada (Bueno *et al*., 2012). Por esse motivo, o uso de *T. pretiosum* tem sido mais comum em cultivos de menor escala ou em ambientes protegidos, como casas de vegetação apesar de sua eficiência também em grandes cultivos. Por exemplo, Figueiredo *et al*. (2015) relataram um aumento de 19,4% na produtividade do milho orgânico após três liberações de *T. pretiosum*.

2.3.2 *Telenomus remus*

Telenomus remus (Figura 5) também é um parasitoide de ovos de pequeno porte, medindo de 0,4 a 0,6 mm de comprimento (Oktaviani *et al*., 2022), com longevidade que varia de 8 a 12 dias (Pomari *et al*., 2013; Bueno *et al*., 2014). O ciclo de vida de *T. remus* a 25 °C dura entre 10 e 15 dias, do ovo ao adulto. Ao final desse período, ocorre a emergência dos adultos, iniciando-se com os machos (fenômeno conhecido como protandria), que permanecem sobre os ovos hospedeiros aguardando a emergência das fêmeas, geralmente entre 17 a 24 horas após a emergência dos machos (Schwartz; Gerling, 1974; Bueno *et al*., 2008). A distinção entre os sexos pode ser feita por características morfológicas, as fêmeas apresentam fêmures e tíbias de coloração escura e antenas clavadas, enquanto os machos possuem fêmures e tíbias mais claros e antenas do tipo filiforme (Cave, 2000; Wengrat *et al*., 2021).

Assim que as fêmeas emergem, ocorre o acasalamento, seguido imediatamente pelo início da busca por novos hospedeiros para oviposição (Schwartz; Gerling, 1974). *Telenomus remus* apresenta reprodução partenogenética arrenótoca, ou seja, na ausência de cópula, as fêmeas geram apenas descendentes machos. Além disso, esse parasitoide tende a depositar um único ovo por hospedeiro (Rojas; Garcia-Roa, 1995).

FIGURA 5 – *Telenomus remus* (barra de escala: 0,20 mm): (A) Vista lateral fêmea. (B) Vista dorsal fêmea. (C) Vista lateral (macho). (D) Vista dorsal (macho).



FONTE: Adaptado de Wengrat et al. (2021).

Telenomus remus originário da região da Malásia Peninsular e Papua-Nova Guiné, foi introduzido em países da América Central e América do Sul com o objetivo de atuar como agente de controle biológico clássico contra espécies do gênero *Spodoptera* (Hernández *et al*., 1989; Wengrat *et al*., 2021). No Brasil, sua primeira introdução ocorreu em 1983, a partir de uma linhagem proveniente da República Dominicana, com foco no controle de *S. frugiperda*. Em 1996, uma nova introdução foi realizada pelo Centro Nacional de Pesquisa de Milho e Sorgo (Sete Lagoas, MG), utilizando espécimes oriundos da Venezuela. Uma terceira entrada do parasitoide no país ocorreu em 2011, promovida pela Universidade Estadual Paulista (UNESP), no campus de Jaboticabal, também com indivíduos da Venezuela (Naranjo *et al*., 2020; Wengrat *et al*., 2021).

Desde sua mais recente introdução no Brasil, *T. remus* tem sido amplamente pesquisado e aplicado em programas de controle biológico. Seu uso ao redor no mundo não se limita ao manejo de *S. frugiperda*, sendo também empregado no controle de outras pragas agrícolas de relevância econômica, incluindo aquelas que afetam culturas como o algodão (Pomari *et al*., 2012; Kenis *et al*., 2019; Colmenarez *et al*., 2022; Li *et al*., 2023a).

Em razão de seu desempenho superior frente a ovos de *Spodoptera* (especialmente *S. frugiperda*), *T. remus* se destaca como um agente promissor no controle biológico de pragas, sobretudo devido à sua elevada capacidade de parasitismo (Pomari *et al*., 2013; Bueno *et al*., 2014). Diferentemente de *T. pretiosum*, *T. remus* consegue superar barreiras físicas normalmente eficazes contra o parasitismo, como as escamas que recobrem as massas de ovos ovipositadas em camadas sobrepostas por mariposas de *Spodoptera* spp. (Dong *et al*., 2021; Li *et al*., 2023b). Além disso, apresenta notável capacidade de dispersão em campo e elevada eficiência na localização de hospedeiros (Pomari *et al*., 2013; Pomari *et al*., 2018). Fêmeas adultas são capazes de parasitar, em média, 121 ovos de *S. frugiperda* nas primeiras 24 horas, podendo alcançar até 220 ovos ao longo de sua vida, que varia entre 8 e 13 dias, dependendo das condições térmicas (Bueno *et al*., 2010; Pomari *et al*., 2013; Bueno *et al*., 2014). Essas características evidenciam o grande potencial de *T. remus* para programas de CBA voltados ao manejo de *Spodoptera* spp.

Apesar do reconhecido potencial de *T. remus* no controle de *Spodoptera* spp., sua adoção em larga escala ainda enfrenta limitações práticas. Diferentemente de *T. pretiosum*, amplamente estudado e já registrado e utilizado comercialmente no Brasil desde de 2013, *T. remus* foi apenas registrado no país em 2024 com ainda poucos estudos realizados e laboratórios produzindo o parasitoide, o que restringe sua inserção em programas oficiais de MIP (MAPA, 2024). O aumento de sua produção e uso de fato no manejo integrado de pragas está parcialmente relacionada a desafios técnicos na criação massal da espécie,

uma vez que não há um hospedeiro alternativo viável, o que encarece a produção e dificulta sua aplicação em programas de controle biológico aumentativo (CBA), exigindo sua criação no hospedeiro natural, *S. frugiperda* (Colmenarez *et al*., 2022).

Espécies de menor custo, como *Corcyra cephalonica* (Stainton, 1866) (Lepidoptera: Pyralidae), amplamente utilizadas na produção massal de *Trichogramma* spp. (Singhamuni *et al*., 2016; Laurentis *et al*., 2019), também foram avaliadas como hospedeiras para *T. remus* (Pomari-Fernandes *et al*., 2016; Queiroz *et al*., 2017). No entanto, os ovos de *C. cephalonica* são consideravelmente menores que os de *Spodoptera* spp., consequentemente há menor disponibilidade de nutrientes, resultando em adultos de *T. remus* de menor porte e longevidade reduzida em comparação àqueles criados em ovos de *S. frugiperda* (Kumar *et al*., 1986; Pomari-Fernandes *et al*., 2016; Queiroz *et al*., 2017).

Outro fator limitante à adoção de *T. remus* em programas de controle biológico é a ausência de uma densidade de liberação padronizada e amplamente validada (Bueno *et al*., 2023). Enquanto para *T. pretiosum* já existem recomendações consolidadas, como mencionado na seção anterior, os programas envolvendo *T. remus* ainda carecem de estudos que estabeleçam de forma precisa as doses ótimas de liberação sob diferentes condições ambientais e níveis de infestação da praga (Colmenarez *et al*., 2022).

A viabilidade e a eficiência de parasitoides após a criação estão diretamente relacionadas às condições de armazenamento e transporte, que influenciam parâmetros essenciais como a longevidade dos adultos, a taxa de emergência e a capacidade de parasitismo (Bueno *et al*., 2023; Bueno *et al*., 2024b). Fatores ambientais, como a ocorrência de chuvas, podem aumentar a mortalidade dos insetos, dificultar sua dispersão e até inviabilizar liberações em campo (Bueno *et al*., 2023). Experiências com *Telenomus podisi* Ashmead, 1893 (Hymenoptera: Scelionidae), como relatado por Roswadowski *et al*. (2024), demonstram que o uso de técnicas de armazenamento dentro de capsulas biodegradáveis pode ser uma estratégia viável para aumentar o tempo de prateleira dos parasitoides (viabilidade após a emergência dos adultos) flexibilizar o calendário de liberação, abordagem que pode ser adaptada ao manejo com *T. remus*, contribuindo para maior eficiência operacional.

2.4 ÓLEOS ESSENCIAIS NO MANEJO DE INSETOS-PRAGA

Óleos essenciais (OEs) são materiais naturais (oriundos de plantas) hidrofóbicos compostos por metabolitos secundários, como monoterpenos, sesquiterpenos e fenilpropenos e se destacam como ingredientes ativos promissores para o desenvolvimento

de biopesticidas (Do *et al*., 2015; Campolo *et al*., 2020). Esses compostos são sintetizados pelas plantas e exercem funções cruciais na defesa contra herbívoros, além de participarem de processos de sinalização, como a atração de polinizadores e insetos benéficos (Asbahani *et al*., 2015; Pavela *et al*., 2015; Zuzarte; Salgueiro, 2015; Giunti *et al*., 2023). Produzidos principalmente por plantas superiores, especialmente as espécies aromáticas (Asbahani *et al*., 2015), os OEs são originados por glândulas endócrinas e/ou exócrinas vegetais, bem como por células epidérmicas (Svoboda; Greenaway, 2003; Campolo *et al*., 2020), podendo ser armazenados em diferentes órgãos da planta, como flores, folhas, raízes, cascas ou sementes (Asbahani *et al*., 2015; Campolo *et al*., 2018).

Para que um óleo essencial (OE) seja caracterizado como tal, é necessário que seja extraído por métodos como hidrodestilação, destilação a vapor ou extração mecânica. Essa característica o diferencia dos extratos botânicos, que são obtidos por meio de solventes e contêm uma gama mais ampla e menos volátil de compostos (Do *et al*., 2015; Ribeiro *et al*., 2023). A ampla utilização dos OEs como estratégia sustentável de manejo deve-se, principalmente, à sua atividade inseticida, ação repelente, deterrência alimentar e outros efeitos adversos sobre a biologia de insetos (Catani *et al*., 2022; Giunti *et al*., 2023), atuando contra pragas de plantas (Isman; Seffrin, 2018; Mudrončeková *et al*., 2019; Soares *et al*., 2019), pragas de produtos armazenados (Campolo *et al*., 2018; Akami *et al*., 2019) e insetos vetores (Baskar *et al*., 2018; Benelli *et al*., 2019; Pavela *et al*., 2019).

Uma das principais vantagens dos óleos essenciais (OEs) é sua baixa toxicidade para humanos e organismos não alvos. Entre os efeitos provocados pelos OEs, destaca-se a ação no sistema nervoso dos insetos, capaz de induzir alterações comportamentais, paralisia e morte (Bendre *et al*., 2018; Youssefi *et al*., 2019; Ayllón-Gutiérrez *et al*., 2024). Além disso, são comuns efeitos subletais relevantes, como ação repelente, inibição alimentar, interferência no desenvolvimento larval e redução da longevidade dos adultos (Giatropoulos *et al*., 2023; Patel *et al*., 2023; Ayllón-Gutiérrez *et al*., 2024). Essas múltiplas ações e atributos reforçam o potencial dos OEs como agentes promissores para programas sustentáveis de manejo de pragas. Apresentam ainda atividade de amplo espectro, atuando sobre diferentes ordens de insetos, o que os torna ferramentas valiosas dentro dos princípios do MIP e compatíveis com práticas da agricultura sustentável (Pascual-Vilalobos *et al*., 2017; Giuliano *et al*., 2024).

No Brasil, existem diversos produtos fitossanitários de baixo impacto registrados no MAPA, com base em extratos e óleos vegetais. Entre eles, destacam-se o óleo de nim (*Azadirachta indica*), o extrato de *Melaleuca alternifolia*, o extrato de alho (*Allium sativum*), o extrato de *Reynoutria sachalinensis*, o extrato etanólico de *Sophora flavescens*, o

cinamaldeído, composto ativo da canela (*Cinnamomum verum*), reconhecidos por suas propriedades inseticidas e repelentes, dentre outros (AGROFIT, 2025).

Além dos compostos já regulamentados, há na literatura científica diversos relatos sobre o uso de outros extratos vegetais e óleos essenciais com potencial inseticida. Entre eles, destaca-se o extrato de moringa (*Moringa oleifera*, Moringaceae), com efeitos positivos no controle de pragas em culturas como feijão (Howladar, 2014; Elzaawely *et al*., 2017), milho (Basra *et al*., 2011) e trigo (Rehman *et al*., 2017). O extrato de alcaçuz (*Glycyrrhiza glabra*) também apresentou bons resultados no feijão (Rady *et al*., 2019) e na ervilha (Desoky *et al*., 2019), assim como o extrato de artemísia (*Artemisia vulgaris*) na cultura da batata (Findura *et al*., 2020). No entanto, esses produtos ainda não possuem registro oficial no Brasil, sendo utilizados apenas em estudos laboratoriais ou experimentações de campo em pequena escala.

Dentre as diferentes famílias botânicas utilizadas para a produção de óleos essenciais (OEs) com atividade inseticida, a família Lamiaceae se destaca pelo elevado potencial pesticida atribuído aos principais compostos presentes, como terpenoides, linalol e timol, que apresentam propriedades neurotóxicas, antialimentares, repelentes e efeitos reguladores do crescimento (Ebadollahi *et al*., 2020; Ali *et al*., 2025). Entre as espécies mais promissoras dessa família está o orégano (*Origanum* spp.), cuja eficácia no manejo de pragas é atribuída à ampla gama de compostos bioativos (Osório *et al*., 2019; Ebadollahi *et al*., 2020; Ali *et al*., 2025).

O orégano tem sido empregado com sucesso no controle de diferentes grupos de pragas ao redor do mundo, como *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), pragas de grãos armazenados, ácaros e espécies do gênero *Spodoptera* (Angliassa; Maffei, 2018; Pinto *et al*., 2022; Plata-Rueda *et al*., 2022; Susurluk, 2023). No entanto, no Brasil, as pesquisas voltadas ao uso do orégano como bioinseticida ainda são incipientes e limitadas a poucos estudos, especialmente no contexto do manejo de pragas de grãos armazenados (Plata-Rueda *et al*., 2022). A prospecção de novas espécies botânicas com potencial inseticida tem se concentrado, principalmente, nas famílias Annonaceae, Asteraceae, Meliaceae, Piperaceae e Solanaceae (Ribeiro *et al*., 2023).

Embora muitos estudos comprovem a eficácia inseticida de compostos vegetais, poucos produtos à base de óleos essenciais (OEs) estão disponíveis comercialmente, devido a limitações relacionadas ao uso em condições de campo (Campolo *et al*., 2020; Isman, 2023). Os OEs são substâncias lipofílicas, geralmente pouco solúveis em água, o que dificulta sua formulação e aplicação eficaz em ambientes agrícolas (Krzyżowski *et al*., 2020). Além disso, muitos desses compostos apresentam fitotoxicidade (Karalija *et al*.,

2020), e seus constituintes são altamente voláteis e suscetíveis à degradação (Pavela; Benelli, 2016). Por um lado, essa rápida biodegradabilidade pode ser vista como uma vantagem ambiental, por não deixar resíduos nos produtos agrícolas ou no ambiente. No entanto, do ponto de vista agronômico, a baixa persistência dos OEs puros pode comprometer sua eficácia no controle de insetos-praga (Campolo *et al*., 2020).

A incorporação de compostos bioativos em formulações nanoestruturadas, como nanopartículas ou nanoemulsões, tem o potencial de aumentar a estabilidade e a eficácia dos óleos essenciais sobre pragas-alvo (Campolo *et al*., 2017; He *et al*., 2019; Giunti *et al*., 2019). No entanto, essa estratégia também pode elevar a toxicidade para organismos não alvo e suscitar preocupações quanto aos impactos ambientais (Giunti *et al*., 2022). O crescente número de estudos sobre o uso da nanotecnologia na agricultura evidencia a oportunidade de aplicar essas inovações no desenvolvimento de biopesticidas (He *et al*., 2019). Tecnologias emergentes como essas podem representar um avanço significativo na implementação de estratégias sustentáveis e favorecer a adoção de óleos essenciais como ferramentas viáveis no manejo integrado de pragas.

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Chapter 1 - Interaction dynamics and field performance of *Telenomus remus* and *Trichogramma pretiosum* for egg parasitoid-based control of lepidopteran maize pests

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Abstract

Biological control using egg parasitoids is a key strategy in the integrated management of lepidopteran pests in large-scale cropping systems. Among these parasitoids, *Trichogramma pretiosum* and *Telenomus remus* are widely used. However, their simultaneous field release raises questions about their potential interactions and overall effectiveness. This study aimed to evaluate the performance and competitive dynamics between *T. remus* and *T. pretiosum* from laboratory to open-field conditions. Four bioassays were conducted: (1) host preference between *Spodoptera frugiperda* and *Helicoverpa armigera* eggs; (2) parasitism capacity (individual and combined); (3) interaction and competition (different densities and release timings); and (4) field releases (individual and combined, using natural and sentinel egg masses). Results consistently showed that *T. remus* outperformed *T. pretiosum* across all experimental conditions, with higher parasitism rates and no negative influence from competition or interspecific presence. In the field, when both species were released together, *T. remus* accounted for nearly all observed parasitism, with minimal contribution from *T. pretiosum,* a pattern consistent with laboratory findings. Under high-density conditions, *T. remus* exhibited a positive density-dependent response, whereas *T. pretiosum* showed reduced performance, likely due to mutual interference. Therefore, the use of *T. remus* as a commercial biocontrol agent needs to be improved not only for managing fall armyworm, but also for other lepidopteran pests in maize cropping systems, to provide farmers with a ready-to-use package.

Keywords: Biological control; field release; *Spodoptera frugiperda*; *Helicoverpa armigera*.

1. Introduction

Biological control is a valuable tool into integrated pest management (IPM), offering sustainable solutions that reduce the reliance on chemical insecticides (van Lenteren et al. 2018; Bueno et al. 2023), which often lead to pest resistance, ecological disruption, and harm to non-target organisms (Serrão et al. 2022; Ngegba et al. 2025). Among natural enemies used in biological control, egg parasitoids are particularly valuable because they target pests at the earliest life stage, preventing larval emergence and subsequent crop damage (Bueno et al. 2023). Their integration into IPM programs is valuable to mitigate yield losses while meeting the growing demand for environmentally responsible agriculture (Masry and El-Wakeil 2020).

The fall armyworm, *Spodoptera frugiperda* Smith, 1797 (Lepidoptera: Noctuidae), is one of the most destructive maize pests globally, causing yield losses in several regions of the world (Kenis et al. 2022; Kumar et al. 2022). Additionally, *Helicoverpa* spp. also threaten maize production, both pest species showing resistance to conventional insecticides and requiring multifaceted control strategies (Laminou et al. 2023). Given the economic and food security implications of those pest outbreaks, identifying effective and scalable biocontrol agents is imperative.

Despite targeting the same pest species and stage, two key egg parasitoids, *Telenomus remus* Nixon (Hymenoptera: Scelionidae) and *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae), have emerged as promising candidates due to their complementary traits (Bueno et al. 2023). *Telenomus remus* excels at parasitizing multi-layered *Spodoptera* egg masses, frequently covered with scales from the moths, with wasp females capable of parasitizing up to 220 eggs during their lifespan (Colmenarez et al. 2022) and achieving parasitism rates above 90% in field trials, being successfully deployed in large-scale IPM programs across South America (García-Roa, 1999; Ferrer, 2001; Figueiredo et al. 2002;

Colmenarez et al. 2022). In contrast, *T. pretiosum*, a more generalist egg parasitoid with broader host plasticity, is easily mass-reared on factitious hosts and performs better against a complex of lepidopteran species that lay their eggs in single layers, such as *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (Parra et al. 2021).

The exclusive use of either parasitoid has limitations. On one hand, *T. remus* is difficult to mass-rear without its target host (*S. frugiperda*), which is, mainly due to larval cannibalism recorded on larvae of *S. frugiperda*. It requires the host to be reared on individualized boxes to reduce larval mortality (Chapman et al. 2000; Silva and Parra, 2013). It increases the costs associated with host production and, consequently, costs associated with parasitoid mass production, which is an important obstacle to *T. remus* large-scale adoption (Colmenarez et al. 2022). On the other hand, *T. pretiosum* struggles to parasitize compact, scale-covered egg masses of *S. frugiperda*, accessing only the upper layers, limiting its success (Goulart et al. 2011; Dong et al. 2021). Thus, the combination or alternation of releases of both parasitoid species can be a strategy not only for managing *S. frugiperda* outbreaks (Lacerda et al. 2023) but also for addressing the multi-pest scenarios typically faced by farmers in field crops (Bueno et al. 2023).

Despite the well-known lower parasitism capacity of *T. pretiosum* on *S. frugiperda* eggs, due to its lower production costs compared to *T. remus*, combining both species may offer practical advantages within an IPM context (Colmenarez et al. 2022; Bueno et al. 2023). Despite overlapping on some host species, in Augmentative Biological Control (ABC), these species are often released against different targets, with *T. remus* especially for *S. frugiperda*, and *T. pretiosum* for *Helicoverpa* spp. (Kenis et al. 2019; Laurentis et al. 2019). It makes the understanding of their interaction particularly relevant in diversified cropping systems or during overlapping pest outbreaks (Bueno et al. 2023). Furthermore, long-term, large-scale releases of a single species may displace native or complementary natural enemies, as observed in

Brazilian sugarcane fields where the dominance of *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae), the most successful biological control case in Brazil, led to the decline of native Tachinid flies (Rossi and Fowler, 2004). Understanding the interactions between *T. remus* and *T. pretiosum* is therefore not only key to maximizing parasitism rates at lower costs in ABC programs but also to maintaining ecological balance and natural occurrence of both parasitoid species in the field, ensuring long-term sustainability of IPM strategies. Therefore, their simultaneous or sequential release in the field may result in outcomes ranging from synergy to antagonism, affecting control success and parasitoid persistence. In this context, the present study investigates the interaction between *T. remus* and *T. pretiosum* under laboratory and field conditions. We assess their host specificity, individual and combined parasitism rates; behavioral interactions and competition dynamics; in addition to field-level efficacy alone and under combined releases.

2. Material and methods

The bioassays assessing host preference (2.2), parasitism capacity (2.3), and interspecific interaction/competition between the parasitoids (2.4) were carried out at the parasitoid laboratory of Embrapa Soybean, in Londrina, Paraná, Brazil. All trials were carried out in a climate-controlled chamber (ELETROLab®, model EL 212, São Paulo, SP, Brazil) set to 25 ± 2 °C, 80 ± 10% relative humidity, and a 14:10 h (L:D) photoperiod. In addition, the combined releases of these egg parasitoids was evaluated under field conditions (2.5) during two maize cropping seasons, first 2022/2023 and second season 2023 at the experimental field station of Embrapa Soybean in Londrina (23◦ 11′ 11.7″ S and 51◦ 10′ 46.1″ W) and on a commercial farm in Sabáudia (23°22'08.8"S 51°35'33.0"W), Paraná, Brazil, respectively.

117 *2.1 Parasitoid and host colonies*
118
119 The parasitoids *T. remus* and *T. pretiosum* and their hosts (*S. frugiperda* and *H.*
120 *armigera*) colonies were kept at Embrapa Soybean, Londrina, State of Paraná, Brazil. Eggs and
121 parasitoids used in all the experiments came from those colonies as briefly described in the
122 followings.
123 *Spodoptera frugiperda* specimens were originally collected from maize plants (C-strain)
124 in Embrapa Field Station, in 2020 and morphologically identified using the Manual for the
125 Identification of Insects and Other Invertebrates of Soybean Crops (Sosa-Gómez et al. 2014).
126 Since then, the insects have been maintained in the rearing during which new field insects had
127 been introduced each year to maintain colony quality. *Helicoverpa armigera* was collected in
128 soybean fields also at Embrapa Field Station, during 2021/22 crop season and morphological
129 and molecular identified performed as described by Pogue (2004) and Behere et al. (2008) and
130 kept the laboratory since then. Larvae of both species were reared under laboratory-controlled
131 conditions (25 ± 2°C, 70 ± 10% RH, and a photoperiod of 14:10 h [L:D]) Initially, neonates
132 were individualized in plastic cups (50 mL) containing an artificial bean-based diet (Greene et
133 al. 1976) until reaching the pupae stage. Then, pupae were separated by sex (Butt,1962) and
134 transferred to acrylic cages (45 × 33 × 35 cm) for eclosion of the adults and their mating and
135 oviposition. The adults were fed with a diet based on honey (10%) and distilled water. The cage
136 was covered internally with white paper as oviposition substrate, and the eggs were collected
137 daily for experiments or colony maintenance.
138 *Telenomus remus* was originally collected in Ecuador and grown at the parasitoid
139 rearing facilities of ESALQ/USP (Luiz de Queiroz College of Agriculture/University of São
140 Paulo), from where some specimens were transferred to Embrapa Soybean in 2008. Since then,
141 *T. remus* has been reared in the laboratory using *S. frugiperda* eggs. Given the prolonged use
142 of the colony and the absence of new introductions of genetic material, colony management

was focused on maintaining performance by monitoring key biological traits such as body size, parasitism efficiency, fly ability and adult longevity, in order to minimize the effects of inbreeding and ensure strain quality.

The laboratory strain of *T. pretiosum* used in this study was sourced from the company JB Biotecnologia Ltda (Paraopeba, Minas Gerais, Brazil) and reared at Embrapa Soybean (only during trials) accordingly to methodology adapted from Parra (1997). Adult parasitoids were placed in cages (one-liter transparent plastic jars, covered with PVC film) with drops of pure-bee honey for feeding of the wasps. Then, eggs of *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Eribidae) (≤ 24-h old) were offered to parasitism for 24 h. Those eggs were glued on cardboard cards (12 × 10 cm). After 24 hours of exposure to parasitism, the cards were removed from the cages and placed in new cages until adult emergence. Newly emerged parasitoids were either used for trials or for maintaining the parasitoid colony.

2.2 Host preference of T. pretiosum (experiment 1) and T. remus (experiment 2) between eggs of S. frugiperda and H. armigera

Two independent bioassays (experiment 1 with *T. pretiosum* and experiment 2 with *T. remus*) were carried out to study parasitoid host preference under choice conditions in a completely randomized design, adopting the arena method described by Thuler et al. (2007), with a total of 15 arenas (replicates) per treatment (eggs of *H. armigera* and eggs of *S. frugiperda*). Each arena consisted of a transparent polyethylene bottle (4 cm in height and 2 cm in diameter) containing two 1.5 mL plastic microtubes placed equidistantly. Each microtube held a cardboard strip (0.9 × 6.0 cm) containing approximately 150 eggs of *S. frugiperda* and 100 eggs of *H. armigera*, all less than 24 hours old (**Fig. 1**). A single mated female parasitoid (newly emerged: ≤ 24 h old), was introduced at the center of the vertical arena, allowing free choice between the two egg types. Eggs were exposed to parasitism for 24 hours. After

exposure, the females were removed and the egg cards were maintained under controlled conditions in Biochemical Oxygen Demand (BOD) climate chambers (ELETROLab®, model EL 212, São Paulo, SP, Brazil) at 25 ± 2 °C, 80 ± 10% relative humidity, and a 14:10 h (L:D) photoperiod. Eggs were checked daily until parasitoid emergence.

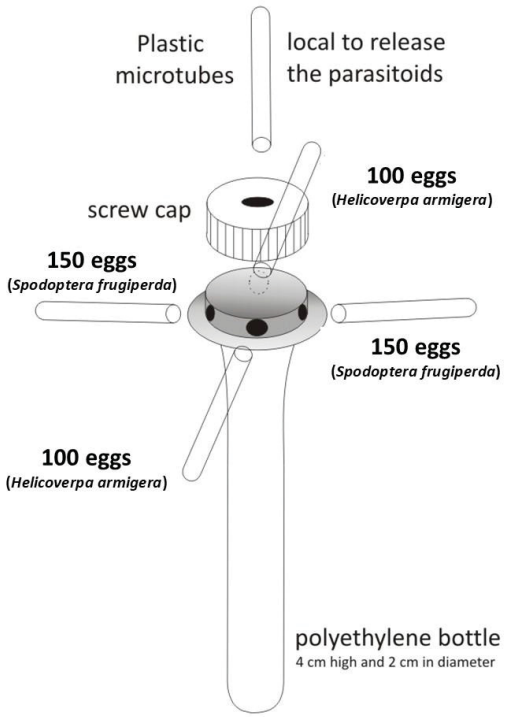

Fig. 1. Arena adapted from Thuler et al. (2007) used in the parasitoid host preference test among two-host species.

The number of parasitized eggs and parasitism rate (%) which was calculated as the number of parasitized eggs / (number of parasitized eggs + number of non-parasitized eggs) × 100. Parasitism was determined by counting the number of emerged adult parasitoids plus the number of fully developed but dead individuals found inside the host eggs, as confirmed by dissection.

2.3 Parasitism capacity of T. remus and T. pretiosum on S. frugiperda and H. armigera eggs of isolated females and in association of both species (experiment 3)

The experiment was conducted in a completely randomized design with six treatments and 20 replicates. The treatments were: (**1**) One *T. remus* + one *T. pretiosum* on *S. frugiperda* eggs, (**2**) One *T. remus* + one *T. pretiosum* on *H. armigera* eggs, (**3**) One *T. remus* on *S. frugiperda* eggs; (**4**) One *T. pretiosum* on *S. frugiperda* eggs; (**5**) One *T. remus* on *H. armigera* eggs, and (**6**) One *T. pretiosum* on *H. armigera* eggs. Each replicate consisted of one or two individualized mated newly emerged parasitoid females (≤ 48h-old *T. remus* and ≤ 24 h-old *T pretiosum*) placed into separate glass tubes (12 mm Ø x 75 mm tall) covered with PVC film. Droplets of pure honey on the walls of the glass tubes were offered to feed the females. Around 150 eggs of S. *frugiperda* (≤ 24 h old), were glued onto a white card board paper (2.5 cm x 5 cm). Similarly, 100 eggs of *H. armigera* (≤ 24 h old) were glued onto different cards also made of white card board paper (2.5 cm x 5 cm). Each paper was previously labeled with the respective treatments. Then, these cards, having the eggs, were offered for parasitism for 24 h and the cards (with the eggs) were replaced daily until the death of the female parasitoid. The eggs daily removed from the glasses were maintained inside the same controlled environmental chamber under the controlled condition until the emergence of the parasitoids. The evaluated parameters were the number of parasitized eggs per day (daily parasitism), lifetime parasitism, emergence (%) and parental female longevity (days). For the combination of *T. remus* and *T. pretiosum* treatments (treatments 1 and 2), the number of parasitized eggs per day (daily parasitism) and lifetime parasitism were total observed per replicate with one parasitoid of each species together and parental female longevity (days) were the average of both females in each replicate.

2.4 Interaction and interspecific competition between T. remus and T. pretiosum on S. frugiperda eggs (experiment 4)

The experiment was carried out in a completely randomized design with nine treatments and 20 replicates. The studied treatments were: (**1**) egg masses first exposed to *T. pretiosum* female for 24 hours, then to *T. remus* female for another 24 hours; (**2**) egg masses first exposed to *T. remus* for 24 hours, then to *T. pretiosum* for another 24 hours; (**3**) simultaneous exposure of the egg masses to both parasitoid species for 24 hours; (**4**) sequential exposure of the egg masses to two different *T. remus* females, each for 24 hours; (**5**) sequential exposure of the egg masses to two *T. pretiosum* females, each for 24 hours; (**6**) simultaneous exposure of the egg masses to two *T. pretiosum* females for 24 hours; (**7**) simultaneous exposure of the egg masses to two *T. remus* females for 24 hours; (**8**) exposure to a single *T. remus* female for 24 hours; and (**9**) exposure to a single *T. pretiosum* female for 24 hours.

Each replicate consisted of a single mated female parasitoid (≤ 48h-old *T. remus* and ≤ 24 h-old *T pretiosum*, with no prior oviposition experience) placed individually inside a glass tube (7.5 cm long × 1.0 cm diameter). Inside each tube, one *S. frugiperda* egg mass (≤ 24 h old, ~120 eggs) was attached to a piece of white cardboard (2.5 cm × 5.0 cm) and offered for parasitism. A drop of pure honey was placed on the inner wall of each tube as a food source for the parasitoid adult. The tubes were sealed with PVC film and maintained in a Biochemical Oxygen Demand (BOD) (ELETROLab®, model EL 212, São Paulo, SP, Brazil) regulated at 25 ± 2 °C, 80 ± 10% relative humidity, and a 14:10 h (L:D) photoperiod.

After the exposure period, parasitoid females were removed, and the egg masses were monitored daily until adult emergence. Emerging *S. frugiperda* larvae from unparasitized eggs were counted and removed daily to prevent cannibalism of adjacent eggs. Emerged parasitoids were separated by species and counted. The following biological parameters were recorded: total parasitism rate (%), parasitism by species (*T. pretiosum* and *T. remus*) and emergence (%).

234

235 *2.5 Field performance of T. remus and T. pretiosum individually or in combined releases of*
236 *both species (experiment 5)*

237

238 The experiment was conducted under field conditions during two consecutive maize
239 seasons (summer crop season of 2022/2023 and autumn/winter crop season of 2023) in the
240 municipalities of Londrina (23°21′19.2″ S, 51°10′16.8″ W) and Sabáudia (23°19′04″ S,
241 51°33′10″ W), in the northern region of Paraná State, Brazil, respectively.

242 In the 2022/2023 season, the trial followed a randomized block design with four
243 treatments (2.7 hectares each treatment) divided into four areas of pseudoreplicates (6,750 m^2
244 each replicate). The treatments were: (**1**) *Telenomus remus* release at 10,000 parasitoids per
245 hectare; (**2**) *Trichogramma pretiosum* release at 100,000 parasitoids per hectare (as per product
246 label recommendation for maize); (**3**) combined release of both parasitoids in a 50%:50%
247 proportion from treatments 1 and 2 (50,000 *T. pretiosum* and 5,000 *T. remus* per hectare); (**4**)
248 Combined release in a 70%:30% proportion from treatments 1 and 2 (70,000 *T. pretiosum* and
249 3,000 *T. remus* per hectare). In the second season (2023), the same experimental setup was used,
250 except for the 70:30 treatment, which was omitted due to limited availability of parasitoids.

251 Releases of *T. pretiosum* were conducted using pupae close to adult emergence, glued
252 onto small release envelopes and hanging in the plants. In contrast, *T. remus* was released as
253 mated and fed adults (48 hours post-emergence) inside biodegradable capsules according to the
254 recommendation, which ensures physiological readiness for host egg location and parasitism.
255 These distinct release strategies were adopted because they reflect the most effective and
256 adopted methods for each species, as reported by previous research (Bueno et al. 2023; Bueno
257 et al. 2024; Roswadoski et al. 2024). Releases were carried out using a minimum of 35 capsules
258 per hectare, corresponding to 35 evenly spaced release points per treatment.

A total of three releases were carried out per treatment, with intervals of six to eight days between them. Releases were performed early in the morning to avoid exposing the parasitoids to high temperatures and allow them to search for shelter. The releases were initiated during the early vegetative stage of the crop (approximately V2). Prior to the first release, a baseline assessment was conducted to determine natural parasitism levels. Parasitism evaluations were performed at 1, 2, and 6 days after each release. At each evaluation, 12 naturally laid egg masses and 12 sentinel egg masses were collected per plot. Sentinel egg masses consisted of *S. frugiperda* eggs from the laboratory colony, glued onto cardboard and attached to marking flags distributed throughout the experimental area (each egg masses in one equidistantly point).

After field exposure, each egg mass was individually placed in a plastic vial and transported to the entomology laboratory. Samples were maintained in climate-controlled chambers (BOD incubators) under standardized conditions. Egg masses were monitored daily to record parasitoid emergence, and any *S. frugiperda* larvae were promptly removed to prevent cannibalism. Parasitism rate (%) was calculated as the number of parasitized eggs / (number of parasitized eggs + number of non-parasitized eggs) × 100

2.6 Statistical analysis

For the laboratory bioassays, the parasitism rate, total number of parasitized eggs, and emergence were analyzed using generalized linear models (GLM) using a binomial error distribution (logit link function). For the field parasitism which include effects such as sampling date or block) was used a generalized linear mixed model (GLMMs), fitted using glmmTMB (package). Multiple comparisons were conducted using estimated marginal means (EMMs), applying Tukey's method for p-value adjustment via the emmeans package (Lenth, 2023).

Female longevity was analyzed using the non-parametric Kruskal–Wallis test, followed by Dunn's post hoc test with Bonferroni correction, as the data did not meet normality assumptions, using the FSA package (Ogle et al. 2023). All statistical analyses were performed in R software (R Core Team, 2024).

3. Results

3.1 Host preference of T. pretiosum (experiment 1) and T. remus (experiment 2) between eggs of S. frugiperda and H. armigera

A clear host specialization was observed by both parasitoids: *T. pretiosum* parasitized significantly more *H. armigera* eggs (20.3 ± 3.5 eggs; parasitism of 13.5%) than *S. frugiperda* eggs (4.8 ± 1.8 eggs; parasitism of 3.2%) ($\chi^2 = 134.1$; $p < 0.001$; df = 1), indicating a strong preference for isolated eggs of *H. armigera* compared to the superposed egg mass of *S. frugiperda* covered with moth's scales (**Table 1**). Conversely, *T. remus* exhibited the opposite trend, showing significantly higher parasitism on *S. frugiperda* eggs (96.6 ± 9.8 eggs; parasitism of 64.4%) compared to *H. armigera* eggs (24.6 ± 1.9 eggs; 16.4%) ($\chi^2 = 716.2$; $p < 0.001$; df = 1). This confirms a distinct host-specific adaptation, with *T. remus* preferentially targeting *S. frugiperda*, aligning with its known efficacy against layered egg masses and *T. pretiosum* preferring eggs in a single layer. Interesting, however, it is the numerically higher parasitism of *T. remus* on *H. armigera* eggs (24.6 ± 1.9 eggs) compared to the parasitism of *T. pretiosum* on the same host (20.3 ± 3.5 eggs) despite the due caution of both results of coming from independent experiments (**Table 1**).

Table 1. Mean (± SE) number of *Helicoverpa armigera* and *Spodoptera frugiperda* eggs parasitized by *Trichogramma pretiosum* (*experiment* 1) and *Telenomus remus* (*experiment* 2) and the parasitism rate on each host species in the preference experiments.

Parasitoid specie	Host	Number of parasitized eggs[a]	Parasitism (%)
T. pretiosum	*H. armigera*	20.3 ± 3.5 a	13.5 ± 1.6 a
T. pretiosum	*S. frugiperda*	4.8 ± 1.8 b	3.2 ± 0.8 b
T. remus	*H. armigera*	24.6 ± 2.0 b	16.4 ± 1.8 b
T. remus	*S. frugiperda*	96.6 ± 9.8 a	64.4 ± 4.45 a

[a]Means ± Standard Error followed by the same letter within columns, for each parasitoid species, are not significantly different from each other ($p > 0.05$) according to Tukey test.

3.2 Parasitism capacity of T. remus and T. pretiosum on S. frugiperda and H. armigera eggs of isolated females and in association of both species (experiment 3)

The parasitism capacity of *T. remus* and *T. pretiosum* differed significantly ($\chi^2 = 319.1$; df = 5; $p < 0.001$) depending on the host species and interaction type (isolated or mixed) (**Table 2**). Overall, *T. remus* parasitism isolated against *S. frugiperda*, 161.0 ± 6.6 eggs/female and 68.4 ± 3.6 eggs/female on *H. armigera*, were values significantly higher than those of *T. pretiosum* on the same hosts (44.3 ± 4.7 and 27.0 ± 2.2 eggs/female, respectively). *Telenomus remus* on *S. frugiperda* eggs also exhibited the longest parental longevity (11.2 ± 0.3 days) and a high emergence rate (72.5 ± 3.0%). For both species the highest parasitism was observed in the first 24 h (**Fig. 2**).

328 **Table 2.** Parasitism capacity of *Telenomus remus* and *Trichogramma pretiosum* on *Helicoverpa*
329 *armigera* on *Spodoptera frugiperda* eggs of isolated females and in association of both species
330 (*experiment* 3).

Treatments	Lifetime number of parasitized eggs/female[1]	Parental longevity of adult females (days)[2]	Emergence (%)[3]	Proportion of parasitized eggs by *T. remus*/*T. pretiosum*
(**1**) One *T. remus* + one *T. pretiosum* on *S. frugiperda* eggs	147.4 ± 6.5 a	7.7 ± 0.7 bc	74.1 ± 3.0 bc	95/5
(**2**) One *T. remus* + one *T. pretiosum* on *H. armigera* eggs	73.4 ± 3.31 b	8.0 ± 0.6 ab	54.4 ± 3.1 c	66/34
(**3**) One *T. remus* on *S. frugiperda* eggs	161.0 ± 6.6 a	11.2 ± 0.3 a	72.5 ± 3.0 bc	-
(**4**) One *T. pretiosum* on *S. frugiperda* eggs	27.0 ± 2.2 c	6.1 ± 0.5 c	82.2 ± 4.1 ab	-
(**5**) One *T. remus* on *H. armigera* eggs	68.4 ± 3.6 b	8.8 ± 0.8 ab	88.0 ± 3.2 a	-
(**6**) One *T. pretiosum* on *H. armigera* eggs	44.3 ± 4.8 bc	4.1 ± 0.4 c	63.2 ± 7.1 cd	-

331 Means ± Standard Error followed by the same letter within columns do not differ significantly from each other
332 [1,3]($p > 0.05$) and [2] was analyzed using the Kruskal–Wallis test, followed by Dunn's post hoc test with Bonferroni
333 correction.
334
335 In mixed treatments, *T. remus* maintained competitive dominance. On *S.*
336 *frugiperda* (combination on *S. frugiperda* eggs), it parasitized 147.4 ± 6.5 eggs/female, with an
337 emergence rate of 74.1 ± 3.0% and a parasitism ratio of 95:5 (*T. remus*/*T. pretiosum*). On *H.*
338 *armigera* (combination on *H. armigera*), *T. remus* accounted for 100% of parasitized

eggs (73.4 ± 3.31 eggs/female), confirming its superior resource exploitation even in the presence of *T. pretiosum*. By contrast, *T. pretiosum* in isolation showed the lowest parasitism levels (27.0 ± 2.2 eggs/female on *S. frugiperda*; 68.4 ± 3.6 eggs/female on *H. armigera*), shorter longevity (6.0–8.8 days), and reduced emergence (82.2–88.0%), highlighting its limited efficacy compared to *T. remus* (**Table 2**).

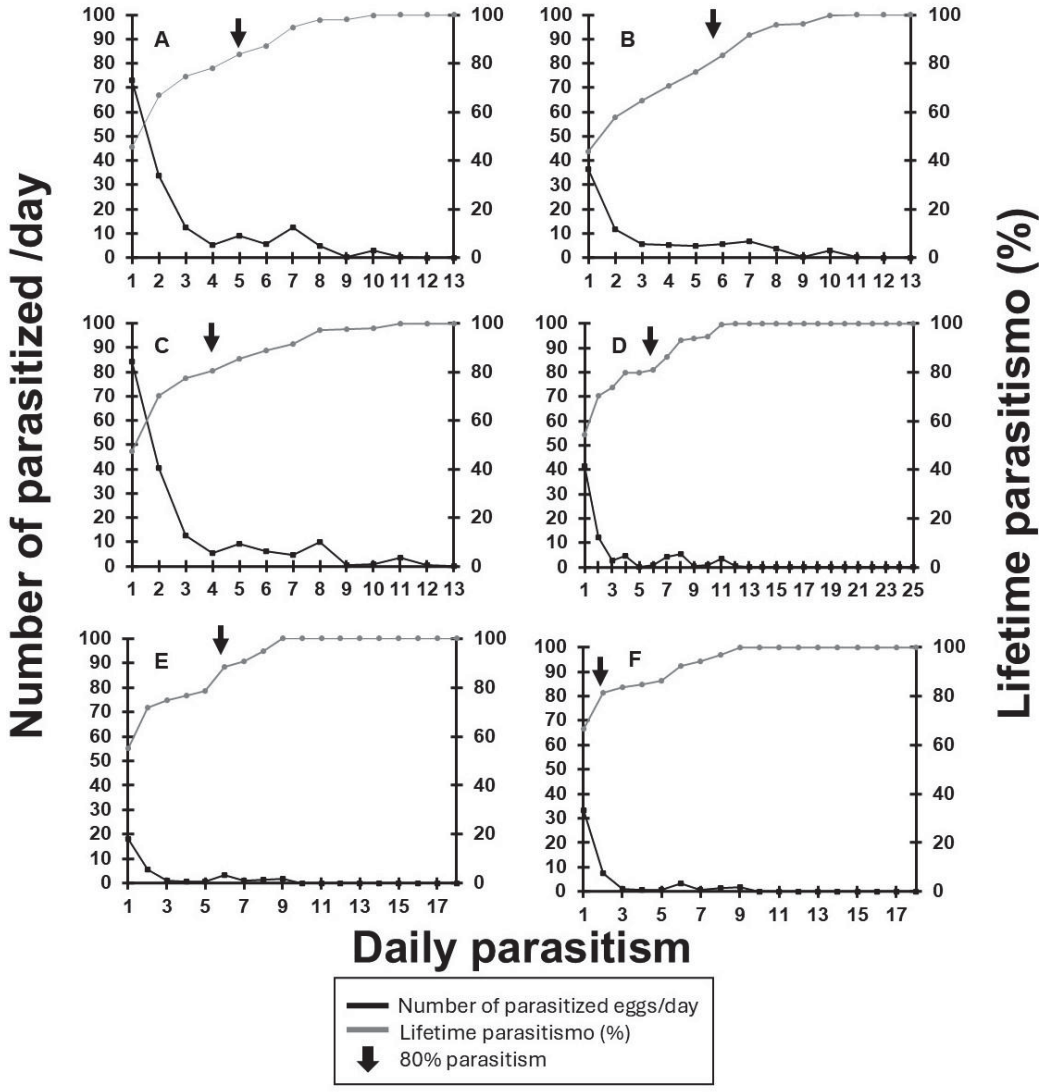

Fig. 2. Daily number and cumulative parasitism rate (%) of *Spodoptera frugiperda* and *Helicoverpa armigera* eggs parasitized by *Telenomus remus* and *Trichogramma pretiosum*, individually or simultaneously. (**A**) both parasitoids on *S. frugiperda* eggs; (**B**) both parasitoids on *H. armigera* eggs; (**C**) *T. remus* on *S. frugiperda* eggs and (**D**) *T. remus* on *H. armigera* eggs; (**E**) *T. pretiosum* on *S. frugiperda* eggs and (**F**) *T. pretiosum* on *H. armigera* eggs (experiment 3).

350 *3.3 Interaction and interspecific competition between T. remus and T. pretiosum on S.*
351 *frugiperda eggs (experiment 4)*
352
353 The total parasitism rate differed among treatments ($\chi^2 = 2403.0$; df = 5; $p < 0.001$). The
354 highest values were recorded in treatment *T. remus* (0-24h) + *T. remus* (24-48h) with 83.4 ±
355 5.4% parasitized eggs during parasitoid lifetime, followed by treatment with two *T. remus*, with
356 77.0 ± 8.5% parasitized eggs (**Table 3**).
357
358 **Table 3.** Mean (± SE) percentage of total parasitism, parasitism by *Trichogramma pretiosum* and
359 *Telenomus remus*, and emergence rate (%) in egg interaction treatments (*experiment* 4).

Treatment (time)	Parasitism (%)			Emergence (%) ± SE
	Total	*T. pretiosum*[1]	*T. remus*[1]	
T. pretiosum (0-24h) +*T. remus* (24-48h)	75.8 ± 6.0 ab	7.6 ± 8.5 B	68.2 ± 1.1 A	80.5 ± 6.3 ab
T. remus (0-24h) + *T. pretiosum* (24-48h)	60.4 ± 6.0 bc	2.3 ± 0.6 B	58.1 ± 2.3 A	68.5 ± 8.6 b
T. remus + *T. pretiosum* (0-24h)	44.7 ± 7.5 b	3.6 ± 2.7 B	41.1 ± 5.3 A	55.5 ± 2.4 c
T. remus (0-24h) + *T. remus* (24-48h)	83.4 ± 5.4 a	—[2]	83.4 ± 5.4	91.2 ± 5.9 a
T. pretiosum (0-24h) +*T. pretiosum* (24-48h)	6.7 ± 1.9 c	6.7 ± 1.9	—	86.1 ± 8.9 ab
Two *T. pretiosum* (0-24h)	13.8 ± 2.1 c	13.8 ± 2.1	—	82.8 ± 4.1 ab
Two *T. remus* (0-24h)	77.0 ± 8.5 ab	—	77.0 ± 8.5	89.3 ± 6.8 a
T. remus (0-24h)	65.2 ± 6.6 abc	—	65.2 ± 6.6	76.8 ± 7.7 b
T. pretiosum (0-24h)	20.3 ± 6.7 c	20.3 ± 6.7	—	86.4 ± 4.5 ab

360 Means ± Standard Error followed by the same small letter within columns for total parasitism and emergence, and capital
361 letter in the line for the comparisons between *T. remus* and *T. pretiosum*, do not differ significantly from each other ($p >$
362 0.05). [1]Parasitism by each species is expressed as a percentage of total host eggs parasitized in the treatment. 2 Non-existent
363 parameters.

In sequential competition treatments, the order of parasitoid release did not affected parasitism outcomes. When *Trichogramma pretiosum* was released prior to *T. remus* [*T. pretiosum* (0-24h) +*T. remus* (24-48h)], the total parasitism rate reached 75.8 ± 6.0%, which was similar to the reverse sequence, [*T. remus* (0-24h) + *T. pretiosum* (24-48h): 60.4 ± 6.0%]. In fact, parasitism in treatments with *T. remus* were consistently higher than those containing *T. pretiosum*. *Trichogramma pretiosum* showed slightly higher parasitism when released before *T. remus* (7.6 ± 8.5%) compared to when released after (2.3 ± 0.6%). In contrast, *T. remus* accounted for the majority of parasitism, reaching 68.2 ± 1.10% when released after *T. pretiosum* and 58.1 ± 2.3% when released first (**Table 3**).

Simultaneous exposure to both species resulted in the lowest parasitism rate among the mixed treatments (44.7± 7.4%) and the lowest emergence rate (55.5 ± 2.4%), compared to the sequential treatments. The treatment with repeated exposure to only *T. pretiosum* [*T. pretiosum* (0-24h) +*T. pretiosum* (24-48h)] showed the poorest overall performance, with only 6.7 ± 1.9% parasitism. Increasing the density of *T. pretiosum* (two releases) led to a parasitism rate of 13.8 ± 2.0% and an emergence rate of 82.8 ± 4.1%, indicating limited improvement despite the increased density (**Table 3**).

In the single-species control treatments, *T. remus* achieved an intermediate parasitism rate (65.2 ± 6.6%) and a high emergence rate (76.8 ± 7.7%). In contrast, a *T. pretiosum* exhibited performance with 20.3 ± 6.7% parasitism and 86.4 ± 4.5% emergence (**Table 3**).

3.4 Field performance of T. remus and T. pretiosum individually or in combined releases of both species (experiment 5)

Parasitism dynamics following the release of *Telenomus remus* and *Trichogramma pretiosum* varied significantly by treatment (sentinel eggs: $\chi^2 = 1226.4$, df = 3, $p < 0.001$; natural eggs: $\chi^2 = 622.6$, df = 3, $p < 0.001$), as well as by season and parasitoid species (**Fig. 3**). In sentinel egg cards (**Fig. 3A**

and C), parasitism peaks consistently occurred within 24 hours post-release, especially after initial applications, in both crop seasons. Maximum parasitism rates reached 43.6 ± 4.5% in 2022/2023 and 42.7 ± 5.38% in 2023. In contrast, parasitism on natural egg masses was much lower, 15.9 ± 1.98% and 6.6 ± 0.92%, respectively, highlighting a higher parasitoid efficiency on sentinel eggs.

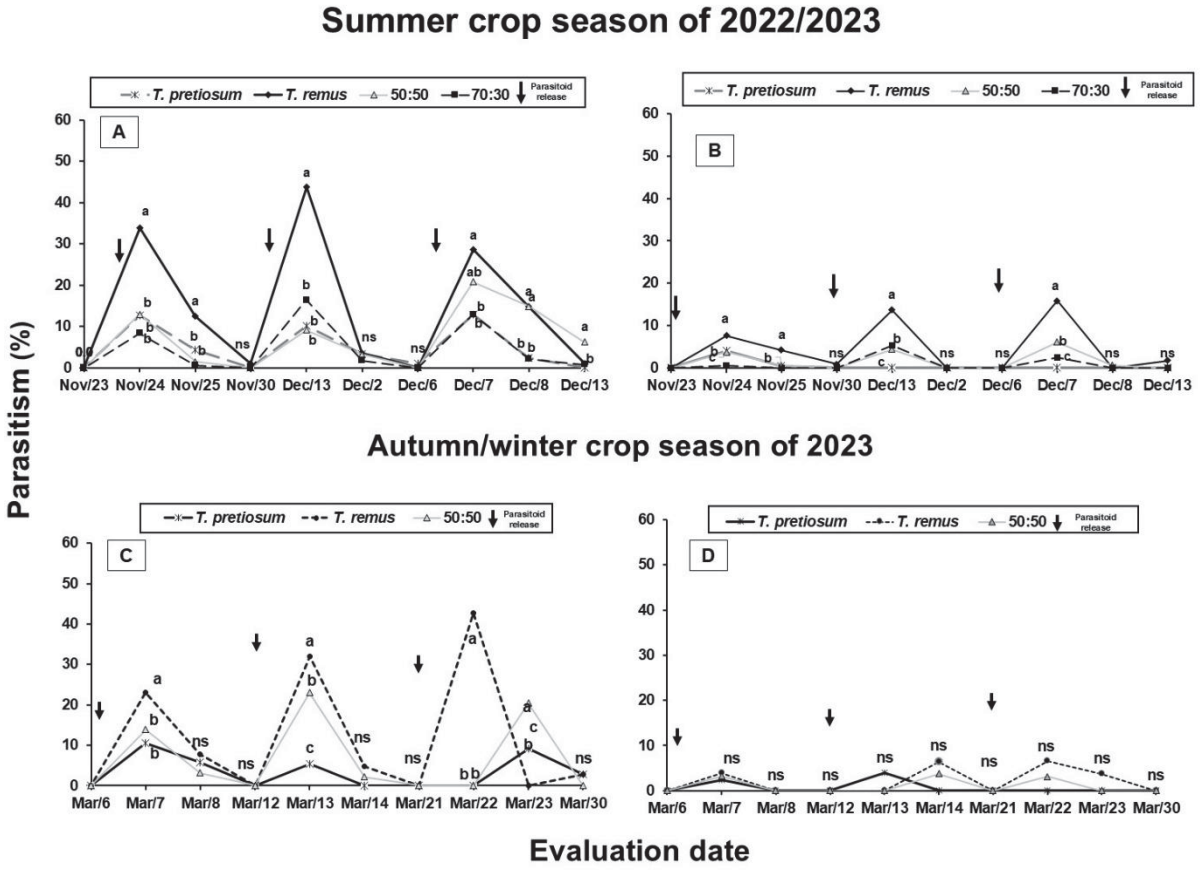

Fig. 3. Parasitism dynamics of *Telenomus remus* and *Trichogramma pretiosum* in maize fields following three augmentative releases spaced at 7-day intervals, during the 2022/2023 and 2023 cropping seasons. (A, C) Percentage of sentinel eggs parasitized 24 hours after each release. (B, D) Percentage of naturally laid eggs parasitized in the field on corresponding dates. Each line represents a treatment: single-species (*T. remus*, *T. pretiosum*) and mixed-species releases at different ratios (proportions of 50%:50% and 70%:30%). Arrows indicate the moment of application of the parasitoids. Letters above bars indicate significant differences among treatments within each date (GLMM, $p < 0.05$).

Telenomus remus consistently outperformed *T. pretiosum* across most sampling dates and seasons. For instance, in 2022/2023, it peaked at 43.6 ± 4.5% on December 1, while *T. pretiosum* remained below 13%. A similar trend was observed in 2023, with *T. remus* reaching 42.7 ± 5.4% on March 22. Combined releases usually over 95% of parasitized eggs were attributed to *T. remus*, suggesting asymmetric competition or dominance in the field (**Fig 3**).

Overall, parasitism rates in 2023 were lower than in 2022/2023. Despite seasonal variation, *T. remus* maintained higher parasitism levels across treatments and years. These results reinforce its superior performance as a field-effective biological control agent, even under mixed-release scenarios where *T. pretiosum* played a minimal role (**Fig 3**).

4. Discussion

The combination of *Telenomus remus* and *Trichogramma pretiosum* for the biological control of *Spodoptera frugiperda* and *Helicoverpa armigera* eggs revealed a complex interaction. Our multiscale approach, spanning from controlled laboratory conditions to open-field trials, demonstrated consistent performance patterns for both parasitoids, highlighting well-defined differences in host preference, parasitism capacity, behavior, and ecological robustness.

In laboratory assays, both species were able to parasitize *S. frugiperda* eggs; however, *T. remus* consistently outperformed *T. pretiosum* across all parameters, including parasitism rate, emergence success, and persistence in oviposition activity. This superiority has also been demonstrated in other studies from literature (Carneiro and Fernandes, 2012; Fortes et al. 2023). The advantage of *T. remus* is linked to its parasitism capacity (Bueno et al. 2010; Colmenarez et al. 2022), facilitated by its physical superiority in removing scales and ability to penetrate deeper layers of egg masses (Carneiro and Fernandes, 2012), efficiently navigating into the egg masses. In contrast, beyond its lower parasitism capacity, the small body size of *T. pretiosum*

limits its ability to parasitize multilayered egg masses (Kasige et al. 2022), common in *S. frugiperda* and often protected by scales (Dong et al. 2021; Hou et al. 2022). This morphological limitation helps to explain the lower parasitism observed, as *Trichogramma* species generally restrict parasitism to the upper egg layers (Beserra et al. 2005; Carneiro and Fernandes, 2012; Jin et al. 2021).

Simultaneous and sequential parasitism trial, the results reinforced the dominance of *T. remus*, which maintained high parasitism rates regardless of release order or if in combination or not with *T. pretiosum*. *Trichogramma pretiosum* showed a sharp drop in parasitism when released after *T. remus*. This suggests behavioral inhibition, possibly due to host discrimination or limited competitive ability, as shown by Carneiro and Fernandes (2012), when *T. pretiosum* avoided parasitizing *S. frugiperda* eggs previously exploited by *T. remus*. It is important to mention that this pattern is due to differences in host age acceptance: *T. pretiosum* typically parasitizes only eggs up to one day old, often rejecting eggs older than 24 hours (Queiroz et al. 2020), whereas *T. remus* parasitizes *S. frugiperda* eggs equally well at one or two days old (Queiroz et al. 2019). As a result, if when releases are done to most eggs on the field are more than 24 hours old, *T. remus* will maintain high efficiency while *T. pretiosum* will significantly reduce parasitism due to recognize as inappropriate for the prole development (Queiroz et al. 2019).

In our field trials, *T. remus* also performed better than *T. pretiosum* when released alone or combined, reflecting its higher effectiveness as already reported in the literature (Goulart et al. 2011; Lacerda, 2022; Fortes et al. 2023; Bueno et al. 2023). Nevertheless, parasitism rates obtained on natural egg masses with *T. remus* rarely exceeded 50%, suggesting that despite its potential, control efficiency may be limited by factors such as release density, adverse environmental conditions, or the spatial distribution of the hosts (Bueno et al., 2023). Results from other studies indicate that large-scale releases, ranging from 40,000 to 200,000 *T. remus* per hectare, can achieve significantly higher parasitism rates, between 60% and 100% (Ferrer,

2001; García-Roa, 1999; Figueiredo et al. 2002). In Brazil, applications of 90,000 to 120,000 parasitoids/ha have already resulted in parasitism rates of up to 82.8% (Figueiredo et al. 2002), indicating that increasing release density may be an effective strategy to maximize the field performance of *T. remus* that should be studied in future trials.

Although *T. pretiosum* is the species most frequently recorded in natural parasitism of *S. frugiperda* egg masses (Beserra et al. 2002; Dequech et al. 2013; Querino et al. 2016), its field performance was quite limited, typically below 20% (Beserra et al. 2002). This matches the parasitism rates by *T. pretiosum* observed in both crop seasons evaluated here, which remained below 20%. In the combined release during the 2022/2023 summer season, only 1.98% of the egg masses were parasitized by *T. pretiosum*. This result reinforces its low competitiveness and effectiveness, even when operating at densities equivalent to individual releases. In addition to the aforementioned, morphological and behavioral limitations, its reduced dispersal capacity, with an average range of just 8.01 m (85.18 m²) (Bueno et al. 2012), compared to 20.31 m (462.80 m²) of *T. remus* (Pomari-Fernandes et al. 2018), may further hinder its ability to access hosts in large and heterogeneous areas.

It was previously believed that combining *T. remus* and *T. pretiosum* could provide complementary biological control by leveraging the strengths of both species. In this model, *T. pretiosum*, a more economically accessible species, would target *Helicoverpa* spp., while *T. remus*, though more costly, would more effectively control *S. frugiperda*, with *T. pretiosum* complementing its action (Bueno et al. 2023). This IPM approach envisioned using *T. remus* in the early stages of the crop and *T. pretiosum* in reproductive phases, aiming at different pests. However, despite the notable parasitism potential on *Spodoptera* eggs, our results challenge the perception that *T. remus* is a specialist parasitoid for *S. frugiperda*. We demonstrated excellent parasitism of *T. remus* on *Helicoverpa armigera* eggs, suggesting that the exclusive release of *T. remus* in maize crops may be a viable alternative for controlling both pests. This finding is

particularly relevant, as it contradicts the previous assumption that *Trichogramma* would be essential for reproductive control of *Helicoverpa* spp. in maize due to supposedly low parasitism by *T. remus* on this pest (Bueno et al. 2023; Bueno et al. 2024).

However, it is crucial that future studies investigate the parasitism capacity of *T. remus* also on *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), a species also common in maize, to strengthen the hypothesis of exclusive use of this parasitoid species. Still, the strategic inclusion of *T. pretiosum* remains relevant within IPM programs when *Helicoverpa* spp. infestations are predominant, especially considering its lower release costs. In other crops such as soybean and cotton, where the lepidopteran pest complex is more diverse (Miranda et al. 2015; Formentini et al. 2015), the importance of *T. pretiosum* may be higher. Therefore, conducting specific studies in these crops is essential to determine the true relative importance of each parasitoid and to optimize IPM strategies, ensuring the efficacy and sustainability of biological control. Still, to improve the efficacy of *T. remus* in field conditions, future programs should refine release strategies, adjusting timing, frequency, and density to align with pest oviposition peaks and environmental suitability. These adjustments are essential to maximize impact and ensure consistent biological control in extensive agricultural systems.

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Chapter 2 - Improving *Telenomus remus* (Hymenoptera: Scelionidae) adoption: contribution of different egg parasitoid densities, fed adults and their storage to biological control success of *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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Abstract

Abstract: Egg parasitoids, such as *Telenomus remus* (Hymenoptera: Scelionidae), face significant challenges after release, as their pupae are exposed to various mortality factors that reduce the efficiency of biological control programs. In this context, this study aimed to evaluate a honey-solid diet that allows feeding adults while still inside the capsules without sticking the wasps on its surface, enabling parasitoid storage and later field release. Three independent bioassays were performed, each with 20 completely randomized replications. The first bioassay evaluated the acceptance of a solid feed, honey soaked in cotton thread, compared to the traditional form, honey droplets. In the second bioassay, the storage periods after emergence of adults in capsules with honey-solid food were analyzed, at 2, 4, 6 and, 8 days post-emergence and the third bioassay was studied the efficacy of different release densities of fed adults under field conditions. Parasitoids fed on the honey-solid diet had 13.3% reduction in parasitism compared to honey droplets. However, the sticky, viscous nature of honey can get parasitoids glued, potentially leading to their death. Differently, *T. remus* feeding on the honey-solid diet resulted in low mortality inside the capsules, living up to six days with only 22.2% reduction in parasitism capacity being a viable alternative to release and transport fed adult parasitoids with an increase around 30% in the released density of parasitoids compared with the fed parasitoids on honey droplets. Furhtermore, this flexibility of releasing *T remus* up until six days after emergence provided a valuable knowledge to establish *T. remus* as a biocontrol agent. Also, the highest tested parasitoid density of 20,000 parasitoids per hectare obtained the highest parasitism of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) eggs but it is still required future studies with higher releasing densities and cheaper ways of massively rearing the parasitoid to make those higher densities economically viable.

Keywords: Biological control; parasitoid nutrition; fall armyworm; *Spodoptera frugiperda*; noctuids.

1. Introduction

Fall armyworm (FAW) *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) is a pest native from the new world [1]. As a highly polyphagous species, FAW larvae feed on leaves, stems and reproductive structures of 353 host plants belonging to 76 botanical families [2], being of key importance to several important staple crops [1], especially maize [3]. Despite the high FAW control triggered by genetically modified maize varieties [4], due to the increased cases of resistance [5,6], insecticides are still widely adopted [7]. However, chemicals overuse has raised global concerns about their negative side-effects over the environment and human health [8,9]. Those concerns have gained increasing attention more recently, especially due to the vast extension of area in which FAW has occurred, invading new regions such as Africa, Asia, Australia and Europe [10,11]. This scenario turns this pest a global threat to food security [12] increasing the demands for more sustainable tools to its management [13].

Among the most eco-friendly alternatives against FAW, biological control is gaining momentum globally [14]. Among the biocontrol agents of FAW, egg parasitoids are noteworthy for controlling the pest in its first stage (egg) before any damage being triggered to its host plants (crops) [15]. Despite different species of the genus *Telenomus*, for instance *Telenomus remus* (Nixon) and *Telenomus dignus* (Gahan), being reported as eggs parasitoids of FAW [16], *T. remus* has emerged as one of the most prominent and widely reported candidates for Augmentative Biological Control (ABC) programs of FAW around the world [17,18,19]. *Telenomus remus* stands out [20] not only due to its high parasitism capacity on *Spodoptera* spp. eggs [18] but also because of the high dispersal capacity of its adults [21] as well as its great host foraging potential [22]. Furthermore, its adults are able to parasitize *Spodoptera* eggs on overlapping layers; including those eggs located in the inner layers of egg masses, frequently covered with scales from the moth's wings [19]. Due to this extraordinary potential, *T. remus* has been one of the most studied egg parasitoids against *Spodoptera* spp., including FAW, in different countries around the world, especially in Latin America

[18,23,24,25,26,27,28]. (Exteckotter et al. 2025, Melo et al. 2025, Mubayiwa et al 2025, Pinto & Romanello 2025, Sampaio et al. 2025, You et al 2025).

First releases of *T. remus* in Latin America were performed manually in 1990s on small scales with overall positive results [18]. Releases of *T. remus* in Venezuela, Colombia, Guyana and Suriname achieved up to 80–100% of FAW egg parasitism [29,30,31,32,33]. In order to scale up the adoption of egg parasitoids as a biological control strategy over large areas, pupae close to adult emergence have been the preferred parasitoid stage to be released by the biological control industry, due to the ease of mechanization of the release process [34]. However, it is important to consider that males of *T. remus* emerge up to 24 hours earlier than females [35]. Therefore, *T. remus* female released as pupae will stay longer in the field before adult emergence. It makes the released parasitoid, especially females, extremely vulnerable to different causes of mortality. Among the most important ones, high temperatures [36,37,38], rainfall [39] and predation [40] can be highlighted as the most frequent and impactful in reducing parasitoid survival in the field immediately after release. Therefore, it is of great theoretical and practical interest to evaluate new strategies to release *T. remus* in field conditions [18]. The refinement of protocols to release biocontrol agents, for instance *T. remus*, in ABC programs is one of the major challenges to improving the use of biocontrol strategies inside integrated pest management (IPM) [41].

Despite the increasing interest in *T. remus* for biological control in Brazil, where it has only recently been officially registered for commercial use, key operational parameters still require to be studied. In South America, release densities of *T. remus* in fields of maize range widely from 5,000 to 200,000 parasitoids per hectare [31,32, 42,43,44,45,46,47,48,49,50]. This undefined clear recommendation protocol of how to release *T. remus* can be the responsible for the variations in the results recorded in the literature. Some Brazilian studies [47,50] reported lower parasitism (from 1.4 to 9.5%) after releasing *T. remus* (≤ 24 hours-old) following single releases of 100,000–200,000 individuals per hectare at the emergence or the first parasitoids. However, considering the males emerge up to 24 hours before females [15], the released parasitoids have been probably mostly males

and the remaining pupae (females) subjected in the field to significant mortality factors for longer periods [18]. Such mortality includes adverse temperature [48] and heavy rainfall [39], or predation of the released pupae [40] as the most important abiotic and biotic mortality factors, respectively, before females` emergence. In addition, not only releasing more parasitoids does not increase parasitism but also on the contrary, can reduce parasitism levels recorded in the field [51]. For most biocontrol agents released in ABC programs, there is an optimal release rate that produces effective control of host pest [52] highlighting the need for better-defined release protocols for *T. remus*. It is also important to point out, that the best biological control practices should not only aim to increase biological control efficacy but also mitigate any risks associated with its careless adoption [53].

Therefore, the present study aimed to improve the recommendations for *T. remus* releases. In order to improve the release of another egg parasitoid of the same genus, *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae) which parasitizes eggs of stink bugs (Hemiptera: Pentatomidae), previous work succeeded developing a honey-solid diet formed by 100% honey soaked in 100%-cotton strings (Tex 378, Ne 8/5 Thread, Charm® Natural Circle) for one minute and left to dry for an hour before being offered to the adult parasitoids into small pieces [54]. This honey-solid diet avoided possible parasitoid mortality caused by honey droplets to the fragile wasps, which due the viscous nature of the honey can make parasitoids to be sticked, potentially leading to their death [54]. In addition, this solid-honey diet prolonged *T. podisi* storage that allowing the parasitoid be kept for up to 14 days under environmental temperature (25ºC), after parasitoid emergence, before being released in the field, without any reduction in its parasitism capacity [54]. However, such honey-solid diet had never being tested for *T. remus*. Consequently, this work (**a**) studied the parasitism capacity of *T. remus* fed with the honey-solid diet compared to the traditional honey-droplet diet to check the suitability of such diets to *T. remus*, (**b**) evaluated the same parameters of the parasitism capacity of *T. remus* fed with the honey-solid diet, but with parasitoids at different times from emergence to check storage impact on the parasitoid quality, and (**c**) tested the parasitism of several parasitoid densities of fed *T. remus* adults (from 24 to 48 hours) with this honey-solid diet, under field

conditions. Together, these approaches aimed to optimize both the quality and quantity aspects of *T. remus* deployment, ultimately enhancing its adoption and effectiveness as a biocontrol agent against FAW.

2. Material and Methods

2.1. Insects rearing

Eggs of *S. frugiperda* as well as *T. remus* females used in the experiments were from insect colonies maintained in laboratory conditions [25 ± 2°C, 80 ± 10% RH and 14h:10h Light:Dark (L/D) photoperiod].

Spodoptera frugiperda specimens were originally collected from maize plants (C-strain) in Embrapa Field Station, Londrina, State of Paraná, Brazil (23°21′19.2″ S, 51°10′16.8″ W), in 2020 and morphologically identified using the Manual for the Identification of Insects and Other Invertebrates of Soybean Crops [41]. Since then, the insects have been maintained in the rearing during which new field insects had been introduced each year to maintain colony quality.

Larvae of *S. frugiperda* were initially individualized in plastic cups (50 mL) containing an artificial bean-based diet [42] until reaching the pupae stage. Then, pupae were separated by sex [43] and transferred to acrylic cages (45 × 33 × 35 cm) for eclosion of the adults and their mating and oviposition. The adults were fed with a diet based on honey (10%) and distilled water. The cage was covered internally with white paper as oviposition substrate, and the eggs were collected daily for experiments or colony maintenance.

Telenomus remus was originally collected in Ecuador and reared at the parasitoid rearing facilities of ESALQ/USP (Luiz de Queiroz College of Agriculture, University of São Paulo). In 2008, a subset of this population was transferred to Embrapa Soybean, where it has since been maintained using individuals exhibiting favorable biological traits. Since then, *T. remus* has been reared in the laboratory using *S. frugiperda* egg masses (approximately 150 eggs each), which were glued onto cards (2 cm × 8 cm) and introduced into tubes together with eggs previously parasitized by *T. remus*.

Small drops of honey were placed inside these tubes to feed the adults as soon as they emerged. The tubes were then closed, and the eggs allowed to be parasitized for 24 h. Adults that emerged from these eggs were used for trials or colony maintenance.

2.2. Experiment 1: Parasitism capacity of Telenomus remus fed on liquid (honey-droplet diet) vs. honey-solid diet

The experiment was carried out in climatized chambers (ELETROLab®, model EL 212, São Paulo, SP, Brazil) set up at temperature of 25 ± 2°C, 80 ± 10% of relative humidity and 14h:10h Light:Dark (L/D) photoperiod in a completely randomized design with two treatments (diets) and 20 replicates (the individualized females ≤ 48 hours with the tested diet). The studied diets were: (1) 100% honey offered in tiny droplets applied to the tube walls every two days (honey-droplet diet) provided *ad libitum*, and (2) 100% honey soaked into cotton strings (honey-solid diet) as proposed for *T. podisi* [54].

Different tubes containing pupae of *T. remus* from the parasitoid rearing received one of tested diet 6 days before the emergence for feeding the recently emerged adults. After 48 hours from emergence of the first adults, enough parasitoid females (20 females of each treatment) were individualized in Duran acrylic tubes (6 cm high and 1 cm in diameter) with the tested diet. Each *T. remus* female (replicate) was provided a card (1.0 × 0.7 cm) with approximately 150 *S. frugiperda* eggs (< 24h old), which were replaced on daily basis until the parasitoid's death. The evaluated biological parameters included: (1) daily parasitism (number of eggs parasitized per day), (2) lifetime parasitism (total number of eggs parasitized per female during its lifetime), (3) emergence rate (%), (4) sex ratio (proportion of females), calculated as sr = number of females / (number of females + number of males), and (5) longevity of parental females (measured in days after release, with "release" defined in this experiment as the day parasitoids first received host eggs).

2.3. Experiment 2: Shelf life of adults of Telenomus remus inside capsules with honey-solid diet

The experiment was carried out in the same climatized chambers and controlled conditions previously described for experiment 1, in a completely randomized design with 4 treatments (different period of storage of *T. remus* inside capsules with honey-solid diet at 25ºC) and 20 replicates. The evaluated periods of storage (treatments) were: 2 (control), 4, 6 and 8 days after the emergence of the first *T. remus* adults inside the capsules (males). A commercial, spherical capsule made of cellulose (Agribela Tecnologias Biológicas®) with a 3.0 cm diameter was used, containing 150 pupae of *T. remus*.

A total of 16 capsules were prepared 6 days before the emergence of the first *T. remus* adults and stored inside the same climatized chambers (ELETROLab®, model EL 212, São Paulo, SP, Brazil). Every 24h, the capsules were handled (turned over) to simulate possible shaking during the transport of the capsules from the production facility (biofactory) to the field. On each evaluation day (treatments), 4 capsules were opened and evaluated for the number of living and dead adult parasitoids inside each capsule. In addition, 20 females from those capsules opened were selected (20 replicates) to perform a parasitism capacity trial. Each replicate was composed of an individual female *T. remus* in a Duran acrylic tube (height 6 cm, diameter 1 cm) with white cards (1.0 × 0.7 cm) containing 150 eggs (≤ 24h old) of *S. frugiperda*, which were replaced daily until the death of the female. The honey droplets were provided in the tube for feeding the adults.

The following biological variables were evaluated: daily (number of eggs parasitized per day) and lifetime parasitism (total number of eggs parasitized per female), emergence (%), sex ratio (female proportion) [sr = number of females / (number of females + number of males)], parental longevity after release (number of days the parasitoid survived after opening the capsules), and number of living and dead adult parasitoids inside each opened capsule.

2.4. Experiment 3: Field performance of honey-solid-fed *Telenomus remus under different release densities*

The experiment was caried out in field conditions (23°21′19.2″ S, 51°10′16.8″ W) in a maize field measuring 270 × 400m at the Brazilian Research and Agricultural Corporation (Embrapa Soja), in Londrina, PR, Brazil during the 2024 maize growing season. The experimental area was divided into four field plots (270 × 100m, totaling 2.7 ha) each assigned to one *T. remus* release density (treatment). Within each treatment plot, four subsampling points (pseudo-replications) were used for parasitism evaluation (67.5 × 100m). In each pseudo-replication (Figure 1) a border area of 10 m was excluded in the evaluation and a used area of 47.5 × 80m was adopted for *T. remus* release *and S. frugiperda* eggs sampling (for parasitism evaluation).

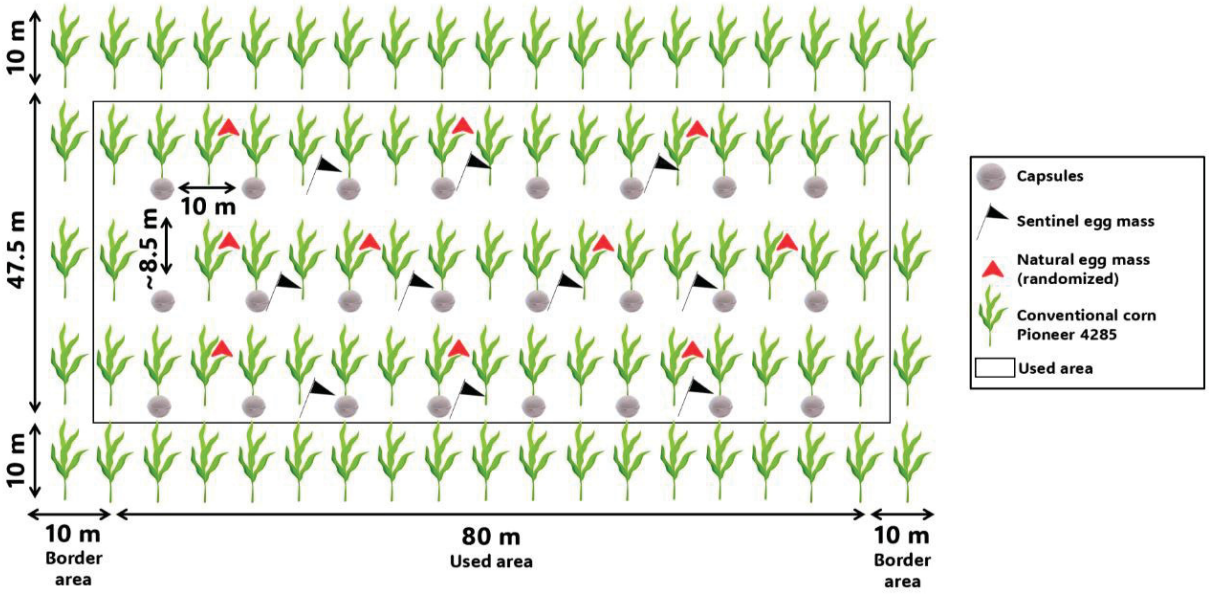

Figure 1. Representation of each pseudo-replication adopted in the release honey-solid-fed *Telenomus remus* field trial carried out in Londrina, Paraná, Brazil in maize.

Treatments consisted of different release densities of fed adults of *T. remus* with the honey-solid diet previously described [40] as following: i) 5.000; ii) 10.000; iii) 15.000; and iv) 20.000 parasitoids per hectare. All parasitoids were obtained from the *T. remus* colony maintained at the Parasitoid Laboratory of Embrapa Soybean, where they were reared on *S. frugiperda* eggs under controlled conditions. Likewise, sentinel egg masses used for parasitism evaluation originated from the same laboratory colony of *S. frugiperda*.

A baseline assessment was carried out before the first parasitoid release to evaluate the presence of natural parasitism in the area. Subsequently, parasitoid was released 3 times for each treatment plot, spaced from seven to ten days apart each release. Parasitoids were released as mated and honey-solid-fed adults, with 24–48 hours post-emergence, ensuring that they were physiologically ready for host searching and parasitism. Adults were released inside biodegradable capsules, which were opened in the field at the time of release to allow the parasitoid dispersion. Capsules were evenly distributed across the field, with 35 release points per hectare [21]. In treatments with higher release densities, the number of capsules per hectare was proportionally increased but the number of releasing points was kept the same (35 points/ha) to maintain homogeneous distribution and prevent capsules from being overcrowded.

Parasitism evaluations were conducted at 1, 2, and 6 days after each release (at 24-, 48-, and 144-hours post-release). In each sampling, 10 naturally laid egg masses and 10 sentinel egg masses were collected per replicate in the used area of each pseudo-replication (Figure 1). Sentinel eggs consisted of laboratory-produced *S. frugiperda* eggs glued onto cardboard strips (2.5 × 5 cm) and fixed on a flag in the field. All collected egg masses were individually transferred to plastic vials and brought to the entomology laboratory of Embrapa Soybean, where they were kept in climate chambers (ELETROLab®, model EL 212, São Paulo, SP, Brazil regulated at 25 ± 2 °C, 80 ± 10% RH, 14:10 h L:D photoperiod). Egg masses were monitored daily to record parasitoid emergence. Emerging *S. frugiperda* larvae were immediately removed to avoid cannibalism of parasitized eggs. Two response variables were analyzed: parasitism rate and proportion of parasitism between egg masses (natural and sentinel) per sampling date.

2.5. Statistical analysis

Parasitism rate was analyzed using generalized linear mixed models (GLMMs) with a binomial error distribution and a logit link function, fitted with the glmmTMB package [58]. The model included treatment, sampling date, and their interaction as fixed effects, and block as a random effect.

When the interaction was significant, we performed pairwise comparisons of treatments within each date using Tukey's test ($p < 0.05$) via the emmeans package. Emergence rate (%) and sex ratio (proportion of females) were analyzed using generalized linear models (GLMs) with binomial error distribution and logit link function. Adult female longevity data were analyzed using one-way analysis of variance (ANOVA) to assess the effects of diet and storage duration on survival. When significant differences were detected, means were compared using Tukey's test ($\alpha = 0.05$). For GLM and GLMM, estimated marginal means and pairwise comparisons were calculated using the emmeans package [59], with Tukey adjustment for multiple comparisons where appropriate also using emmeans package. All statistical analyses were performed in R version 4.3.2 [60].

3. Results

3.1. Experiment 1: Parasitism capacity of Telenomus remus fed on liquid (honey-droplet diet) vs. honey-solid diet

An overall better parasitoid performance was recorded for adult parasitoids that received honey-droplet diet. Although the difference was statistically significant, the lifetime number of *S. frugiperda* eggs parasitized was only 15.34% higher in parasitoids fed with this diet (165.4 eggs) compared to those fed with the honey-solid diet (143.4 eggs) ($\chi2= 914.21$, df=13, $p < 0.0002$, Table 1).

Significant differences in the emergence (%) of parasitoids were also observed between diets ($\chi2= 44.51$; df = 1; $p < 0.001$) but again with only 4% of difference. It was recorded 77.7% emergence (F2) from parasitism from adults fed with honey-droplet diet compared to 73.7% emergence (F2) from parasitism from adults fed with honey-solid diet. In contrast, no difference among *T. remus* diet was recorded for the progeny sex ratio ($\chi2= 0.0023$; df= 2; $p <0.724$) or the parental longevity of adult females (days) ($F_{(1, 8)} = 1.01$; $p <0.300$), which were not influenced by the tested diets (Table 1).

Table 1. *Telenomus remus* parasitism capacity on eggs of *Spodoptera frugiperda* with adult parasitoid feeding on different diets.

Diet	Lifetime number of parasitized eggs/female[1]	Emergence (%)[2]	Progeny sex ratio[2]	Parental longevity of adult females (days)[3]
100% honey in tiny droplets (honey-droplet diet)	165.4 ± 5.88 a	77.7 ± 1.84 a	0.69 ± 0.04 a	10.4 ± 0.71 a
100% honey in macerated cotton strings (honey-solid diet)	143.4 ± 4.57 b	73.7 ± 1.01 b	0.70 ± 0.02 a	10.1 ± 0.40 a

Means ± Standard Error (N=64) followed by the same letter within columns did not statistically differ from each other by 1(GLMM $p > 0.05$), 2(GLM $p > 0.05$) and 3(ANOVA $p > 0.05$).

The distribution of the number of parasitized eggs per day over parasitoid lifetime was similar between parasitoid fed with honey-solid and honey-droplet diets, with parasitoids in both diets having the highest number parasitized eggs on the first day of parasitism and 80% parasitism being reached at the 5th day (Figure 2). In the first 24 hours of parasitism, *T. remus* fed with honey-solid diet parasitized the average of 86.1 eggs (Figure 2A) while the parasitoids fed with honey-droplet diet parasitized 79.2 eggs (Figure 2B).

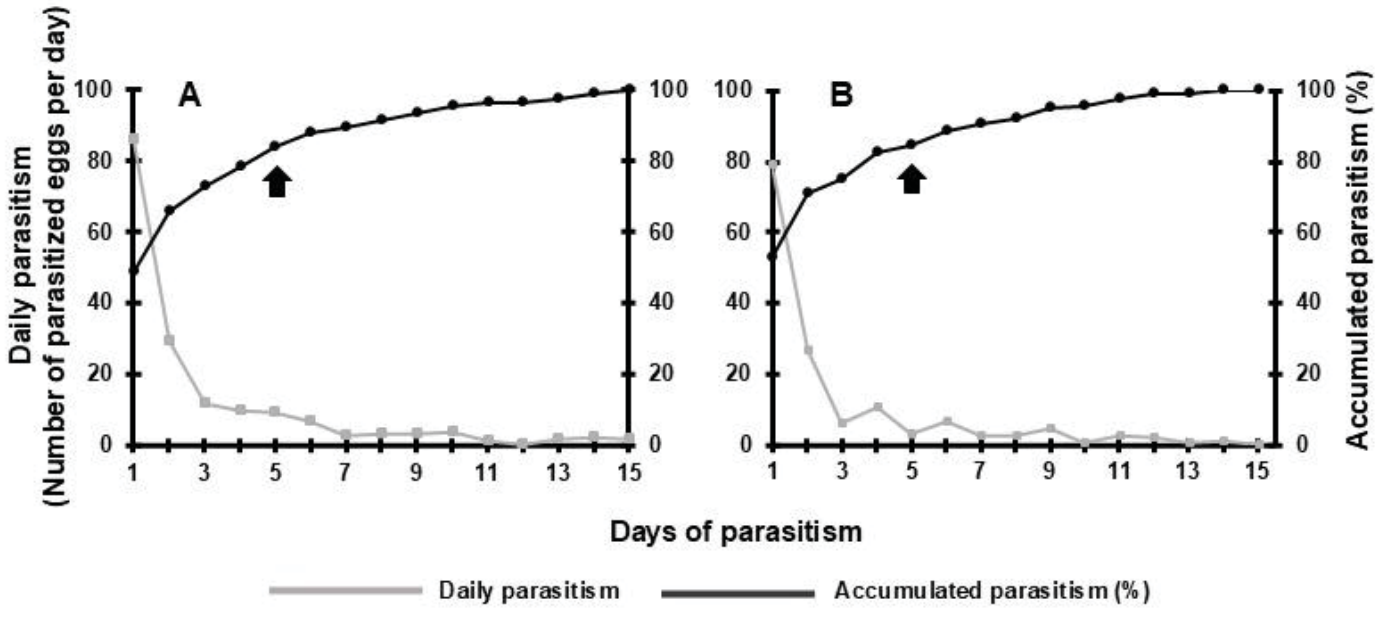

Figure 2. Parasitism capacity of *Telenomus remus* fed with honey-solid diet (A) and honey-droplet diet (B) on eggs of *Spodoptera frugiperda*. Arrows indicate 80% of total parasitism.

3.2. Experiment 2: Shelf life (storage period in days) of adults of Telenomus remus inside capsules with a honey-solid diet

The highest number of parasitized *S. frugiperda* eggs was observed during the first 24 hours of parasitism (83.4, 75.5, 102.2 and 62.0 eggs) by *T. remus* storage during 2, 4, 6 and 8 days, respectively (Figure 3). Eighty percent parasitism was recorded at 9 days of parasitism (*T. remus* females stored ≤ 2 days) (Figure 3A), 4 days of parasitism (*T. remus* females stored ≤ 4 days) (Figure 3B), 4 days of parasitism (*T. remus* females stored ≤ 6 days) (Figure 3C) and only 3 days of parasitism (*T. remus* females stored ≤ 8 days) (Figure 3D). 80% parasitism was reached faster after the longest storage periods.

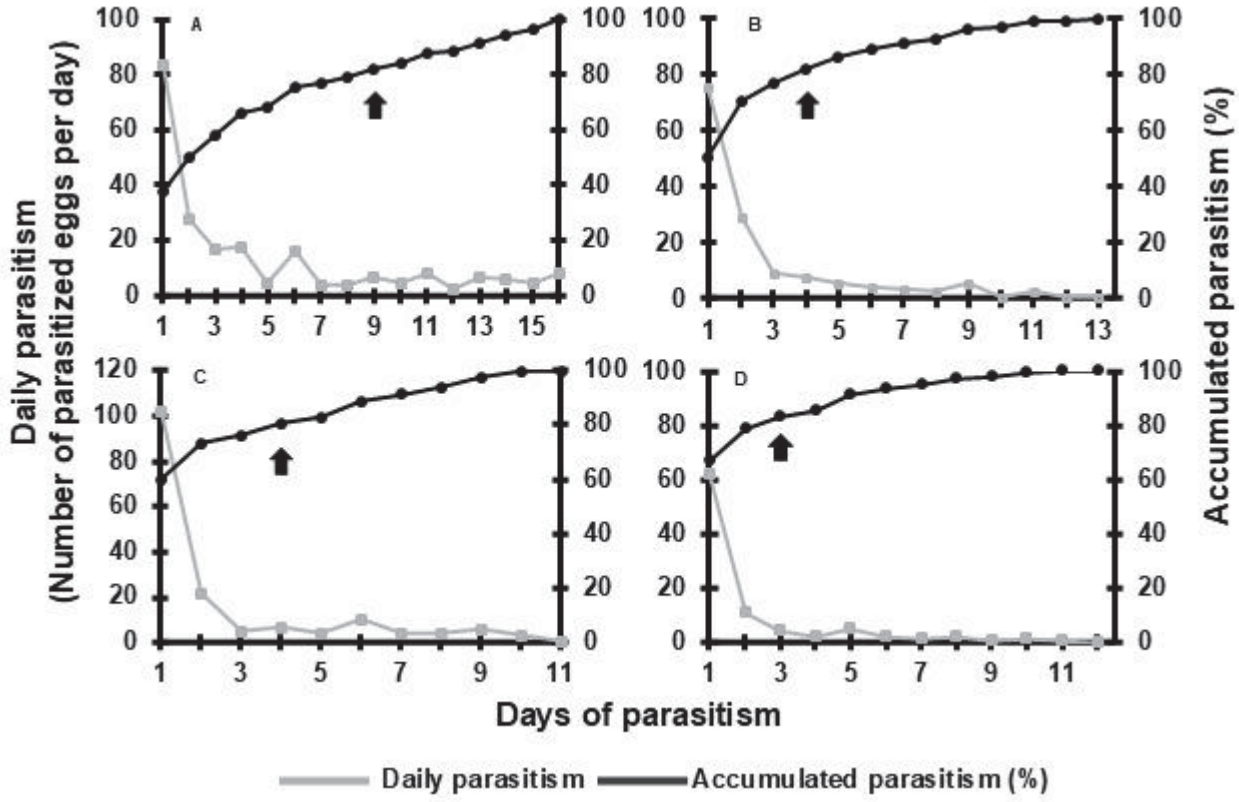

Figure 3. Parasitism capacity of *Telenomus remus* fed with honey-solid diet at different periods after parasitoid emergence. (A) two days, (B) four days, (C) six days, (D) eight days after adult parasitoid emergence. The arrows indicate 80% parasitism.

297

298 The lifetime number of eggs parasitized was significantly influenced by the storage periods,
299 decreasing as storage time increased with no differences among 4 and 6 days of storage ($\chi 2$= 773.41;
300 df = 3; p <0.002). The highest number of eggs parasitized during the females' lifetime occurred in
301 the shortest storage period 193.4 (storage ≤ 2 days) followed by 150.4 (storage ≤ 6 days) and 143.6
302 (storage ≤ 4 days) and lowest lifetime number of parasitized eggs 86.6 was recorded for females'
303 storage of 8 days (Table 2). This showed that the longest storage time had a marked impact on the
304 parasitism capacity of *T. remus* with a reduction of 42.4% in the parasitism at eight days of storage
305 compared to six days of storage.

306

307 **Table 2.** *Telenomus remus* parasitism capacity on eggs of *Spodoptera frugiperda* on different days
308 after emergence, stored inside release capsules with honey-solid diet.

Days of storage after parasitoid emergence	Lifetime number of parasitized eggs/female[1]	Emergence (%)[2]	Progeny sex ratio[2]	Parental longevity of adult females (days)[3]
2	193.4 ± 8.82 a	79.9 ± 1.78 a	0.74 ± 0.02 a	11.5 ± 0.63 a
4	143. 6 ± 4.35 b	74.4 ± 1.75 b	0.80 ± 0.01 a	10.0 ± 0.35 a
6	150.4 ± 14.79 b	76.5 ± 1.79 b	0.56 ± 0.04 b	7.4 ± 0.69 b
8	86.6 ± 5.63 c	80.5 ± 1.88 a	0.71 ± 0.08 a	7.1 ± 0.54 b

309 Means ± Standard Error followed by the same letter within columns did not statistically differ from
310 each other by 1 (GLMM p > 0.05), 2(GLM p > 0.05) and 3(Log rank test p > 0.05).

311

312 Despite statistically significant differences among some treatments, emergence rates were not
313 only higher than 74% for all evaluated periods of storage (treatments) but also varied only slightly
314 among treatments, ranging from 74.4% to 80.5% (χ^2 = 53.6; df = 3; p < 0.001), and the longevity of
315 parental adult females ranged from 7.1 to 11.5 days [F(3, 27) = 33.07; p < 0.001], without exhibiting
316 any clear trend with emergence of the progeny of *T remus* stored for 2 and 8 days being statistically
317 similar. Likewise, the results show progeny sex ratio ranging from 0.56 to 0.80 (Deviance = 63, df=
318 3, p < 0.006).

The evaluation of parasitoids stored during the opening of the capsules revealed similar emergence rates of the adults put inside the capsules 6 days before emergence together with honey-solid diet, regardless of the storage duration, and overall low mortality. The percentage of dead parasitoid adults found inside the capsules ranged from 2.1% (2 days post-emergence) to 5.9% of the total adults per capsule (6 days post-emergence), with significant differences among treatments (Table 3). However, this difference was mainly driven by the lower mortality observed in the 2-day group, which differed significantly from the others. No significant differences were found among the remaining treatments. Moreover, the mortality of emerged adults was always below 6%.

Table 3. *Telenomus remus* emergence (%) and dead adults (%) trapped inside capsules on different days after adult emergence from *Spodoptera frugiperda* eggs, with parasitoid adults feeding on a honey-solid diet.

Days after parasitoid emergence	Emergence (%) of adults from pupae inside capsules	Dead adults (%) trapped inside capsules
2	69.8 ± 2.76 a	2.1 ± 0.61 a
4	74.6 ± 2.56 a	5.2 ± 0.50 b
6	73.3 ± 3.58 a	5.9 ± 1.03 b
8	75.0 ± 0.97 a	5.5 ± 0.80 b

Means ± Standard Error followed by the same letter within columns did not statistically differ from each other (GLM $p > 0.05$).

3.3. Experiment 3: Field performance of honey-solid-fed *Telenomus remus under different release densities*

The field parasitism rate of *S. frugiperda* eggs recorded after the releases of different densities of *T. remus* positively responded to the increase in numbers of parasitoids ($\chi^2 = 3616.1$, df = 3, $p < 0.001$). Overall, parasitism rates were higher in the treatment of 20,000 parasitoids/ha than parasitism recorded with 15,000 parasitoids/ha and 10,000 parasitoids/ha ($\chi^2 = 62.74$, df = 3, $p < 0.001$) while parasitism of 10,000 and 15,000 parasitoids/ha did not differ between themselves ($\chi^2 = 5.82$, df = 3, $p = 0.12$). Also, the highest parasitism was recorded in the treatment with 20,000 parasitoids/ha,

reaching a peak of 49.1 ± 6.1% 24 hours after the first release (Figure 4). The highest parasitism observed in the 15,000/ha treatment was 17.9 ± 2.6%, which, which despite being close in the parasitoid release density to the 20,000 parasitoids/ha treatment, was substantially lower in effectiveness. In contrast, the 5,000 parasitoids/ha treatment consistently showed the lowest parasitism levels, often near zero throughout the entire evaluation period.

Regardless of treatment, parasitism consistently peaked at 24 hours after each release and dropped sharply afterward. In the first release, the parasitism rate in the 20,000/ha treatment fell from 49.1% to only 8.0% on the second day. In the second release, the decline was from 42.1% to 11.0 ± 1.6%.

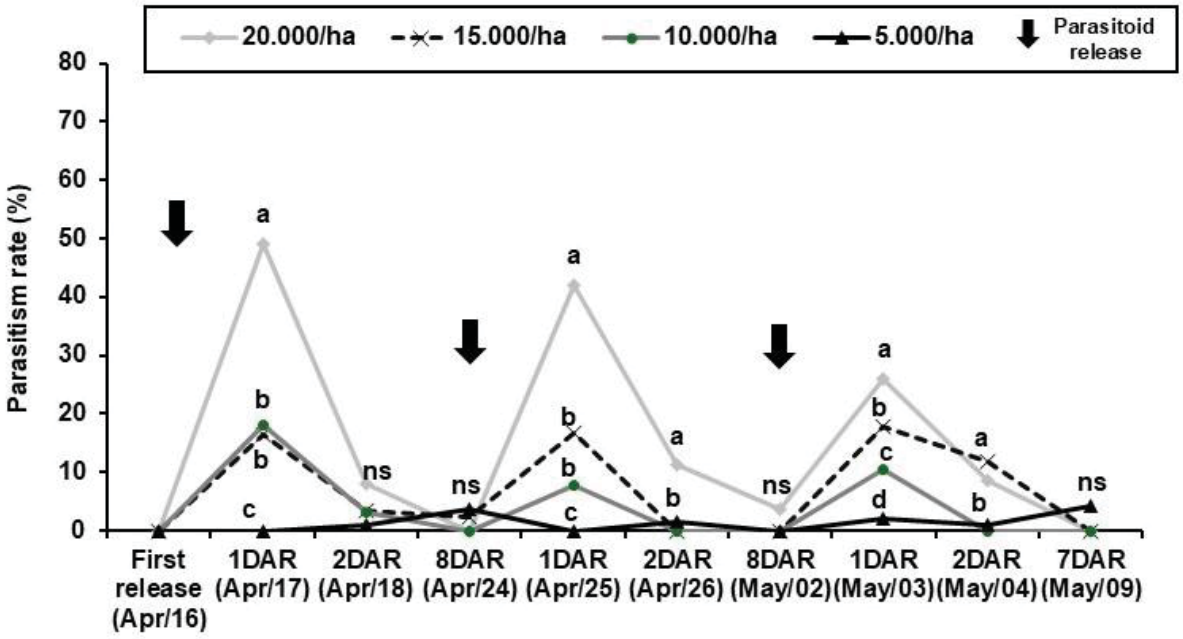

Figure 4. Release of *Telenomus remus* at different densities (parasitoids/ha) in maize during the second cropping season of 2024, conducted at the Embrapa Soja experimental field (Londrina, Paraná, Brazil) (experiment 3). The x-axis shows the number of days after each parasitoid release, indicated as "DAR" (e.g., 1DAR = 1 day after release, etc.). Letters indicate significant differences among treatments within each date, based on generalized linear mixed models (GLMMs) with binomial distribution and logit link, followed by pairwise comparisons using Tukey's test on estimated marginal means ($p < 0.05$).

Nearly all parasitism recorded in the 5,000, 10,000, and 15,000 parasitoids/ha treatments occurred in sentinel egg masses (Figure 5). In contrast, parasitism in the 20,000/ha treatment occurred approximately in a 70%:30% ratio between sentinel and natural egg masses, indicating a broader dispersal and activity of the parasitoids at higher release densities.

Parasitism trends over time clearly highlight the superior performance of the 20,000 parasitoids/ha treatment, with pronounced peaks and greater temporal impact. Lower-density treatments maintained minimal and stable parasitism rates throughout the study period.

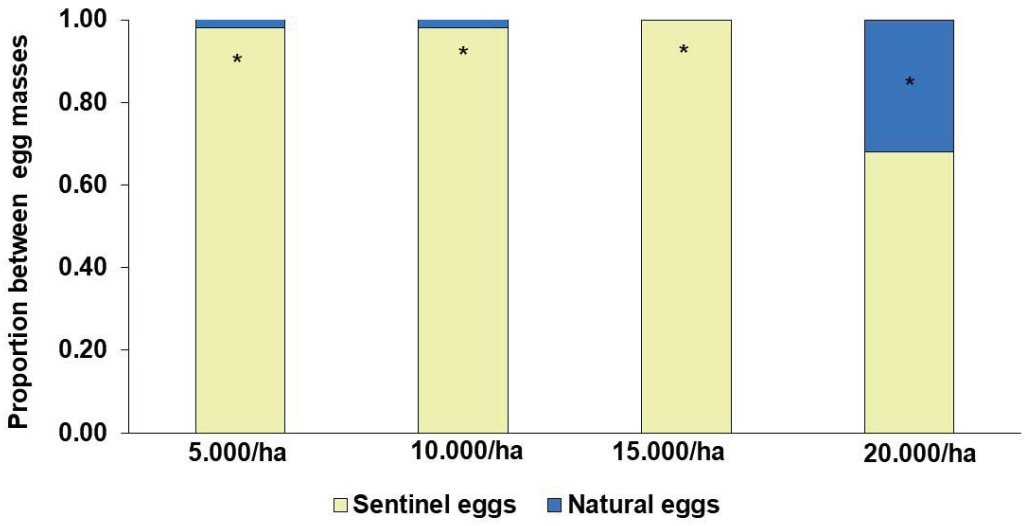

Figure 5. Proportion of parasitized egg masses, sentinel and natural, following releases of *Telenomus remus* at different densities in maize during the second cropping season of 2024, conducted at the Embrapa Soja experimental field, Londrina, Paraná, Brazil. *Represent parasitoid densities with statistical difference between the proportion of sentinel eggs and natural eggs.

4. Discussion

Overall, the results herein reported illustrate that the release of fed adult parasitoids can be adopted with the use of the tested honey-solid diet previously described in the literature [54]. Being able to release *T. remus* as an adult fed has great advantages compared to the most common strategy

currently adopted of releasing egg parasitoid pupae in bulk [34,61] increasing the parasitoid survival after released [54]. The survival of parasitoids released as pupae can be reduced by predation [40], temperature [62], and rainfall [39], among other mortality causes. It is especially important for scelionids (Hymenoptera: Scelionidae), whose males emerge up to 24 hours before females, making females then, to be exposed for longer periods of time in the field to the different causes of mortality if released as pupae [63]. Previous results reported in the literature prove that when exposing parasitoid pupae of another *Telenomus* species (*T. podisi*) in soybean fields for only 24 hours, it was observed a significant reduction in adult emergence from 76% (control) to close to 20% when pupae were exposed directly to sunlight between soybean rows (hotter spots) [62].

Furthermore, the herein results also allow to claim that *T. remus* fed with honey-solid diet can be storage up to 6 days before release with low reduction in lifetime parasitism, which can be compensate by increasing the number of released parasitoids in the field by around 30%. In addition, the recorded sex ratio was always higher than 0.50, which is similar to previous recorded results published in the literature [18, 64]. Having a similar or higher proportion of *T. remus* females in the parasitoid population is important since they are responsible for the parasitism [18].

Different from storing *T. remus* up to 6 days, eight days of parasitoid storage should be avoided due to the significant reduction in both lifetime number of parasitized egg per female and in the parental longevity of females to only 7.1 days. Despite apparently short storage of 6 days, prolonging the shelf life of the *T. remus* bioinsecticide from zero to 6 days is a significant advancement that might prove beneficial in cases of rainy or extremely hot days, or even short delays in the transport of the parasitoid from production sites to the field, still preserving the efficacy of the parasitoid [54].

Thus, the possibility of using the honey-solid diet to feed *T. remus* adults is an important innovation in pro of the success of the parasitoid release. As a synovigenic parasidoid, *T. remus*, females store few to no mature oocytes in their abdomen at the time of emergence [54, 65, 66]. Thus, parasitoid adult females continue to produce and mature eggs throughout adult life, requiring them to regularly acquire nutrients for egg production [67]. Therefore, fed *T. remus* can minimize food

foraging efforts focusing parasitoid energy into locating and parasitizing its hosts. Additionally, parasitoids with access to sugar sources in the field have longer lifespans and higher parasitism rates compared to those experiencing food deprivation [68]. Furthermore, once depleted of mature eggs, fed parasitoid females have also been reported to contribute to greater non-reproductive host mortality [69]. However, nectar or honeydew, the most common natural food sources for parasitoids, are usually scarce in large commodity crops such as maize, for example [68,70]. Low availability and accessibility of food sources for parasitoids strongly reduce parasitoid retention in the field and host-finding efficacy [71].

In light of these physiological and ecological constraints, look for immediate shelter likely can enhance initial efficacy, since parasitism rates at the field level was consistently highest within the first 24 hours post-release. Previous results published in the scientific literate also reported *T. remus* parasitism peaking on the first day [72] and starving parasitoids tending to prioritize foraging over host hunting [66].

Despite the high potential for technological innovation brought by T. remus release of fed adults, field parasitism is also strongly tied to the right parasitoid release density [41]. For the success of biological IPM programs with *T. remus,* releases at the density of 20,000 parasitoids/ha, S. frugiperda will probably require additional control strategies to keep *S. frugiperda* under the economic injury level since *T. remus* parasitism in the field did not reach 80% of the eggs. This release density is higher than previous proposal of 5,000 to 10,000 wasps per hectare per season [18] and previously successfully adopted in Venezuela during the 1990s [21,42,44]. On the other hand, 20,000 parasitoids/ha is lower than release density proposed in the *T. remus* registration in Brazil, which happened on July 2024, suggesting 3 releases of 40,000 parasitoids /ha [73]. Those divergent results indicate that higher densities of *T. remus* should be further evaluated in future field trials, in addition to cheaper massive rearing protocols which still need to be develop to make this higher release density commercially viable.

However, it is important to highlight that our trial was carried out in the second season (autumn/ winter) while most of the previous published trials were carried out in the first crop season of maize (summer season), what completely differs in weather conditions. Furthermore, despite some field trials carried out in Brazil, only one study has reported successful parasitism rates above 70% so far. This exception involved the release of 90,000 to 120,000 parasitoids/ha, achieving rates of 72.4% and 82.8% [45], a significantly higher density than the 20,000/ha tested in our field trial. Despite those higher *T. remus* release density being limited by rearing`s costs at the moment due to the costs involved in rearing of its hosts [18,74], artificial eggs are being intensively studied [75]. The potential of artificial rearing technologies can revolutionize biological control reducing today`s agriculture reliance on chemical pesticides, emphasizing the role of biological control as a cornerstone of modern sustainable agriculture [76].

The limited parasitism observed in natural egg masses at lower release densities may be explained also by a combination of biological and ecological factors. First, sentinel egg masses were placed in accessible and exposed positions, which greatly facilitated host detection, despite its great parasitoid dispersion capacity [21]. In contrast, natural *S. frugiperda* eggs are often laid in concealed locations, such as between the base of the leaves of maize plants [77], where physical access is more difficult for the parasitoid. Second, *T. remus* exhibits a narrow host age window, with the highest parasitism success occurring in eggs 24 to 48 hours after released, and negligible parasitism beyond 72 hours after released [78], and it is likely that some of the natural eggs present at the time of parasitoid release were too old to be parasitized. Finally, the increase in parasitism of natural egg masses observed only at the highest release density can indicate a clear density-dependent effect. This pattern is consistent with previous report from literature [22,45,79], which also describes a trend needing a high number of active females per egg mass to ensure sufficient parasitoid coverage, reaching until 45 *T. remus* females per 300 eggs of *S. frugiperda*. Such coverage is essential to overcome spatial and temporal limitations that often occur under field conditions and may vary depending on the crop's structure and phenology.

455 It is important to highlight that release intervals of 7 to 10 days may not be sufficient to maintain
456 adequate control pressure, particularly under conditions of high infestation or pest migration. As
457 noted previously published [42], a higher number of releases at shorter intervals extends the period
458 in which active parasitoid populations are present in the field, thereby reducing the risk of pest
459 resurgence from either survivors or new incoming individuals. Therefore, future studies should
460 consider evaluating strategies that involve more frequent releases and/or higher parasitoid densities,
461 aiming to enhance the overall effectiveness of biological control and to ensure longer-lasting
462 protection over vulnerable pest stages.
463
464 **5. Conclusions**
465 The storage period after the emergence of the first adults of *T. remus* can be done up to 6 days
466 after parasitoid emergence using the honey-solid diet herein studied, making the release process of *T.*
467 *remus* more flexible and attractive to farmers. Such an increased shelf life can be extremely helpful
468 when the release of *T. remus* must be delayed for one or two days, for instance, due to bad weather
469 conditions or other reason. In addition, the release of fed adults should also reduce predation and
470 other causes of mortality to which immobile *T. remus* pupae are more susceptible than adults.
471 The release density of 20,000 parasitoids per hectare seems to be the most effective among the
472 studied rates (from 5,000 to 20,000) and could be recommended as part of an integrated pest
473 management (IPM) strategy, given that it resulted in approximately 50% control of *Spodoptera*
474 *frugiperda* egg masses under field conditions considering the second season (autumn/ winter) in
475 Brazil. However, this level of suppression may still be insufficient as a stand-alone strategy.
476 Therefore, future studies should aim to evaluate the efficacy of higher *T. remus* release densities,
477 perform cost-effectiveness analyses, and explore shorter release intervals to optimize the practical
478 use of egg parasitoids in field conditions. In addition, it is important to research a cheaper massive
479 production protocol for *T. remus* in order to make higher than 20,000 parasitoids/ha to be
480 economically feasible in field conditions.

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Data availability: Raw data available at supplementary materials.

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Chapter 3 - Insecticidal potential of oregano essential oil nanoemulsion to control *Spodoptera littoralis*

Abstract

Spodoptera littoralis (Boisduval) (Lepidoptera: Noctuidae) is a significant agricultural threat widely distributed over Africa, Mediterranean Europe and the Middle East. The main management approaches targeting this pest mainly rely on synthetic insecticides. Despite their efficacy, chemical insecticides can have notable drawbacks, including the development of resistance, environmental contamination, consumer concerns, and adverse effects on non-target organisms. In response to these challenges, in this study, we developed and evaluated an oregano EO-based nano-emulsion with a high EO concentration (15%) and low surfactant content to enhance efficacy against *S. littoralis* eggs and larvae. Laboratory bioassays demonstrated high egg mortality, with embryos failing to hatch at EO concentrations of 3, 4.5, and 6%, and larvicidal activity, with EO Lethal Concentration 50% (LC50) and 90% (LC90) ranging from 4.10% to 8.20%, respectively. In addition, a significant decrease in larval feeding activity was observed when leaves were previously treated with the EO nanoemulsion. In terms of fold reduction, the treatments resulted in 2.0, 2.7, and 6.2 times less leaf area eroded than the untreated treatment, by LC5, LC30 and chemical, respectively. This study underscores the potential of the newly developed nano-formulated oregano EO as a sustainable botanical insecticide to control *S. littoralis*.

Keywords: antifeedant; biopesticides; cotton leafworm; botanicals.

Introduction

The cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), native to Africa (ElShahed et al. 2023), is a destructive pest capable of triggering significant economic losses to several crops (El-Sayed et al. 2024) across the Middle East, Southern Europe, Western Asia and some islands of the Pacific Ocean (ElShahed et al. 2023; Durand et al. 2025). Being highly polyphagous, the species feeds on 130 different host plants belonging to 56 botanical families (Pogue 2002), including more than 80 important cultivated crops such as cotton, corn, peanut and soybean, among others (El-Sheikh et al. 2018).

Spodoptera littoralis has been commonly controlled by the adoption of traditional chemical insecticides as the main component of Integrated Pest Management (IPM) packages against the pest (Moustafa et al. 2023). However, the overdependence on chemicals has raised concerns about their lasting impact on the environment (Gong et al. 2023). The overuse of insecticides has been leading to negative side effects to the agroecosystem (Jacquet et al. 2022), impacting the ecological services by pollinators and biocontrol agents (Lisi et al. 2024), in addition to selecting resistant *S. littoralis* populations (Moustafa et al. 2024; Durand et al. 2025). Consequently, replacing the use of harmful chemicals to more sustainable tools is of great theoretical and practical interest and has become an increasing demand and an important global policy issue (Lee et al. 2019). Among the eco-friendly management tools available, essential oils (EOs) have been recognized as an effective pest control option (Damalas and Koutroubas 2020) with lower persistence in the environment (Ntalli et al. 2022), faster degradation (Isman 2000; Batish et al. 2008) and lower impact on non-target organisms (Benelli et al. 2020) compared to the most common traditional chemical insecticides (Ebadollahi et al. 2020).

Among the different botanical families used to produce EOs with insecticide activity, the Lamiaceae family stands out due to the innumerous reports on the high pesticidal potential due to the major components present such as the terpenoids 1,8-cineole, linalool, terpinene, thymol, α-pinene, and β-pinene, which can cause neurotoxic, antifeedant, repellent properties, and growth regulatory effects (Ebadollahi et al. 2020; Ali et al. 2025), having *Origanum* spp. among the most promising

plants for pest management also due to the wide range of compounds (Osório et al. 2019; Ebadollahi et al. 2020; Ali et al. 2025). Despite the benefits of botanical insecticides, some constraints of their adoption include their low stability during storage and transportation processes in addition to their short persistence, as well as the need for a large amount of active ingredient to be efficient against the target pest (Isman 2016). An alternative to those limitations is the use of nanoformulations (Luneja and Mkindi, 2025).

Nanoformulations have proven to be an innovative tool (Giunti et al. 2025), enabling controlled release, increased stability, and reduced dosage (Marín et al. 2025). An average gain of 20% to 30% in efficacy of botanical insecticides in nano-systems in relation to conventional products has been recorded (Kah et al. 2018), illustrating the importance of developing nanoformulated botanical insecticides. A few previous studies have demonstrated the insecticidal potential of essential oil (EO) derived from *Origanum vulgare* in pest management, particularly against species such as *Anobium punctatum* (De Geer) (Coleoptera: Anobiidae), where it showed repellent activity (Palla et al. 2020), *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae), with evidence of oviposition deterrence and repellency (Pinto et al. 2022), the mite *Tetranychus urticae* Koch (Acari: Tetranychidae) (Susurluk 2023) and *Spodoptera littoralis*, where the EO induced oxidative stress and reduced feeding activity (Agliassa and Maffei 2018).

In light of its potential for pest management and the chemical diversity of *Origanum vulgare*, this study investigated the mortality caused by a nano-formulated oregano essential oil on *Spodoptera littoralis* eggs and larvae, along with its antifeedant effects on the larval stage.

Material and Methods

The following analyses described in Sections Chemical characterization of the oregano essential oil and Formulation and physical characterization of the oregano nanoemulsion were conducted at the Department of Agriculture, University of Reggio Calabria (Italy). Sections Biological material, Ovicidal effect of the oregano EO nanoformulation), Larvicidal effect of the

oregano EO nanoformulation, and Antifeedant effects of the oregano EO nanoformulation on larvae were performed at the Department of Agriculture, Food and Environment, University of Catania, Sicily, Italy.

Chemical characterization of the oregano essential oil

Origanum vulgare EO was purchased from Esperis S.p.A. (Milan, Italy) and chemically analyzed using a gas chromatography-mass spectrometry (GC-MS) device, following the methods described by (Giunti et al. 2019). Briefly, a Thermo Fisher TRACE 1300 GC with a MEGA-5 capillary column (30 m × 0.25 mm; coating thickness = 0.25 μm) and a Thermo Fisher ISQ LT mass detector (ionization mode: EI; scan time: 1.00 s; scan mass range: 30–300 m/z) were used setting injector and transfer line at 250 and 240 °C, respectively, and a temperature ramp from 60 to 240 °C at 3 °C min−1 (carrier gas: He 1 mL min−1). The pure EO was diluted (1:10 v:v) in hexane (95%, Sigma Aldrich, Munich, Germany), and 0.2 μL were injected at a split ratio of 1:30. The identification of peaks was made using computer matching against the commercial libraries (NIST 05, Wiley FFNSC and ADAMS) comparing linear retention indices (LRI). The LRIs were calculated using the formula of van den Dool and Kratz (1963) by comparing the retention times of the compounds to be identified with those of a standard mixture of alkanes (C8-C20 saturated alkanes standard mixture, Supelco®, Bellefonte, PA, USA) which was analysed in GC-MS under the identical operating conditions as the sample (van den Dool and Kratz 1963; Masada 1976; Davies 1990).

Formulation and physical characterization of the nano-emulsion or oregano

The oregano EO nanoemulsion was developed using a high-pressure microfluidization technique as described by Modafferi et al. (2024). In detail, a homogeneous organic phase was obtained by mixing EO and sorbitan laurate 20® (ratio 3: 1 w: w) for 5 minutes at 6000 rpm. Then, a raw emulsion was obtained by adding the double-distilled water to the organic phase (ratio 4: 1 w: w) and subsequently homogenized using a high-pressure microfluidizer (HMP) device (LM20 Microfluidizer™ Processor, USA). In order to obtain a homogeneous oregano EO-based nano-

emulsion, the microfluidization process was repeated three times at 30.000 psi. In addition to avoiding the EO degradation during the process, the interaction chamber, coupled with a heat exchanger, was submerged in an ice bath. Then, the obtained oregano EO nanoemulsion was collected inside an aluminum bottle and stored at room temperature (25±2°C) until use.

The physical characteristics (i.e., particle size and polydispersity index PDI) of the developed oregano EO nanoemulsion were evaluated using a Zetasizer device (Zetasizer Nano, Malvern®). The analysis was performed 24 hours after the nanoemulsion preparation and was conducted by diluting the oregano EO nanoemulsion in double distilled water (ratio 1: 200 v: v).

Biological material

Spodoptera littoralis rearing was carried out under controlled constant conditions (25 ± 2 °C, 50 ± 10% RH, photoperiod: 14:10 h (L:D)). The laboratory colony of *S. littoralis* used in this study originated from a laboratory colony from the Department of Agriculture of the University of Naples (Portici, Italy). Larvae were fed with an artificial diet (41.4 g/L wheat germ, 59.2 g/L brewer's yeast, 165 g/L cornmeal, 5.9 g/L ascorbic acid, 1.53 g/L benzoic acid, 1.8 g/lmethyl 4-hydroxybenzoate and 29.6 g/L agar), while the adults were provided with a sugar solution (1:1) (Giuliano et al. 2024). Larvae of third instar and eggs (≤ 24 hours) of the following generations were then used for trials.

Antifeedant trials were conducted by providing larvae with leaves of bell pepper plants (*Capsicum annuum* L., cv Makko F1). The plants used in the study were grown from untreated seeds and planted in pots filled with organic soil. The plants were regularly watered throughout the growth period and received the required nutrients inside an experimental greenhouse at natural environmental conditions (late winter/early spring in Catania, Italy). Leaves from plants having 4-6 definitive leaves were used for trials.

Ovicidal effect of oregano EO nanoformulation

The direct ovicidal effect of the studied nanoformulated EO of oregano was assessed on *S. littoralis* eggs (≤ 24 hour-old) collected from the laboratory rearing. Egg masses containing from 150-

200 eggs oviposited in superposed layers (3-5 layers) were selected. After that, each egg mass was exposed topically to the respective nanoformulated EO concentrations, [0 (untreated control), 0.125, 0.25, 1.0, 2.0, 3.0, 4.5, and 6%], diluted in distilled water using the dipping method previously described by Valizadeh et al. (2015) with few adaptions as described in the followings.

The experiment was performed with 5 replications per tested concentration, consisting of 1 egg-mass per replicate. Egg masses were dipped into 10 mL of each tested dilution for 10 seconds and then, left to dry under paper towel inside a laminar flow hood for 45 minutes. After that, each egg mass was individually placed inside a specific arena (plastic box) of 100 mL, containing a circular ventilation area on top (diameter: 5 cm), which was covered with a net. Eggs were then kept in controlled conditions (25 ± 2 ºC, 50 ± 10% RH, photoperiod: 14:10 h (L:D)). Two days after dipping the eggs, a piece (2.5 x 2.5 cm) of artificial diet described by Giuliano et al. (2024) was placed into the arena. After emergence, the number of eggs with *S. littoralis* embryo without emergence (dead eggs) and the number of alive larvae were recorded for each replicate. Moreover, four healthy larvae per replicate were individualized with diet and evaluated until pupal to assess larval development time and pupal weight.

Larvicidal effect of oregano EO nanoformulation

Ten different concentrations of oregano EO nanoformulation [0 (untreated control), 1.5, 3.0, 3.5, 4.0, 4.5, 6.0, 9.0, 12.0, and 15%] were tested. Based on preliminary observations, the tested concentrations were chosen to identify the active ingredient concentrations resulting in mortality levels ranging from 5 to 90%. This mortality range was selected to meet the assumptions of Probit analysis, which was used to estimate the concentrations of essential oil (EO) required to kill 5% (LC_5), 30% (LC_{30}), 50% (LC_{50}), and 90% (LC_{90}) of the exposed 3rd instar larvae.

Untreated controls was performed by applying distilled water with Tween 80® (Biolife Italiana S.r.l., Monza MI, Itália) 0.5% v/v and, as a treated control, a commercial insecticide, namely deltamethrin (Dorotrin® 25EC, Gowan, Faenza, Itália; deltamethrin, emulsifiable concentrate, 25

grams/liter) at the recommended label rate against *Spodoptera* sp., consisting of 50 mL of commercial product/hL of water. 1 µL/caterpillar of the different concentrations was applied to the dorsal region using a single-channel micropipette (Sartorius Proline Plus 0.1 – 3µL). Larval mortality was assessed daily, starting 24 hours after the application of treatments. Each larva was gently stimulated using a fine paintbrush to evaluate responsiveness. Larvae that did not exhibit any movement after repeated gentle touches were considered dead. The experiments were carried out under controlled environmental conditions (25 ± 2 ºC, 50 ± 10% RH, photoperiod: 14:10 h (L:D)).

After 48 hours, the time point used for mortality evaluation, the concentrations corresponding to LC_5, LC_{30}, a chemical control, and an untreated control were selected for continued monitoring until the larvae reached the pupal stage. Larval development duration (in days) and pupal weight (in grams) were then measured to assess sublethal effects.

Antifeedant effects on larvae of oregano EO nanoformulation

The antifeedant activity of the tested oregano EO nanoformulation was assessed exposing third instar larvae of *S. littoralis* to treated leaves in a no choice experiment. In detail, fresh bell pepper-leaf discs with a diameter of 5.2 cm were dipped into dilutions of the oregano EO nanoformualation, corresponding to LC5 and L30 previously estimated for larvae (section 2.5). Water + Tween 80 0.5% v/v was tested as untreated control and deltamethrin as treated control. Leaf discs were dipped into 10 ml of the dilutions for 10 s and then, left to dry under paper towel inside a laminar flow hood for 45 minutes. After drying, each treated leaf disc was carefully placed in a sterile plastic arena (5.5 × 3.5 cm). Afterward, one larva was released into each arena using a fine paintbrush. Once the larvae were placed inside the arena, the arenas were covered with a mesh to avoid any escape, allow ventilation, and prevent fumigation effect. For each treatment, 12 replicates (arenas) were provided. The leaf surface eroded by larvae during the experiments was evaluated after 24 hours of feeding by ImageJR v.1.53 software (National Institutes of Health, Bethesda, MD, USA) and expressed as a percentage of eaten leaf disc.

182

183 **Statistical Analyses**

184 The percentage of mortality (egg and larval) was analyzed using a generalized linear model
185 (GLM) with a quasibinomial error distribution to account for overdispersion. One-way ANOVA
186 followed by Tukey's HSD post hoc test ($p < 0.05$) was used to analyze developmental time, pupal
187 weight, and eroded leaf surface. Kaplan–Meier survival analysis was used to evaluate the effects of
188 sublethal concentrations on larval survival, and survival curves were compared among treatments
189 using the Log-Rank (Mantel–Cox) test. Probit analysis was performed to estimate lethal
190 concentrations after 48 h after the topical exposure; concentrations were considered significantly
191 different when their 95% fiducial limits did not overlap (Finey 1952). Kaplan–Meier and Probit
192 analyses were carried out using IBM SPSS Statistics version 19, while GLM, ANOVA, and post hoc
193 tests were performed in the software R version 4.4.1 (R Core Team 2024).

194

195 **Results**

196 **Characterization of EO of oregano and its nanoemulsion**

197 The chemical characterization of oregano EO is shown in **Table 1**. Forty-seven out of seventy-
198 two compounds were detected, accounting for 98.72% of the total area. The EO was rich in carvacrol,
199 thymol, and o-cymene, representing 25.39%, 18.71%, and 14.26% of the total area, respectively.

200

201 **Table 1.** Tested oregano EO chemical composition.

COMPOUND	C-LRI	L-LRI	RT	Area %
Tricyclene	922	926	4.97	0.04
α-Thujene	926	926	5.06	0.25
α-Pinene	933	939	5.24	2.95
β-Fenchene	937	940	5.35	0.01
d-Camphene	946	948	5.57	0.17
Camphene	948	948	5.62	1.48
2,4-Thujadiene	953	955	5.75	*
β-Pinene	977	976	6.36	1.82
3-Octanone	986	985	6.6	0.29
β-Myrcene	991	991	6.72	1.72
3-Octanol	996	994	6.85	0.13
3-Carene	1011	1010	7.32	0.03
α-Terpinene	1017	1016	7.53	0.90
β-Cymene	1022	1025	7.7	0.26
o-Cymene	1027	1025	7.87	14.26
D-Limonene	1030	1029	7.97	2.21
Eucalyptol	1032	1031	8.05	2.19
γ-Terpinene	1058	1056	8.94	3.13
trans-Sabinene hydrate	1067	1068	9.24	0.07
cis-Linalool oxide	1072	1072	9.44	0.05
Linalool	1102	1101	10.47	8.19
1-Terpinenol	1134	1137	11.76	0.06
Pinocarveol	1139	1139	11.95	0.03
(-)-Camphor	1144	1145	12.17	1.80
Isoborneol	1156	1152	12.64	0.37
Borneol	1166	1166	13.03	2.67
Terpinen-4-ol	1177	1175	13.49	1.09
p-Cymenol	1186	1187	13.82	0.02
α-Terpinol	1192	1190	14.06	1.68
Borneol, formate	1228	1232	15.56	0.03
Propanal, 2-methyl-3-phenyl-	1240	1244	16.06	0.03
Anisole	1244	1235	16.25	0.39
Anethole	1285	1284	17.98	0.33
Thymol	1296	1290	18.45	18.71
Carvacrol	1312	1307	19.12	25.39
α-Terpinyl acetate	1351	1350	20.74	0.07
Eugenol	1359	1356	21.06	0.25
α-Copaene	1375	1377	21.74	0.06
Isocaryophyllene	1406	1405	23.02	0.05
Caryophyllene	1419	1418	23.54	3.43
Humulene	1452	1452	24.9	0.65
Ledene	1494	1490	26.58	*
β-Bisabolene	1508	1509	27.16	*
γ-Cadinene	1513	1511	27.34	*
δ-Cadinene	1523	1524	27.72	0.03
Caryophyllene oxide	1582	1583	30.02	1.19
Humulene epoxide II	1608	1609	31.01	0.14
Total Area				**98.72**

202 *It was not possible to quantify.

The physical characterization of the developed oregano EO-based nanoemulsion revealed a narrow and uniform particle size distribution, indicating homogeneity and stability, as shown in **Figure 1**. In detail, the nanoemulsion exhibited particle sizes ranging in the nanoscale with average values of 217.5±2.66 nm. In addition, the developed oregano EO-based nanoemulsion showed an excellent homogeneity of the particle size due to the very low PDI values achieved (0.07±0.03).

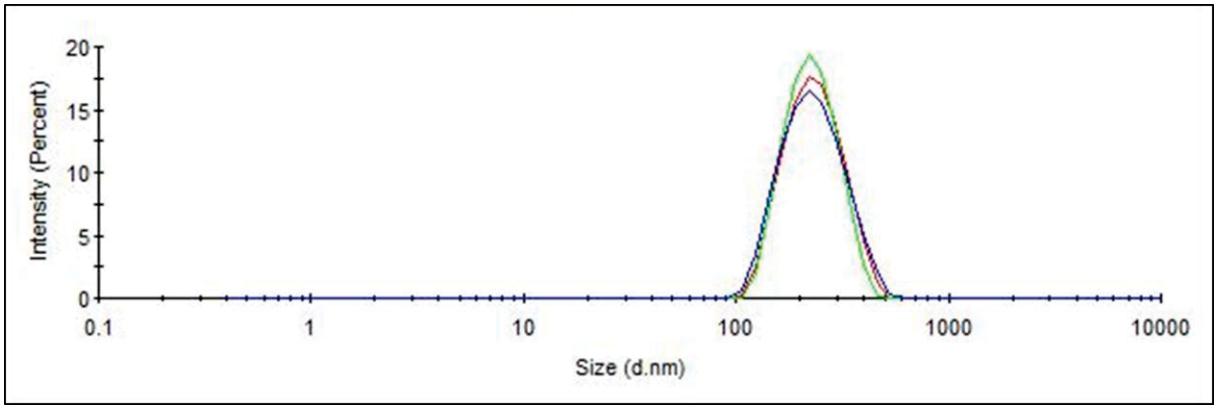

Figure 1. Size distribution by intensity of the developed oregano EO-based nano-emulsion

Ovicidal effects oregano EO nanoformulation

The EO was able to kill the embryo during development avoiding emergence of larvae. Mortality of *S. littoralis* embryo was inversely proportional to tested EO dose with mortality of all EO treated eggs masses being significantly higher to the mortality recorded in the untreated control (4.80 ± 0.96) ($\chi^2 = 4010.08$, df = 7, $p < 0.001$) (**Table 2**). Overall, the tested EO at 3% reached the highest percentage level of mortality (96.82 ± 2.61), not statistically different from 4.5% (98.89 ± 0.72) and 6% (100.00 ± 0.00). From the larvae emerged from treated eggs no sublethal effects on larvae development (days) neither on pupal weight (g) were recorded (**Table 2**).

Table 2. Impact of the oregano EO nano-emulsion tested at different EO concentration on *Spodoptera littoralis* embryos, development time of hatched larvae and weight of pupae (Mean ± SE). Within each column, means (±SE) with different letters are significantly different, in the column, according to Tukey test ($p < 0.05$).

Concentration	Mortality (%)	Larval development time (days)	Pupal weight (grams)
0	4.80 ± 0.96 f	18.43 ± 0.37 a	0.366 ± 0.010 a
0.125	19.53 ± 2.16 e	18.05 ± 1.11 a	0.366 ± 0.003 a
0.250	35.52 ± 3.23 d	18.33 ± 0.13 a	0.341 ± 0.017 a
1.000	47.46 ± 2.15 c	19.10 ± 0.80 a	0.359 ± 0.017 a
2.000	58.60 ± 3.27 b	19.66 ± 0.12 a	0.382 ± 0.007 a
3.000	96.82 ± 2.61 a	-	-
4.500	98.89 ± 0.72 a	-	-
6.000	100.00 ± 0.00 a	-	-

Larvicidal effect of oregano EO nanoformulation

The oregano EO nanoformulation exhibited high toxicity against third-instar larvae of *S. littoralis* (**Table 3**). No mortality was recorded in the untreated control during the entire observation (48 h), whereas 100% mortality was recorded in the treated control.

Table 3. Toxicity of oregano essential oil nanoemulsion against third instar *Spodoptera littoralis* larvae after 48 hours of topical exposure, and its effects on development duration (L3 to pupal stage) and pupal weight. Means (±SE) of data on developmental time and on pupal weight did not differ significantly according to Tukey test ($p < 0.05$).

Treatments	Estimate (LC%)	Fiducial limits (%)	Developmental time (3rd instar-pupae) (days)	Pupal weight (grams)
Control	-	-	15.27 ± 0.32 ^{ns}	0.501 ± 0.019 ^{ns}
LC_5	1.673	1.315-1.983	15.41 ± 0.17 ^{ns}	0.484 ± 0.016 ^{ns}
LC_{30}	3.086	2.741-3.383	15.17 ± 0.15 ^{ns}	0.505 ± 0.034 ^{ns}
LC_{50}	4.109	3.784-4.438	-	-
LC_{90}	8.274	7.530-9.792	-	-

Significant differences were observed in mortality levels caused by the tested formulation among the different application rates, 48 h after exposure (χ^2 = 213.43, df = 8, $p < 0.001$). The mortality data fitted the Probit model (**Table 3**), and the LC values indicate that most of the larvicidal activity occurred within the first 24 h after treatment. Indeed, the LC values highlighted no statistical differences among the two different exposure times (i.e., fiducial limits did not overlap). The amounts of EO required to kill 5, 30, 50 and 90% of the exposed larvae after 24 h were 1.67, 3.0, 4.10, and 8.20%, respectively (**Table 3**).

The survival analysis (**Figure 2**) further confirmed the toxic effects of the oregano EO nanoemulsion on *S. littoralis* larvae. Kaplan–Meier survival curves showed a significant reduction in survival probability over time in treated groups compared to the untreated control. Larvae exposed to LC30 exhibited the fastest decline, with complete mortality recorded within 5 days, whereas the LC5 group reached 100% mortality by day 8. In contrast, the untreated control group maintained high survival throughout the observation period, with only a gradual decline beginning after day 7.

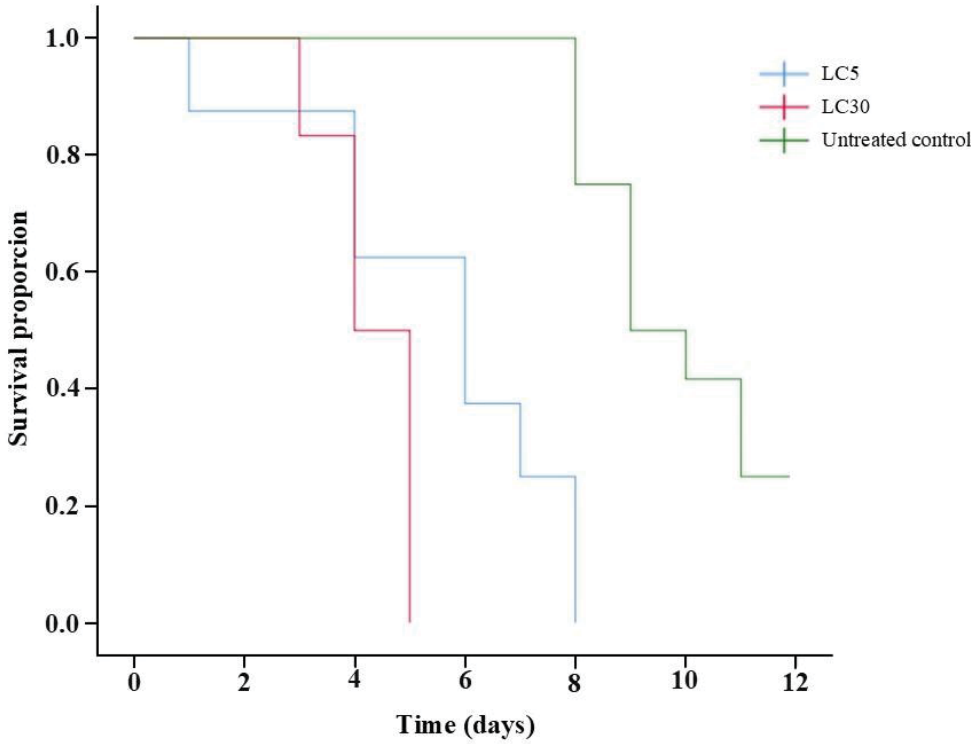

Figure 2. Survival analysis of *Spodoptera littoralis* larvae after topical application of the oregano EO nanoemulsion at LC5 and LC30 previously estimated for larvae.

Antifeedant effects on larvae of nano-formulated EO of oregano on *S. littoralis*

The antifeedant effect of the oregano EO-based formulation is showed in **Figures 3A and 3B**. The eroded leaf surface after the treatment followed a dose-dependent pattern ($F_{3,21} = 44.45$, $p < 0.001$), where larvae were able to feed on more than 3.43±0.36 % of the untreated leaf area. Indeed, larvae treated with LC5 and LC30 of EO exhibited a significant reduction of the feeding activity, with the eroded leaf areas ranging from 1.75±0.17 % to 1.29±0.21 %, respectively, compared to the control.

Larvae provided with leaves treated with LC5, LC30, and the chemical standard exhibited 49.0±1.89%, 62.4±2.15%, and 84.0±2.24% less leaf consumption, respectively, compared to the untreated control (3.43% erosion). In terms of fold reduction, the treatments resulted in 2.0, 2.7, and 6.2 times less leaf area eroded than the untreated treatment, by LC5, LC30 and chemical, respectively.

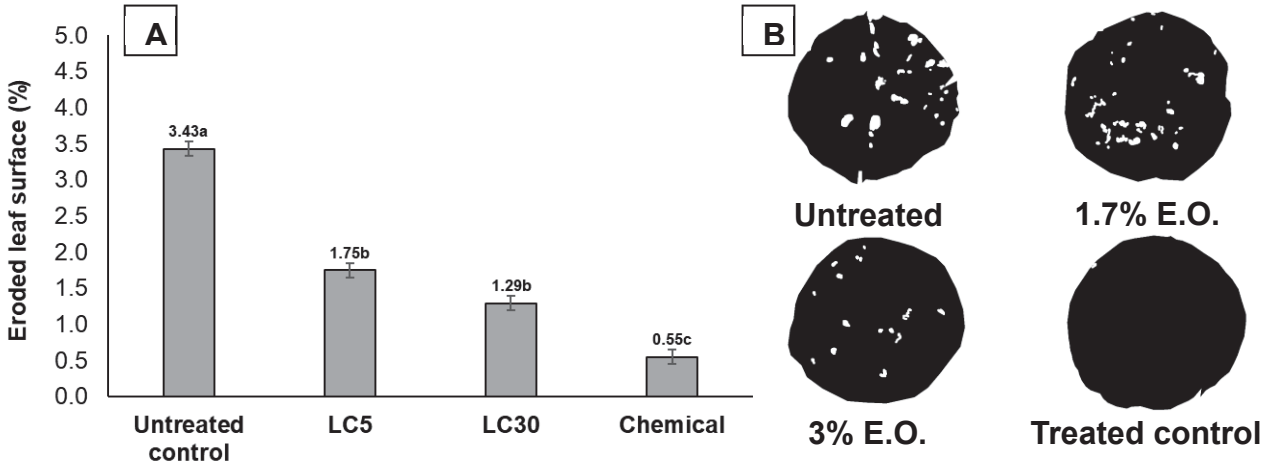

Figure 3. (A) Percentages (±SE) of disc leaf eroded by *Spodoptera littoralis* third instar larvae 24 h after exposure. Different letters indicate significant differences among the groups according to Tukey's test at $p < 0.05$. **(B)** Example of disc leaves eroded by *Spodoptera littoralis* larvae and previously treated with different doses of oregano EO nanoemulsions. Pictures processed with ImageJ v. 1.53 software.

Discussion

Current pest management approaches are increasingly focusing on innovative and environmentally friendly tools to ensure agro-ecosystem stability. Essential oils (EOs) are being

integrated into IPM packages as part of this shift. This study provides new insights into the potential of a newly developed oregano EO nanoemulsion for controlling *Spodoptera littoralis*, a significant pest. The tested EO nanoemulsion significantly impacted pest offspring development and demonstrated repellent activity against larvae. These findings suggest its high potential as a sustainable alternative for managing *S. littoralis*.

The major components of the tested oregano EO, i.e., carvacrol (25.39%), thymol (18.71%), and o-cymene (14.26%), have also been reported in previous studies (Gong and Ren 2020). Despite those chemicals being frequently main in the composition of EO of oregano, variation in their amounts is frequently reported due to endogenous factors (related to the plant itself) and exogenous factors (environmental conditions and processing methods) (Barra 2009; Qian et al. 2024). For instance, Russo et al. (1998) reported thymol (from 36.61% to 40.11%) and carvacrol (from 24.13% to 30.77%) while in another studies, Martino et al. (2009) and Sarikurkcu et al. (2015) reported thymol 26.75% and 58.31%, respectively, as major components of oregano EOs. More recently, Leyva-López et al. (2017) globally reviewing EOs of oregano, reported a range for carvacrol from 1.7% to 92.6% of their composition. Carvacrol has five-folds higher effect than that of oregano EO against *H. armigera*, indicating that hydroxyl group (-OH) plays an important role in its recorded insecticidal activity (Gong and Ren 2020). Therefore, reported variations in the amounts of major components can significantly impact the control efficacy of the EO against pests (Luz et al. 2022). EO of *Origanum vulgare* was toxic to 2nd instar of *Spodoptera frugiperda* (Bibiano et al. 2022). Nevertheless, in this study there is more than one active substance in the EOs or else, synergism and/or an additive effect between the substances can be important to pest management (Bibiano et al. 2022).

Overall, major components of EOs and their interaction are in fact supposed to be the active ingredients mainly impacting on the pest survival. Consequently, knowing and understanding their action is important to the proper potential inclusion of those botanical insecticides in IPM programs (Acheuk et al. 2022). Although the mode of action of EOs from plants of the Lamiaceae family is not

yet fully known, inhibition of acetylcholinesterase (AChE), adenosine triphosphatases (ATPases) and butyrylcholinesterase (BuChE) and action on octopamine and gamma-aminobutyric acid receptors (GABArs) have been reported (Ebadollahi et al., 2020). With neurotoxic action, the intoxication symptoms include agitation, hyperactivity, paralysis, and knockdown (Ahmadi et al. 2022).

Overall, EO of oregano is a rich source of polyphenols (Awad et al. 2024), which has been used in several studies as natural insecticides against several pest species such as *Pediculus humanus* (Psocodea: Pediculidae) (Yang et al. 2009), *Blattella germanica* (Blattodea: Ectobiidae) (Jang et al. 2005) and *S. littoralis* (Pavela 2004). This broad-spectrum bioactivity against agriculture and urban pests (Benelli et al. 2019) is due to the lipophilic nature of those major chemical components that interfere with the normal physiological metabolism of insects (Jankowska et al. 2017; López et al. 2018). Despite this insecticidal potential, the efficacy of different EOs on pest control is highly dependable on their formulation (Campolo et al. 2020).

The physical characteristics of the developed nano-formulated EO of oregano indicated good quality of the formulation both in terms of size (≈200 nm) and PDI (0.07) what is essential for a good stability of the developed botanical insecticide over time and its solubility in water (Campolo et al. 2020). Overall, the widespread adoption of EOs in the field is usually hindered due to the limitations related to high volatility, poor solubility in water, and instability of their formulation (Marín et al. 2025). Therefore, the formulation quality reported in this study is crucial to the success of the first step in the introduction of an EO-based nano-emulsions into field application (Campolo et al. 2020). Similar results were previously reported in literature with different EOs formulated using the same self-emulsification process combined with sonication (Ricupero et al. 2022; Giuliano et al. 2024) highlighting the enormous potential application of this methodology for EO formulation (Modafferi et al. 2024). More recently, nanomaterial-based platforms for the formulation of bioactive compounds derived from EOs have attracted considerable attention due to their potential to enhance not only the stability but also to control the release, and consequently efficacy of these natural compounds (Marín et al. 2025).

335 The negative impact recorded in this study when testing the developed botanical insecticide
336 on *S. littoralis* eggs suggests a positive characteristic of this nanoemulsion based its potential to
337 control the pest in a stage (egg) before being capable of triggering any damage to the plant.
338 Consequently, this results in an important feature consisting in the disruption of the reproductive cycle
339 of pest, preventing future generations from causing damage (Valizadeh et al. 2021). Several plant EOs
340 have been reported to have ovicidal effects against pests (Soonwer et al. 2022) including oregano, for
341 instance, against *Helicoverpa armigera* (Hübner) (Gong and Ren 2020), *Xanthogaleuro luteola*
342 (Mull.) (Valizadeh et al. 2021) and mosquitos (Chen et al. 2023) but as far as we know, this is the first
343 report of the ovicidal effect of an oregano EO against eggs of *S. littoralis*.

344 Despite having embryo development after dipping the egg masses (≤ 24h-old) in the EO
345 tested, those *S. littoralis* larvae fail to emerge. It has been reported that the phenolic compounds of
346 EOs affect the movement and vital systems of the insect embryo, and they have inhibitory effects on
347 gas exchange inside the egg. This action could have leads to hardening of the crust and direct
348 influence on protoplasm, causing the death of the insect embryo inside the egg (Al-Murmidhi and Al-
349 Hasnawi 2019) what might explain the reported harmfulness of the EO on pest eggs.

350 Regarding the insecticidal efficacy of the studied oregano EO-based nano-formulation, our
351 findings suggest its potential for the control of *S. littoralis*. Indeed, LC90 was achieved at the
352 concentration of 8.2% with topical application. Previous results reported variation in the toxicity of
353 EOs of oregano to *S. littoralis*. For instance, Pavela (2005) reported LD50 (µL/lavae) from 0.004 to
354 0.06 and, after, Pavela (2015) reported LD30 of EOs of oregano ranging from 13 to 73 µg/larvae.
355 However, without a description of the chemical composition of the studied EOs, those results have
356 lower contribution to the *S. littoralis* management (Isman and Grieneisen 2014). More recently, Awad
357 et al. (2024) reported results demonstrating the potential use of EO of *O. majorana* in the management
358 of *S. littoralis* either as an alternative to or in combination with chemical insecticides.

359 Not only did the studied oregano EO-based nano-formulation demonstrate lethality against *S.*
360 *littoralis* eggs and larvae, but it also showed promising antifeedant potential. Both LC_5 and LC_{30}

361 significantly reduced *S. littoralis* feeding compared to the control. It is important to highlight that
362 botanical insecticides can deter insect feeding through various mechanisms, such as rendering treated
363 plants unpalatable or interfering with the insect's ability to detect and ingest food. Thus, their mode
364 of action does not necessarily rely solely on acute or chronic toxicity. The use of plant-derived
365 antifeedants, capable of significantly reducing food intake in phytophagous insects, represents a
366 promising approach in pest management (Pavela et al. 2025). In agreement with our findings, Agliassa
367 and Maffei (2018) also reported reduced feeding in *S. littoralis* due to oregano terpenoids, particularly
368 γ-terpinene and carvacrol, which exert both behavioral and toxic effects on the larvae.

369 In conclusion, the newly developed essential oil-based oregano nano-emulsion caused high
370 mortality levels (15%) not only of larvae, but also of *S. littoralis* eggs, together with significant
371 antifeeding effects triggered by the EO at its LC5 (1.75±0.17%) and LC30 (3.43±0.36%). This
372 strongly suggests that the developed nano-emulsion of EO oregano can potentially be considered
373 when developing pest management programs. However, further studies should be conducted to
374 evaluate the potential of this EO nanoemenulsion under field conditions, as well as to assess its
375 potential effects on natural enemies.

376 Overall, the results found in the present study provide important insights for the adoption of nano-
377 emulsion of EO of oregano for *S. littoralis* management. In addition to its insecticidal properties, the
378 observed reduction in larval feeding suggests a promising antifeedant effect, which should be further
379 investigated and the effects of the major components of the EO of oregano determined in this research
380 should also be studied in future trials as possible alternative insecticides. The metabolite profile of the
381 developed EO showed that the major compounds were carvacrol (25.39%), thymol (18.71%) and o-
382 cymene (14.26%). Understanding how these compounds modulate herbivore-plant interactions may open
383 new avenues for developing behavior-based pest management strategies, particularly through the
384 integration of antifeedants into push-pull systems or as feeding deterrents in IPM programs.
385

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CONSIDERAÇÕES FINAIS

Os resultados deste trabalho representam um avanço significativo para o uso de bioinsumos no manejo de lepidópteros-praga na cultura do milho, especialmente no que se refere à aplicação de parasitoides de ovos e inseticidas botânicos. Este é o primeiro estudo a avaliar, em condições de campo, a interação entre *Telenomus remus* e *Trichogramma pretiosum*, tema até então explorado apenas em ambientes controlados. A interação entre esses parasitoides é suportada pelos dados, dessa forma, não é vantajosa para o manejo de *Spodoptera frugiperda*, direcionando para a recomendação do uso isolado de *T. remus*, cujo desempenho foi superior. Ainda assim, *T. pretiosum* mantém relevância em diferentes contextos produtivos. O produtor ainda dispõe de um excelente, como hortaliças, cultivos orgânicos e áreas de menor escala, embora sua adoção em grandes monocultivos, como milho e soja, seja mais restrita.

A possibilidade de armazenamento de *T. remus* por até quatro dias em cápsulas biodegradáveis contendo alimento solidificado, amplia sua viabilidade logística avanço importante em direção ao estabelecimento de um programa de controle biológico no Brasil. Essa inovação responde a uma das principais limitações operacionais do uso de parasitoides de ovos em larga escala: o curto tempo de vida dos adultos com as dificuldades de transporte em um país de dimensões continentais bem como os fatores de mortalidades outrora discutidos como exposição de pupas do parasitoides a elevadas temperaturas e/ou a predadores. Apesar disso, diversos avanços ainda precisam ser superados para isso. Esse é o caso da definição de uma densidade ideal para liberação, visto que as utilizadas nesse estudo não foram suficientes para fornecer o suporte necessário para tal definição, mas indicam a necessidade de estudos adicionais em condições reais de campo para definir densidades operacionais mais eficazes e economicamente viáveis, capazes de garantir níveis consistentes de supressão populacional de *S. frugiperda*.

No caso dos inseticidas botânicos, este trabalho reforça o potencial de óleos essenciais no manejo de lepidópteros. O óleo de orégano demonstrou atividade larvicida, ovicida e antialimentar contra *Spodoptera littoralis*, configurando-se como uma alternativa sustentável alinhada à demanda por redução do uso de pesticidas químicos. Ensaios futuros deverão investigar sua formulação, estabilidade, seletividade a inimigos naturais e eficácia contra *S. frugiperda* em condições de campo no Brasil, visando sua integração a programas de Manejo Integrado de Pragas (MIP).

Em síntese, esta tese sustenta o papel dos bioinsumos no manejo de lepidópteros-praga, mostrando caminhos práticos para aumentar a eficiência do controle biológico com

parasitoides de ovos e destacando o potencial de inseticidas botânicos como alternativas complementares. A incorporação desses resultados em programas de MIP pode reduzir a dependência de inseticidas químicos, mitigar impactos ambientais e favorecer uma agricultura mais sustentável e alinhada às políticas públicas de incentivo ao uso de bioinsumos.

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