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FUNGOS ENTOMOPATOGÊNICOS PODEM ALTERAR O COMPORTAMENTO ALIMENTAR DE *Euschistus heros* EM SOJA?

Autor: Guilherme Pereira de Oliveira
Orientador: Frederico Antonio Loureiro Soares

RIO VERDE - GO
março – 2026

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BANCA EXAMINADORA DE DEFESA DE TESE

Aos cinco dias do mês de março do ano de dois mil e vinte e seis, às 08:00h, reuniram-se os componentes da Banca Examinadora: sob a presidência do primeiro, Dr. Frederico Antônio Loureiro Soares (Presidente); Dr. Pablo da Costa Gontijo (Avaliador interno); Dr. Flávio Gonçalves de Jesus (Avaliador externo); Dra. Simone Graziele Moio Velozo (Avaliadora externa) e Dra. Heloiza Alves Boaventura (Avaliadora externa) em sessão pública realizada no IF Goiano – Campus Rio Verde, para procederem a avaliação da defesa de Tese, em nível de Doutorado, de autoria de **GUILHERME PEREIRA DE OLIVEIRA**, discente do Programa de Pós-Graduação em Ciências Agrárias – Agronomia do Instituto Federal Goiano – Campus Rio Verde. A sessão foi aberta pelo presidente da Banca Examinadora, Prof. Dr. Frederico Antônio Loureiro Soares (Presidente), que fez a apresentação formal dos membros da Banca. A palavra, a seguir, foi concedida a autora da Tese para, em 40 min., proceder à apresentação de seu trabalho. Terminada a apresentação, cada membro da banca arguiu a examinada, tendo-se adotado o sistema de diálogo sequencial. Terminada a fase de arguição, procedeu-se a avaliação da defesa. Tendo-se em vista as normas que regulamentam o Programa de Pós-Graduação em Ciências Agrárias – Agronomia, e procedidas às correções recomendadas, a Tese foi APROVADA, considerando-se integralmente cumprido este requisito para fins de obtenção do título de **DOUTOR EM CIÊNCIAS AGRÁRIAS-AGRONOMIA,** na área de concentração Produção Vegetal Sustentável no Cerrado, pelo Instituto Federal Goiano – Campus Rio Verde. A conclusão do curso dar-se-á quando da entrega na secretaria do PPGCA-AGRO da versão definitiva da Tese, com as devidas correções. Assim sendo, esta ata perderá a validade se não cumprida essa condição, em até **60** (sessenta) dias da sua ocorrência. A Banca Examinadora recomendou a publicação dos artigos científicos oriundos dessa Tese em periódicos de circulação nacional e/ou internacional, após procedida as modificações sugeridas. Cumpridas as formalidades da pauta, a presidência da mesa encerrou esta sessão de defesa de Tese de Doutorado, e para constar a presente Ata, que, após lida e achada conforme, será assinada pelos membros da Banca Examinadora.

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Ainda, expresso minha gratidão ao meu orientador, professor Frederico Antonio Loureiro Soares, aos coorientadores Anderson Rodrigo da Silva e André Cirilo de Sousa Almeida e aos demais coorientadores. A orientação e a dedicação de todos foram essenciais para a conclusão desta pesquisa.

BIOGRAFIA DO AUTOR

Guilherme Pereira de Oliveira, natural de Patos de Minas–MG, filho de Liliane Cristina Oliveira e Moacir Rogério Pereira.

Graduou-se em Biotecnologia pela Universidade Federal de Uberlândia (2011–2017).

Em 2018, iniciou no Mestrado em Biotecnologia na mesma instituição, concluído em 2020, com dissertação voltada ao uso de fungos do gênero *Trichoderma* no controle da podridão vermelha da cana-de-açúcar.

Em 2022, iniciou no Doutorado no Programa de Pós-Graduação em Ciências Agrárias – Agronomia, no Instituto Federal Goiano – Campus Rio Verde.

No âmbito profissional, iniciou sua carreira na Lallemand Plant Care Brasil, atuando como Estagiário, Analista de Pesquisa e posteriormente como Coordenador de Projetos.

Em 2024, assumiu a Gerência de Marketing de Produto na Nooa Ciência e Tecnologia Agrícola, sendo responsável pelo posicionamento estratégico e desenvolvimento de soluções biológicas.

Desde agosto de 2025, atua na Vitalforce como Gerente de Marketing e Produto, liderando as áreas de desenvolvimento de mercado, comunicação, inteligência de mercado, P&D e gestão de portfólio, com foco na construção de soluções sistêmicas e na consolidação da empresa como referência em bioinsumos de alto valor.

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Símbolo / Sigla	**Significado**	**Unidade**
%	Percentage	%
±	Plus or minus	-
√	Square root transformation	-
$\leq$	Less than or equal to	-
×	Multiplication factor	-
α	Significance level	-
$\alpha = 0.05$	Significance threshold adopted	-
AIC	Akaike Information Criterion	-
AC	Alternating Current	-
AC-DC EPG monitor	Electropenetrography recording equipment	-
CI	Confidence interval	-
conidia mL^{-1}	Concentration of fungal propagules	conidia/mL
cv.	Cultivar	-
DC	Direct Current	mV
df	Degrees of freedom	-
δ	Effect size (multiplicative factor)	-
Eh1	Stylet penetration waveform	-
Eh2	Xylem sap ingestion waveform	-
Eh3a	Seed disruption (laceration/maceration) waveform	-
Eh3b	Seed ingestion waveform	-
Eh4	Ingestion from unknown location waveform	-
EPG	Electropenetrography	-
et al.	And others	-
Fig.	Figure	-
FTLP	Final Time of the Last Probe	h
g	Gram	g
GAMLSS	Generalized Additive Models for Location, Scale and Shape	-
GLM	Generalized Linear Model	-
h	Hour	h
Hz	Sampling frequency	Hz

Símbolo / Sigla	Significado	Unidade
INFEST	Insect Feeding Behavior Statistics Software	-
IPM	Integrated Pest Management	-
log	Logarithmic transformation	-
logit	Logit link function	-
LRT	Likelihood Ratio Test	-
MASS	Statistical package for GLM and distributions	-
mL	Milliliter	mL
min	Minute	min
μ	Mean parameter	-
μL	Microliter	μL
Mean ($\bar{x}$)	Arithmetic mean	-
n	Sample size	-
nlme	Linear and nonlinear mixed-effects models package	-
N	Total number of events	-
NBSB	Neotropical Brown Stink Bug (*Euschistus heros*)	-
NWEi	Number of Waveform Events per Insect	events/insect
obs.	Observations	-
p	Probability value	-
$p < 0.05$	Statistical significance criterion	-
PDA	Potato Dextrose Agar	-
Pr( )	Probability function	-
R	Statistical computing environment	-
RH	Relative humidity	%
RMSE	Root Mean Square Error	-
R5.5	Soybean reproductive stage (pod filling)	-
s	Second	s
SE	Standard error	-
sp.	Species (singular)	-
spp.	Species (plural)	-
σ	Dispersion parameter	-
T	Total recording time	h
Tab.	Table	-
Tukey	Tukey’s multiple comparison test	-
Tween 80	Polysorbate 80 surfactant	% (v/v)
v/v	Volume/volume ratio	%
vs.	Versus	-
WDEi	Waveform Duration per Event per Insect	min
y	Vector of waveform events	-
Z	Non-probing waveform	-
°C	Temperature	°C
mV	Electrical potential	mV

RESUMO

OLIVEIRA, GUILHERME PEREIRA DE. Instituto Federal Goiano – Campus Rio Verde– GO, março de 2026. **Fungos entomopatogênicos podem alterar o comportamento alimentar de *Euschistus heros* em soja?** Orientador: Frederico Antonio Loureiro Soares. Coorientadores(as): Anderson Rodrigo da Silva, Andre Cirilo de Sousa Almeida, Juliana de Fatima Sales e Pablo da Costa Gontijo.

O percevejo-marrom *Euschistus heros* é uma das principais pragas da cultura da soja, causando perdas significativas por meio da alimentação em vagens e sementes. O entendimento do comportamento alimentar desse inseto é fundamental para o desenvolvimento de estratégias de manejo mais eficientes. Nesse contexto, fungos entomopatogênicos têm sido amplamente estudados como agentes de controle, porém seus efeitos sobre o comportamento alimentar ainda são pouco compreendidos. Esta tese teve como objetivo avaliar se fungos entomopatogênicos são capazes de alterar o comportamento alimentar de *E. heros* em soja. Para isso, foram conduzidos três estudos complementares, integrando modelagem estatística, análise comportamental por eletropenetrografia (EPG) e avaliação de dano em sementes. O primeiro estudo abordou a modelagem de dados de EPG, caracterizados por contagens correlacionadas e sobredispersas. O modelo multinomial apresentou melhor desempenho, com controle da taxa de erro tipo I próximo ao nível nominal ($\alpha = 0{,}05$) e maior poder estatístico ($> 0{,}80$), enquanto o modelo de Poisson apresentou taxas infladas de erro tipo I ($> 0{,}40$), indicando inadequação para esse tipo de dado. O segundo estudo avaliou o comportamento alimentar de *E. heros* exposto a *Cordyceps javanica* e *Metarhizium anisopliae*. Foram observadas diferenças significativas ($p < 0{,}05$) em variáveis de EPG, incluindo redução na frequência de eventos de penetração do estilete (Eh1) e ingestão (Eh2 e Eh3b), aumento do tempo em não alimentação (Z) e redução do tempo final da última prova (FTLP), que passou de 44,09 ± 4,13 h no controle para aproximadamente 31 h nos tratamentos com fungos. O terceiro estudo relacionou essas alterações comportamentais ao dano em sementes. A análise por raios X evidenciou redução significativa ($p < 0{,}05$) do dano interno, de 65,5% no controle para 27,8% em *Metarhizium anisopliae* e 34,4% em *Cordyceps javanica*. De forma integrada, os resultados demonstram que fungos entomopatogênicos alteram o comportamento alimentar de *Euschistus heros*, reduzindo a frequência, duração e continuidade dos eventos de alimentação. Como contribuição científica, esta tese demonstra, de forma consistente e quantitativa, que fungos entomopatogênicos interferem diretamente no comportamento alimentar de insetos-praga, ampliando a compreensão dos seus efeitos além da mortalidade.

PALAVRAS-CHAVE: Eletropenetrografia; imagens termais; modelagem de dados e raios X.

ABSTRACT

OLIVEIRA, GUILHERME PEREIRA DE. Instituto Federal Goiano – Campus Rio Verde– GO, março de 2026. **Can entomopathogenic fungi alter the feeding behavior of *Euschistus heros* in soybean?** Advisor: Frederico Antonio Loureiro Soares. Co-advisors: Anderson Rodrigo da Silva, Andre Cirilo de Sousa Almeida, Juliana de Fatima Sales e Pablo da Costa Gontijo.

The Neotropical brown stink bug, *Euschistus heros*, is one of the main pests of soybean, causing significant losses through feeding on pods and seeds. Understanding its feeding behavior is essential to develop effective pest management strategies. Although entomopathogenic fungi have been widely studied as biological control agents, their effects on insect feeding behavior remain poorly understood. This thesis aims to evaluate whether entomopathogenic fungi can alter the feeding behavior of *E. heros* in soybean. To address this question, three complementary studies were carried out, integrating statistical modeling, behavioral analysis using electropenetrography (EPG), and seed damage assessment. The **first study** focused on the statistical modeling of EPG data, which are characterized by correlated and overdispersed count variables. A multinomial-based approach showed superior performance, with appropriate control of type-I error rates (≈ 0.05) and higher statistical power (> 0.80), whereas conventional models such as Poisson exhibited inflated error rates (> 0.40). The **second study** evaluated the feeding behavior of *E. heros* exposed to *Cordyceps javanica* and *Metarhizium anisopliae* using EPG. Significant changes ($p < 0.05$) were observed, including reductions in stylet penetration (Eh1) and ingestion events (Eh2 and Eh3b), increased non-feeding time (Z), and a reduction in the final time of the last probe (FTLP), from 44.09 ± 4.13 h in the control to approximately 31 h in fungal treatments. The **third study** linked these behavioral changes to seed damage. X-ray analysis revealed a significant reduction in internal seed damage ($p < 0.05$), from 65.5% in the control to 27.8% for *M. anisopliae* and 34.4% for *C. javanica*. Overall, the results demonstrate that entomopathogenic fungi can alter the feeding behavior of *Euschistus heros* in soybean by reducing the frequency, duration, and continuity of feeding events.

KEYWORDS: Data modeling, electropenetrography, thermal imaging and X-ray imaging.

1. INTRODUÇÃO

A intensificação dos sistemas agrícolas nas últimas décadas tem sido acompanhada pelo aumento da complexidade fitossanitária, impulsionada por monocultivos extensivos, sucessão contínua de culturas e pressão seletiva decorrente do uso recorrente de inseticidas sintéticos(Chen *et al.*, 2021, 2023). No Brasil, maior produtor mundial de soja(USDA - United States Department of Agriculture, 2024), a sustentabilidade do sistema produtivo depende da capacidade de manejar pragas-chave de forma eficaz e ambientalmente responsável.

Entre as principais pragas da soja destaca-se o percevejo-marrom *Euschistus heros* (Hemiptera: Pentatomidae), cuja importância econômica tem aumentado em função da expansão territorial da cultura e da adaptação do inseto a diferentes sistemas produtivos (Ecco *et al.,* 2020; Tibola *et al.,* 2021). A espécie apresenta hábito alimentar sugador e atua principalmente na fase reprodutiva, provocando danos internos às sementes por meio da inserção do estilete e injeção de saliva enzimática, resultando em redução de peso, qualidade e viabilidade das sementes(Pinto *et al.*, 2009; Rohr *et al.*, 2023).

O manejo predominante de *E. heros* baseia-se na aplicação de inseticidas químicos, estratégia que, embora eficiente em curto prazo, tem favorecido a evolução de resistência populacional em diferentes regiões produtoras (Tibola *et al.,* 2021). Estudos recentes indicam que a resistência a múltiplos modos de ação representa um dos principais desafios globais no controle de insetos-praga (Wang *et al.*, 2021), reforçando a necessidade de integração de estratégias biológicas no contexto do Manejo Integrado de Pragas (MIP).

Nesse cenário, os fungos entomopatogênicos têm recebido crescente atenção como alternativas sustentáveis para o controle de insetos-praga (Maluta; Castro; Spotti Lopes, 2023; Resquín-Romero *et al.*, 2020). Espécies como *Metarhizium anisopliae* e

Cordyceps javanica apresentam elevada capacidade de infectar insetos por contato direto, promovendo alterações fisiológicas progressivas que culminam na morte do hospedeiro (Battisti *et al*., 2022). Contudo, a ação desses agentes é caracterizada por progressão temporal distinta dos inseticidas neurotóxicos químicos, e demanda critérios avaliativos que transcendam a mortalidade imediata.

A literatura recente tem destacado a importância dos efeitos subletais na avaliação de agentes de controle biológico (Maluta; Castro; Spotti Lopes, 2023; Porras *et al*., 2021). Alterações comportamentais, fisiológicas e reprodutivas podem reduzir o impacto agronômico mesmo quando a mortalidade não ocorre de forma imediata (De Oliveira *et al*., 2025). Em insetos sugadores, cujo dano está diretamente relacionado à intensidade e duração da alimentação, a modificação do comportamento alimentar constitui um ponto biologicamente relevante (Backus *et al*., 2019; Ebert; Backus; Rogers, 2018).

A eletropenetrografia (EPG) consolidou-se como ferramenta essencial para a análise detalhada do comportamento alimentar de insetos com aparelho bucal picador-sugador, permitindo quantificar eventos de penetração de estilete, ingestão e interrupção alimentar (Backus *et al*., 2019; Backus; Lin; Chang, 2015). Estudos recentes demonstraram que fungos entomopatogênicos podem reduzir significativamente a frequência e duração de sondagens alimentares em *E. heros*, evidenciando comprometimento funcional do inseto antes da morte (Almeida *et al*., 2025; De Oliveira *et al.,* 2025).

Sob a perspectiva fisiológica, a interação patógeno-hospedeiro envolve alterações metabólicas detectáveis por mudanças térmicas e comportamentais (Santos, 2023). Imagens termais têm sido empregadas como ferramentas não invasivas para monitoramento de estresse fisiológico em insetos, permitindo inferir alterações metabólicas associadas à infecção (Raju Ahmed *et al*., 2020). Evidências recentes indicam que percevejos tratados com fungos entomopatogênicos apresentam padrões térmicos compatíveis com distúrbios fisiológicos induzidos pela infecção (De Oliveira *et al*., 2026). Paralelamente, técnicas de imagem como raios X têm sido amplamente utilizadas para avaliação não destrutiva de danos internos em sementes. A integração dessas técnicas pode auxiliar na compreensão de forma mais abrangente dos mecanismos pelos quais fungos entomopatogênicos afetam o inseto (De Oliveira *et al.,* 2026; Rohr *et al*., 2023).

Apesar dos avanços recentes, a literatura ainda carece de modelo integrativo que relacione infecção fúngica, resposta fisiológica, modificação comportamental e redução efetiva do dano agronômico. Estudos tendem a abordar esses componentes de forma isolada (Backus; Lin; Chang, 2015; Ebert; Backus; Rogers, 2018; Filho *et al.*, 2022a; Tessmer *et al.*, 2022). Assim, a eficácia do controle não deve ser avaliada apenas pela mortalidade do inseto, mas, sobretudo, pela redução efetiva do dano à cultura. Essa perspectiva funcional está alinhada aos princípios atuais do MIP, que priorizam a proteção da produtividade e a sustentabilidade do sistema produtivo.

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2. OBJETIVOS

- Desenvolver uma abordagem estatística adequada para análise de dados de eletropenetrografia (EPG), considerando contagens correlacionadas e sobredispersas;
- Comparar o desempenho de modelos estatísticos quanto ao ajuste, taxa de erro tipo I e poder estatístico;
- Avaliar o efeito de *Cordyceps javanica* e *Metarhizium anisopliae* sobre o comportamento alimentar de *Euschistus heros* em soja;
- Quantificar alterações na frequência, duração e proporção dos eventos de alimentação e não alimentação (Z, Eh1, Eh2 e Eh3b);
- Determinar o efeito dos tratamentos sobre o tempo final da última prova (FTLP) como indicador da duração da atividade alimentar;
- Avaliar o dano interno em sementes de soja causado por *Euschistus heros* por meio de análise por raios X;
- Relacionar as alterações no comportamento alimentar com o dano em sementes de soja.

3. CAPÍTULO I

(Normas de acordo com a revista Acta Scientiarum.Agronomy)
(https://periodicos.uem.br/ojs/index.php/ActaSciAgron/article/view/71591)

Alternatives for simulating and modeling simplified insect feeding eletropenetrography discrete data

ABSTRACT

The study of insect feeding behavior using electropenetrography (EPG) typically involves analyzing complex data. EPG data comprises a temporal sequence of behaviors summarized using a collection of counts, durations, and sequential variables. These variables can be counts, means, percentages, or linear combinations of behaviors. This results in numerous variables being correlated to a certain degree. Consequently, statistical analysis is rendered complex, particularly in terms of model fitting and selection. This study proposed a statistical approach to simulate overdispersed correlated count data based on a previous comparative experiment to monitor the feeding behavior of untreated *Euschistus heros* versus *E. heros* treated with an entomopathogen. The waveforms included non-feeding (Z), pathway (Eh1), laceration/maceration of endosperm tissue (Eh3a), short ingestion events of lacerated/macerated endosperm tissue (Eh3b), xylem sap ingestion (Eh2), and ingestion from an unknown location (Eh4). Simulated scenarios involved the creation of differences between groups of insects based on the total number of events or in the proportion of Z events. Several statistical models were then fitted to the simulated data and evaluated based on goodness-of-fit, type-I error rate, and power analysis. The multinomial model exhibited the lowest type-I error rate and was more sensitive in detecting higher (>1.35x) differences between groups. Only the multinomial model achieved power greater than 0.8. Conversely, models such as the Poisson and normal models exhibited limitations such as inflated type-I error rates in the presence of overdispersion. Among the univariate models, the mixed model exhibited the best fit.

Keywords: EPG; multinomial model; double-poisson.

3.1 INTRODUCTION

Several pests affect soybeans, among them the neotropical brown stink bug (Euschistus heros (Fabricius), Hemiptera: Pentatomidae) has garnered attention because of its high losses (Sosa-Gómez et al., 2020). The feeding damage caused by sucking insects on pods can result in losses exceeding 30% (Antúnez et al., 2022). There are numerous challenges to understanding the biology of sucking insects, and elucidating this information will enable the development of more efficient management tools.

In sucking insects, the observance of feeding activities with the naked eye is challenging because they occur inside the opaque host tissue. This makes the identification of initial symptoms difficult. Through a technology called electropenetrography (EPG) (Mclean & Kinsey, 1964), detailed insights into the feeding behavior of this group of insects can be obtained by analyzing voltage pattern data from an electrical circuit that includes both insects and plants (Backus et al., 2019).

In experiments with E. heros using EPG, we can monitor and characterize EPG waveforms, to determine specific feeding sites, and ascertain the biological significance of waveforms based on their electrical characteristics and histological correlations (Lucini & Panizzi, 2018). However, although these feeding events are intrinsically related, they are generally treated separately. This facilitates the exploitation or underutilization of data.

The EPG generates a large amount of data with various types of variables of duration and number of waveform events. It is common for these data to have excess zeros, which renders statistical analysis complex, particularly in terms of model fitting and the selection of the most important variables (Ying et al., 2021; Hu et al., 2020; Lucini & Panizzi, 2017). Several programs are used to read the data generated by EPG, such as Backus 1.0 (Backus et al., 2007) EPG-Calc (Giordanengo, 2014), Sarria Workbook

(Sarria et al., 2009), Ebert 1.0 (Ebert et al., 2015), and INFEST (Silva et al., 2022). The data read by these programs can be used to build statistical models to elucidate issues related to pest biology (Ying et al., 2021; Hu et al., 2020 and Lucini & Panizzi, 2017).

There are numerous approaches to evaluating this large volume of data, ranging from simpler analyses, such as linear models and their transformations, to complex models, such as generalized linear models (GLM), generalized additive models for location, scale, and shape (GAMLSS), and even multinomial models (Freitas & Duarte, 2023; Schmidt et al., 2022; Rigby & Stasinopoulos, 2005). To assess the accuracy of these models, the Akaike Information Criterion (AIC), power analysis, and type-I error rate must be examined (Sakamoto et al., 1986).

One method to evaluate the model performance and importance of variables involves data simulation based on sets of rules or probabilistic distributions observed in previous experiments (Tyralis & Papacharalampous, 2024). However, studies involving simulations of EPG data are scarce. This may be because of the nature of the raw data generated by the equipment, which comprises recordings with voltage information (Backus & Shih, 2020). Consequently, the identification and classification of voltage patterns in specific types of waves are time-consuming, and the process requires a highly trained and skilled user. Moreover, variables generated by EPG generally exhibit a correlation, which must be considered in the simulation process.

Thus, this study aimed to simulate and model discrete feeding EPG data for Euschistus heros and compare these models based on goodness-of-fit, type-I error rate, and power analysis.

3.2 MATERIAL AND METHODS

3.2.1 Simulation strategy and scenarios

The simulation scenarios were based on an EPG study (Rodrigues, 2023) to monitor the behavior of Euschistus heros (Fabricius) (Hemiptera: Heteroptera) feeding on soybean pods in two groups of 16 insects each (control and treatment: application of the entomopathogenic fungus Metarhizium anisopliae). The recordings were completed after 72 h. The following waveform events were recorded: Z (non-feeding), Eh1 (pathway), Eh3a (laceration/maceration of the endosperm), Eh3b (short ingestion event of lacerated/macerated endosperm tissue), Eh2 (xylem sap ingestion), and Eh4 (ingestion from an unknown location). From the experimental data on the number of waveform events by insects, 10,000 data simulations were performed.

Let y = [y1 y2 … yk] represent the k-dimensional vector of the number of events per waveform by an insect (NWEi), which is mutually exclusive. Let N = $\sum_{k=1}^{K} y_k$ be the total number of insect events during the entire recording period. As previously reported (Terza & Wilson, 1990; Schmidt et al., 2022), we considered the conditional probability distribution of y given N as

y|N~Multinomial(N,π)

where: yk = 0, 1, 2,…,∞; and is π = [π1 π2 … πk] the k-dimensional vector of parameters representing the multinomial probabilities, with $\sum_{k=1}^{K} \pi_k$ = 1.

Because N is not expected to be homogeneous across insects, we considered it a random variable that could be modelled by a discrete probability model, say, h(.),

N~h(μ,σ)

where: μ represents the mean and σ the dispersion parameter; N = 0, 1, 2,…,∞. Here, a natural choice for h(.) is Poisson(μ,σ=1). Because N can be affected by factors that may vary among insects, such as recording time and treatment, it may be overdispersed. Thus, its observed variability is greater than that captured by parametric models such as Poisson. Alternatively, examples of models that can accommodate overdispersion are Double-Poisson(μ,σ) and Gamma-Poisson(μ,σ), because both have σ > 0. We computed the estimates of the maximum likelihood (EMV) of μ and σ and used Akaike's information criterion (AIC) to select the best-fitting model.

From the joint distribution of y and N, we obtained:

Pr(y,N | μ,σ,π) =Pr(y | N,π) Pr(N |μ,σ)

We simulated 10,000 data matrices Y with dimensions 32×16 (n insects × K waveforms) under the null hypothesis of no difference between experimental groups, that is, H0: θ1= θ2; where θ: {μ,σ,π}. This was done to evaluate the type-I error rate of statistical tests as one criterion to evaluate and select regression models. The next section describes this calculation and that of the power analysis.

3.2.2 Regression models, type-I error rate and power analysis

After simulating Y, the univariate regression models were fitted to the selected response variable y_1, corresponding to waveform Z (no feeding activity), as presented in Table 1.

Table 1. Regression models.

Model	Type*	Mean/Linear Predictor**	Dispersion***
Normal	Linear	$\mu i=\beta 0+\tau i$	σ
Normal with sqrt transformation	Linear	$\sqrt{}\mu i =\beta 0+\tau i$	σ
Normal with log transformation	Linear	$\log(\mu i)=\beta 0+\tau i$	σ
Poisson	GLM	$\log(\mu i)=\beta 0+\tau i$	--
Negative Binomial type II	GLM	$\log(\mu i)=\beta 0+\tau i$	σ
Gamma	GLM	$\mu i)-1=\beta 0+\tau i$	σ
Gamma-Poisson	GAMLSS	$\log(\mu i)=\beta 0+\tau i$	$\log(\sigma i) = \alpha 0$
Poisson-inverse Gaussian	GAMLSS	$\log(\mu i)=\beta 0+\tau i$	$\log(\sigma i) = \alpha 0$
Mixed effects	Linear	$\mu i=\beta 0+\tau i$	$\sigma\tau + \sigma$
Mixed effects with heteroscedasticity	Linear	$\mu i=\beta 0+\tau i$	$\sigma 1,\sigma 2,\sigma$

*GLM: generalized linear model; GAMLSS: generalized additive model for location, scale, and shape. **μ_i: expected mean of Group i (i = 1, 2) for the response y_1, β_0: intercept; τ_i: effect of Group i. ***α_0: intercept for the dispersion parameter σ.

After fitting the regression models, type-I error rate was calculated as the proportion of the p-values of the F-test or likelihood ratio test (LRT) (depending on the regression model) for the group factor that was lower than the nominal significance level, $\alpha=0.05$.

A power analysis was performed by simulating the data matrices Y under the alternative hypothesis $Ha:\theta \neq \theta 2$, employing 2 methods.

1) A multiplicative effect size δ was applied to the mean of Group 2, $\mu 2=\delta\hat{\mu}$, where $\hat{\mu}$ is the maximum likelihood estimate (MLE) of the general mean of the total number of events by insect; y1 (waveform Z) was selected as response variable. The following values of effect size were used: $\delta = \{0.2, 0.3, 0.5, 0.7, 0.8, 0.9, 1.1, 1.2, 1.3, 1.5, \text{and } 1.8\}$. The proportion of p-values of the F-test or LRT lower than $\alpha=0.05$ was considered as the power.

2) A multiplicative effect size δ was applied to the probability of y1 (waveform Z) of Group 2, that is, $\pi 12=\delta\hat{\pi}1$; where $\hat{\pi}1$ is the MLE of the general probability of y1. To maintain the constraint $\sum_{k=1}^{K}\pi_k = 1$ of the multinomial model, we subtracted the value $(\pi 12 -\hat{\pi}1)/(K-1)$ from all the other probabilities ($k\neq 1$) uniformly. The following values of effect size were used: $\delta = \{1.1, 1.25, 1.5, 1.75, \text{and } 2.0\}$. The proportion of p-values of the F-test or LRT lower than $\alpha = 0.05$ was considered as the power.

In addition to the regression models described in Table 1, the multinomial regression model was also used. Consequently, the type-I error rate and power analysis (multiplicative effect size δ to the probability of (waveform Z) of Group 2) were calculated. The multinomial regression model is a generalization of the logistic y1 regression model wherein the mean is modelled by the linear predictor using the following equation:

$$\mathrm{logit}(\mu i)=\beta 0+\tau i$$

where: μi represents the vector of proportions of waveform events for the Group i.

3.2.3 Computing

EPG recording files were processed using INFEST® (Silva et al., 2022). Statistical analysis and computing were performed in R (R Core Team, 2023), using the packages MASS, nnet (Venables & Ripley, 2002), gamlss (Rigby & Stasinopoulos, 2005), nlme (Pinheiro et al., 2021), and extraDistr (Wolodzko, 2020).

3.3 RESULTS

Table 2 lists the total number of events and waveform proportions from the experimental data used in the simulations. The experimental groups showed large numerical differences in the number of waveform events, such as Eh3b (74 vs. 218) and Eh2 (16 vs. 95). Moreover, a low frequency was observed in Eh4 for Group 1, which corresponded to one or fewer events per insect. This resulted in zeros in the data to be subjected to statistical analysis. In contrast, waveforms such as Eh3a exhibited a considerably higher number of events.

Table 2. Total number of waveform events (N) and proportions from the data used for simulations.

			Waveforms					
Group	#Insects		Z	Eh1	Eh3a	Eh3b	Eh2	Eh4
1	16	N	201	206	239	74	16	15
		Proportion	0.2676	0.2743	0.3182	0.0985	0.0213	0.0200
2	16	N	255	306	424	218	95	44
		Proportion	0.1900	0.2280	0.3159	0.1624	0.0708	0.0328

Z = non-feeding, Eh1 = stylet penetration, Eh3a = seed disruption, Eh3b = ingestion from seeds, Eh2 = xylem sap ingestion, and Eh4 = ingestion from unknown location.

The total number of activities per insect (N) was over dispersed, and distribution models with a dispersion parameter greater than one presented a better fit (Figure 1). The three models correctly estimated a general mean of approximately 65.5 events; however, the Poisson model underestimated the data dispersion. The double-Poisson estimate for the dispersion parameter was 29.67, with a slightly better fit (lower AIC) than that of the Gamma–Poisson, whose dispersion estimate was 47.31. Thus, a double-Poisson model was used to simulate the data using the proposed approach based on a multinomial distribution.

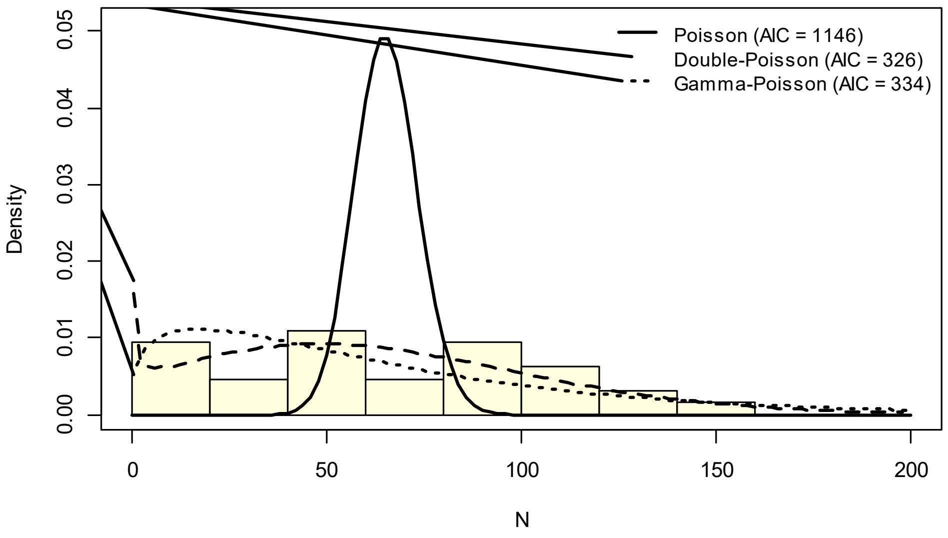

Figure 1. Goodness-of-fit of the probability distribution models to the total number of activities per insect (N).

To check whether the simulation strategy could maintain the correlation structure among the waveforms, it was calculated a coincidence index between each resulting correlation matrix from the simulated data and that from the original dataset. The index is based on the average of the absolute differences between the correlation values. We obtained index values ranging as 0.65–0.81, with a mean coincidence of 0.73.

The type-I error rate for 10,000 simulations is shown in the level plot (Figure 2). It was calculated for all fitted regression models, including the multinomial model, which considered all waveform events simultaneously.

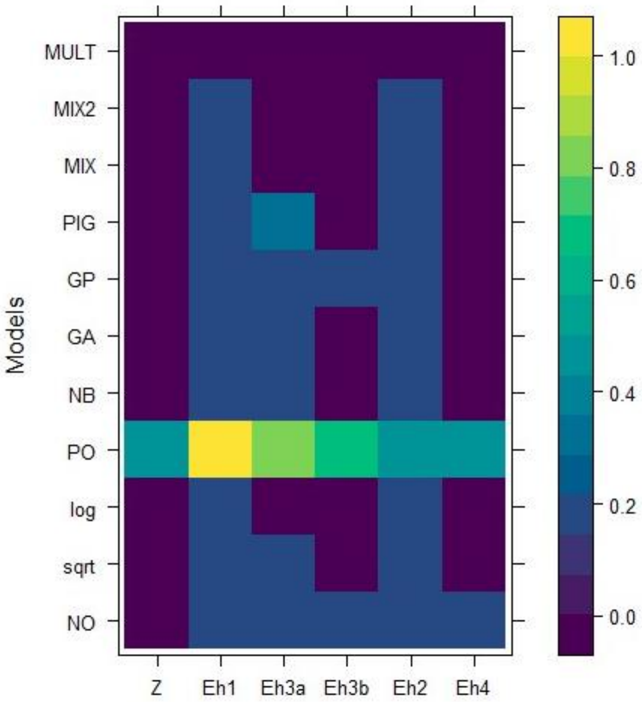

Figure 2. Type-I error rate of likelihood ratio tests (α = 0.05) performed on regression models fitted to simulate data on the number of events per waveform by insect. NO = normal, sqtr = normal with sqrt transformation, log = normal with log transformation, PO = Poisson, NB = negative binomial type II, GA = Gamma, GP = Gamma–Poisson, PIG = Poisson-inverse gaussian, MIX = mixed and MIX2 = Mixed with heteroscedasticity. Z = non-feeding, Eh1 = stylet penetration, Eh3a = seed disruption, Eh3b = ingestion from seeds, Eh2 = xylem sap ingestion, and Eh4 = ingestion from unknown location.

The type-I error rate must be measured because it is undesirable for a test to reject a true hypothesis. The multinomial model had the lowest type-I error rate. In general, the other models identified non-existent differences that did not exist, primarily for the waveforms Eh1, Eh3a, and Eh2. The Poisson model exhibited the highest type-I error rate for all waveforms (above 0.4). Except for waveform Z, the Normal model exhibited a type-I error exceeding 0.2.

The power of tests and the root-mean-squared-error (RMSE) are shown in Figure 3, which is based on the effects size of μ and the mean number of total activities by insect. Figure 4 shows the power based on the effect sizes of the probability of the waveform Z.

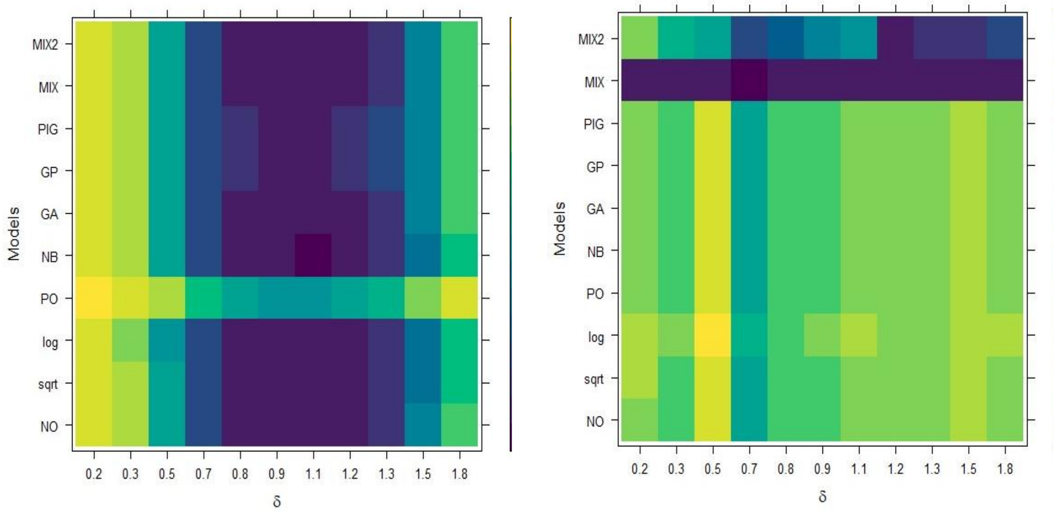

Figure 3. Power analysis (left side) and RMSE (right) based on the effect size of μ and the general mean of the total number of events by insect. NO = normal, sqtr = normal with sqrt transformation, log = normal with log transformation, PO = Poisson, NB = negative binomial type II, GA = Gamma, GP = Gamma–Poisson, PIG = Poisson-inverse gaussian, MIX = mixed, MIX2 = Mixed with heteroscedasticity, and RMSE = root mean-square error.

Power analysis can quantify the extent to which the model can detect statistical differences, and RMSE measures the accuracy of the model. Through the application of a multiplicative effect size δ to the mean of Group 2, the Poisson model could detect differences between treatments with the highest power. The Gamma–Poisson and Poisson-inverse Gaussian models exhibited higher power for small differences (30% and 20%, respectively). In general, the models identified differences with at least 80% power from 70% difference or greater. Between the two transformations, the log transformation performed slightly better.

The mixed (MIX) model was the most accurate, with RMSE below 1.5, regardless of the effect size of μ. This was followed by the mixed model with heteroscedasticity (MIX2). The other models exhibited similar values.

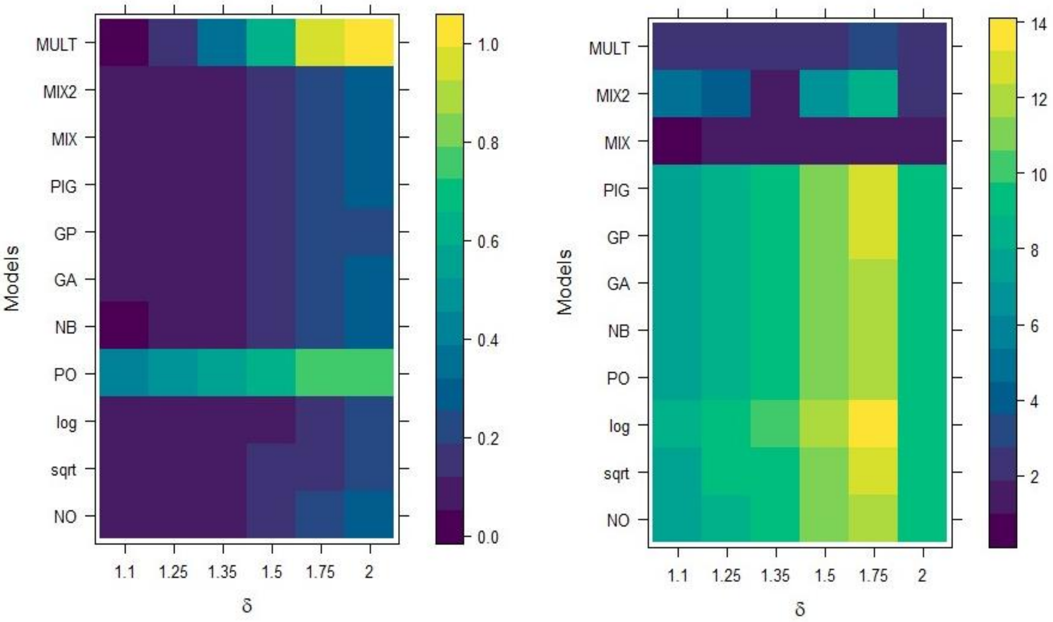

Figure 4. Power analysis (left side) and RMSE (right) based on the effect size δ of the probability of the waveform Z. NO = normal, sqtr = normal with sqrt transformation, log = normal with log transformation, PO = Poisson, NB = negative binomial type II, GA = Gamma, GP = Gamma–Poisson, PIG = Poisson-inverse gaussian, MIX = mixed, MIX2 = Mixed with heteroscedasticity and MULT = multinomial, and RMSE = root mean-square error.

Through the application of a multiplicative effect size δ to the probability of waveform Z for Group 2 in simulations, the Poisson model was observed to be the most sensitive in terms of detecting small differences between Groups. In contrast, the multinomial model was more sensitive in detecting higher (>1.35 ×) differences between the groups. Only the multinomial model achieved 80% power. The multinomial and the mixed models (MIX) exhibited the lowest RMSE regardless of the difference in effect size δ applied to the probability of waveform Z.

3.4DISCUSSION

Overdispersion, as observed in Figure 1, is common in count data, such as EPG experiments (Coly et al. 2016). This is attributable to several factors, such as excess of zeros (Avci et al., 2015). Excess zeros may be caused by a treatment effect, such as pesticide application, or may be a result of insufficient recording time. In the E. heros data, Group 1 presented only 15 events of waveform Eh4 throughout the recording (Table 3), which indicated the presence of zeros; although the way that they were distributed in the data was not indicated.

There are many specific models for overdispersion that can be divided into two classes: (i) models that assume more general forms for the variance function, possibly including additional parameters, such as the binomial model, and (ii) models wherein the response model parameter itself has certain distribution, such as the negative binomial model (Hinde & Demétrio, 1998).

Several statistical models were fitted and evaluated based on goodness-of-fit, type-I error rate, and power analysis. Models used for continuous data were also applied, such as normal data, because there may be asymptotic normal approximations from the count data. Moreover, transformations were evaluated. This is because data that not satisfy the assumption of normality is common and prevents the use of classical regression models (Silva et al., 2019).

The multinomial model exhibits the lowest type-I error rate. Furthermore, it facilitates the analysis of complex and interrelated relationships between waveforms (El-habil 2012; Schmidt et al., 2022). For the power analysis based on the effect size δ of the probability of the waveform Z, the model was the best for detecting differences between groups greater than 1.35x. Nonetheless, no published studies were found in the literature involving the use of the multinomial model to analyze EPG data.

Poisson regression is commonly used to count data from the EPG, as performed by Almeida et al. (2025), to analyze the feeding behavior of Euschistus heros treated with Metarhizium anisopliae. However, in our study, the Poisson model presented high type-I error rates (above 0.5) for most response variables. This is probably owing to the model's assumption of equidispersion when the variance is equal to the mean, which is a strong limitation (Freitas & Duarte, 2023) to EPG data. In modeling, inappropriate assumptions may result in invalid hypothesis tests (Gourieroux et al., 1984). Thus, the higher power observed with Poisson's law was likely associated with a higher type-I error rate. For data that present overdispersion, models such as negative binomial or Gamma–Poisson are typically a better choice (Hausman et al., 1984).

The Gamma–Poisson model presented type-I error rates of approximately 0.2 for most variables, which is smaller than the Poisson model. The combination of Gamma and Poisson distributions facilitated the highest flexibility as it did not assume equidispersion, resulting in a lower type-I error rate for overdispersed data (Freitas & Duarte, 2023). Greenwood and Yule (1920) considered that the number of events of the response variable followed a Poisson distribution, with parameter λ that varied according to a Gamma distribution with parameters α and β. Thus, it is considered that the conditional $Y \mid \lambda \sim \text{Poisson}(\lambda)$, and the parameter λ itself followed a Gamma distribution $\lambda \sim \text{Gamma}(\alpha, \beta)$.

The PIG model is also a derivative of the Poisson distribution proposed by Holla (1967) as an alternative to the Poisson distribution for cases with overdispersion; therefore, lower type-I error rates compared to the Poisson model were expected. Furthermore, the PIG distribution has been considered a better alternative than the Gamma–Poisson distribution to model data with long-tail overdispersion (Putri et al., 2020). In terms of general performance, that is, considering the type-I error, power of tests, and goodness-of-fit, PIG and Gamma–Poisson exhibited similar results, representing good alternatives to model the EPG count data.

The classical normal model is most used to analyze EPG data. However, in our study, the model exhibited high type-I error rates (approximately 0.2). This type of distribution is often used in works involving continuous data and counts (Krithikadatta, 2014), including EPG data (Wayadande et al., 2020; Guedes et al., 2018). For EPG data, particularly from experiments with pesticides, in case of early stops in insect feeding activities, or in cases of different recording times, certain response variables are likely to be heteroscedastic in terms of the experimental factor levels. Thus, the assumption of homoscedasticity is strong and the unique estimate of variance used to test for significant differences may be underestimated, thereby increasing the type-I error rate. In certain situations, the insecticide effect of a treatment can prevent the insects from performing certain feeding activities that the untreated insects usually do. This causes certain response variables to be zero-inflated or, more generically, overdispersed. The classical normal model cannot incorporate this, thereby capturing only part of the observed variability. This renders the related statistical tests more susceptible to type-I error.

In certain studies the count data was assumed to follow a normal distribution, and the t-test was applied to compare treatments (Ebert et al., 2018; Tariq et al., 2017). In our study, data transformation, particularly the logarithm, reduced the type-I error rate close to the nominal level (0.05) for the variables Eh3a, Eh3b, and Eh4.

Thus, probably because of their flexibility in capturing different data structures (Harrison, 2014; Dixon, 2016; Giesselmann & Schmidt-Catran, 2020), mixed models exhibited the lowest RMSE values and relatively low type-I error rates.

3.5 CONCLUSION

This study proposed a comprehensive approach for simulating correlated overdispersed count EPG data and conducted a comparative examination of statistical models. The multinomial model emerged as a robust choice, presenting low values of root-mean-square error, excelling in controlling the type-I error rate, and exhibiting the highest power for the detection of simulated differences between the means. Conversely, the Poisson model and classical normal distribution exhibited inflated type-I error rates in the presence of overdispersion, leading to erroneous conclusions. Among univariate models, the mixed model exhibited the best fit.

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4. CAPÍTULO II

(Normas de acordo com a revista Scientific Reports)
(https://www.nature.com/articles/s41598-025-31096-w)

Entomopathogenic fungi disrupts the feeding behavior of *Euschistus heros* in soybeans

ABSTRACT

This study evaluated the effects of the entomopathogenic fungi on the feeding behavior of the Neotropical brown stink bug, *Euschistus heros*, using electropenetrography (AC-DC) technology. Twenty female insects per treatment were reared under controlled conditions and exposed to soybean pods treated with: fungal suspensions (2 µl on the pronotum with 5×10^6 and 6.15×10^8 conidia mL^{-1} for *Cordyceps javanica* and *Metarhizium anisopliae*, respectively), chemical insecticide (Thiamethoxam + Lambda-cyhalothrin, 0.025/100 mL), and aqueous solution of Polysorbate 80 (0.01% v/v) (control). Response variables associated with count and duration of feeding behavior events over 72 hours of recording were modelled using GAMLSS (generalized additive models for location, scale, and shape) to assess the statistical significance of treatments and for pairwise comparisons of means ($p < 0.05$). The fungal treatments and chemical insecticide significantly reduced the frequency and duration of feeding events, especially stylet penetration and seed ingestion, compared to untreated controls. Additionally, insects treated with fungi spent more time in non-feeding behavior, indicating disrupted feeding behavior. Both fungi also shortened the duration of the final feeding probe by about 13 hours. Furthermore, electropenetrography enables assessment of pest–plant interactions and biocontrol efficacy beyond mortality.

Key words: Biological Control, *Cordyceps javanica*, *Metarhizium anisopliae*, Electropenetrography, Integrated Pest Management.

4.1 INTRODUCTION

The Neotropical brown stink bug (NBSB), *Euschistus heros* (Fabricius, 1798) (Hemiptera: Pentatomidae), is an important pest in South America, that cause serious damage to crops, such as soybeans [*Glycine max* (L.) Merr.] and maize (*Zea mays* L.)[1]. Egg-adult development can vary from 25 to 32 days (5 to 7 days in the egg stage and 20 to 25 days in the nymphal stage), and the adult phase can vary from 75 to 116 days[1].

E. heros begins the infestation of soybean plants in the vegetative phase and is harmful in the reproductive phase as a pod feeder. Their feeding can have direct effects on yield and/or other parameters related to grain quality during pod development and seed filling, when the stylet penetrates the plant tissues, injects saliva, and ruptures cells[2,3,4,5]. Furthermore, the punctures left by the stylets can facilitate infection by pathogenic microorganisms[6].

The most common method used to control the NBSB is chemical application of insecticides[7]. Using only insecticides to control *E. heros* can lead to undesirable effects on the environment, human health, and non-target organisms, as well as favoring the selection of insects resistant to insecticide[8,9]. Therefore, incorporating alternative control methods compatible with Integrated Pest Management (IPM) is necessary. Biological control stands out as a key strategy for pest control, representing a practical and ecologically friendly tactic[10].

Entomopathogenic fungi are a diverse group of organisms that have garnered significant attention for their potential in biological pest control. They have been extensively studied for their ability to infect and kill a wide range of insect species[11]. The fungal species *Metarhizium anisopliae* (Metsch.) Sorok., *Beauveria bassiana* (Bals.) Vuill., and *Cordyceps javanica* (Frieder. & Bally) have been widely applied to control *E. heros*[12,13]. The mode of action, efficacy, and virulence of the species have been studied for the control of *E. heros*[14]. Recently, a study verified the probing behavior of *E. heros* treated with *M. anisopliae,* demonstrating that adults of insects treated with *M. anisopliae* exhibited an 86% reduction in probing behavior by the fourth day after exposure, and probing activity ceased entirely by the fifth day ($p < 0.05$)[15]. This behavioral disruption, monitored via electropenetrography (EPG), led to a measurable decrease in seed damage even before insect mortality occurred[16].

EPG has emerged as a powerful tool to study piercing-sucking insects, offering real-time insights into their probing and non-probing activities. The insect and plant become part of the same electrical circuit to record voltage changes that represent otherwise invisible stylet penetration behaviors inside the plant, and the system generates waveforms on a computer screen[16,17]. This technology may be useful in the applied field of NBSB management, for example, to evaluate the selection of insect-resistant plants and resistance inducers[18], the action of insecticides[2], and recently, the effect of entomopathogenic fungi[15].

Results evaluating the probing behavior of *Dalbulus maidis* (DeLong &Wolcott) (Hemiptera: Cicadellidae) on corn, after spraying of *Cordyceps javanica,* indicate a significant disruption of the probing activities in 48 h[19]. This reduction in insect feeding activities, reported by Almeida[15] and Maluta[19], is important information for managing pests in the field, as it shows that, even if the insect is still alive, its feeding is compromised, and, consequently, the damage caused to the crop is mitigated. Therefore, the slower lethal effect of entomopathogenic fungi compared to synthetic insecticides can be minimized by changes in the pest's probing behavior[6].

The feeding behavior of *E. heros* treated with different fungal species has not been verified. We hypothesized that NBSB treated with different fungal species could exhibit different probing behaviors. Therefore, we carried out this study to ascertain whether different fungal species can alter the probing behavior of *E. heros* feeding on soybean pods.

4.2 MATERIALS AND METHODS

4.2.1 Rearing of *Euschistus heros*

E. heros was maintained at the Integrated Pest Management Laboratory of the Instituto Federal Goiano, Urutaí, Goiás, Brazil, under controlled environmental conditions (25 ± 2 °C, relative humidity of 60 ± 10%, and a photoperiod of 14 h). Adults were housed in plastic containers (25×20×10 cm) lined with filter paper to provide a clean and stable environment. Their diet consisted of green bean pods (*Phaseolus vulgaris L.*), soybean seeds (*Glycine max L.*), and raw peanut kernels (*Arachis hypogaea L.*), which were replenished every 48 h. Containers were cleaned regularly to ensure hygienic rearing conditions.

A voile fabric was used as the oviposition substrate, where eggs were collected and transferred to plastic containers (Gerbox 11×11×3.5 cm) with moistened soybean pods and filter paper to maintain optimal humidity. Early instar nymphs (first and second) were fed exclusively on soybean pods, whereas from the third instar onward, their diet was expanded to include the same food sources provided to adult insects. When the nymphs reached the fifth instar, they were relocated to plastic containers to continue their development.

4.2.2 Soybean cultivation and plant preparation for EPG analysis

Soybean plants (cv. BRS 7470 IPRO) were cultivated in pots containing a soil-to-sand mixture at a 2:1 ratio. Seeds of a commercial cultivar were sown in 18-liter plastic pots and grown in a greenhouse (27 ± 10 °C, relative humidity of 60 ± 20%, and a photoperiod of 13 hours), preventing pest infestation without insecticide applications. Fertilization was performed at sowing (3 g per pot of monoammonium phosphate – MAP) and later supplemented with 5 g of urea + 5 g of potassium chloride per pot. To ensure a continuous supply of plants for the EPG trial, seeds were sown weekly in batches of five pots until a total of 75 pots was reached. Irrigation was carried out as required to support proper plant growth.

Once the plants reached the R5.5 (pod filling) reproductive stage, stem sections containing pods were excised and transferred to 0.2-liter pots with moistened soil. These plant sections were used for monitoring *E. heros* probing activities during the EPG recordings.

4.2.3 Suspension preparation

To prepare the fungal suspensions, there were used commercial products and commercial doses. First, 0.65 g of *Cordyceps javanica* (isolate BRM 27666) and 8.13 g of *Metarhizium anisopliae* (isolate IBCB 425) were weighed and then mixed into 900 mL of distilled water, followed by the addition of 100 mL of an aqueous Polysorbate 80 (Tween 80) solution (99.9 ml of distilled water + 0.1 ml of Tween 80 - 0.01% v/v). The mixture was homogenized in a 1-L beaker to ensure even distribution of fungal propagules. The final suspension concentrations were 5×10^6 and 6.15×10^8 conidia

mL^{-1} for *C. javanica* and *M. anisopliae*, respectively. The conidial concentrations were enumerated by hemocytometer (Brightline Improved Neubauer, New OptikR, São Paulo, SP, Brazil) at 400× magnification. Conidial germination was verified (exceeding 98%) on potato dextrose agar (PDA) after 18 h at 26 °C. Only conidia with germ tubes greater than the conidial diameter were considered germinated.

For chemical insecticide treatment, Thiamethoxam + Lambda-cyhalothrin was prepared by diluting 0.025 mL of the product in 100 mL of water, using a 100 mL volumetric flask for precise measurement and standardization of the solution. This dose corresponds to a quarter of the commercial dose of the product[20], which was adjusted to cause only a sublethal effect on the insect and not kill the NBSB during the recording.

4.2.4 Electropenetrography experiment

The experiment was carried out in a controlled environment of 25 ± 2 °C, 70 ± 10% relative humidity, and a 14 hours photoperiod. Adult females (10–12 days after the emergence of adults) (females were used because they exhibit a longer feeding period) were subjected to four different treatments: fungal suspensions (*C. javanica* and *M. anisopliae*), the chemical insecticide, and a control group consisting of an aqueous solution of Polysorbate 80 (0.01% v/v)[15]. NBSB were topically treated (2 μL) on the pronotum. The insect activities on plant sections were assessed 4 h post-treatment (a fasting period was used to stimulate insect probing during the recording) using an AC-DC EPG monitor (EPG Technologies, Inc., Gainesville, FL, USA). The study was structured as a randomized block design with four treatments and twenty replicates per treatment. Each replicate consisted of a single insect, and the experiment was temporally blocked to account for variations over time.

Before the start of the recordings, insects underwent a 4-h fasting period. They were then immobilized for gold wire attachment following the methodology proposed by Lucini and Panizzi [21], using a thin strip of tape for stability. The pronotum was gently abraded with dental sandpaper to enhance adhesion of the silver glue [made with silver flake (Sigma Aldrich, St. Louis, MO, USA), white glue (Cascorez, Jundiai, SP, Brazil) and water (1:1:1 wt/vol/vol)], which was used to attach a gold wire (0.127 mm in diameter and 3.5 cm in length; Sigma Aldrich, Barueri, SP, Brazil) to a copper wire (3 cm in length) soldered to a brass nail. A small loop was made at the other end of the gold wire to increase the contact area with the insect and improve electrical conductivity[22].

After the glue dried, the insects were connected to the EPG monitor, where their activities were recorded for 72 hours. Twenty adults were successfully recorded (recordings without any insect probing were not used) per treatment in a completely randomized design.

All recordings were performed inside a Faraday cage to eliminate external electrical noise. Recordings were conducted using an input impedance of 10^7 Ohms for each of the four channels of the EPG monitor, with a voltage of 50 mV in alternating current (AC) across the plant electrode, and a gain set to 400x (actual gain of 400x with a 1x multiplier)[23]. An offset control was used to prevent rectifier folding and to preserve the native waveform polarity after rectification. Output voltages were amplified and captured at a sampling rate of 100 Hz per channel using the WinDaq DI-710 system (Dataq Instruments, Akron, OH, USA) connected to a computer running WinDaq Lite software version 3.11 (Dataq Instruments, Akron, OH, USA).

At the end of each recording, the NBSB treated with fungi were separated to confirm mortality due to fungal infection. NBSB were transferred to Petri dishes (60 mm) with a wet cotton and maintained at room temperature. Insects were considered infected by the fungus when mycelial and conidial growth was observed in the insect cadaver. Recordings of insects that did not exhibit fungal infection were excluded from the analysis.

The waveform events were identified and named according to a waveform library described in the literature[23]: Z (non-probing), Eh1 (stylet penetration), Eh2 (xylem sap ingestion), Eh3a (cellular laceration and enzymatic maceration of seed endosperm), Eh3b (short ingestion of macerated tissues of seed endosperm), Eh4 (ingestion from unknown location – probably phloem sap), without identifying treatments to reduce any bias. Three variables were calculated to study the feeding behavior: NWEi (Number of Waveform Events per Insect), WDEi (Waveform Duration per Event per Insect), and Final Time of the Last Probe (FTLP).

4.2.5 Data analysis

The raw data generated by the EPG monitor were processed and analyzed using the INFEST v.2.0 software[24] based on R v.4.2.1 and the gamlss package v.5.4-12. Statistical modeling was based on GAMLSS – Generalized Additive Linear Models for Location, Scale, and Shape[25,26], testing fourteen distribution models for each count and duration response variable. The selection of the best-fitting model and probability distribution was based on the Akaike Information Criterion (AIC). The standardized Pearson's residuals were graphically examined through a worm plot to check for fitting problems. The significance of the treatment effect was verified by the likelihood ratio test. Estimated marginal means were estimated for group comparisons, and pairwise tests were conducted by applying Tukey's procedure at 5% significance.

4.3 RESULTS

The groups presented significant ($p < 0.05$) differences in the means of the Number of Waveform Events per Insect (NWEi), Waveform Duration per Event per Insect (WDEi), and Final Time of the Last Probe (FTLP) as shown in Table 1. For the non-probing (Z) waveform, insects in the Control group exhibited an NWEi 3 times higher than the sublethal doses of Chemical treatment. *C. javanica* and *M. anisopliae* presented similar and intermediate values. However, the WDEi of Z was 3 times higher with the Chemical treatment than the untreated, indicating less time upon probing activities. A similar result was observed for NWEi associated with stylet penetration (Eh1). On average, untreated insects performed about 50 more probing events of stylet penetration than chemically treated insects. *C. javanica* promoted the lowest duration of Eh2 (xylem sap ingestion) and Eh3b (short ingestion of macerated tissues of seed endosperm), almost 4 times lower ($p < 0.05$) than the control group. However, no statistical differences were found for WDEi in Eh1, and for NWEi and WDEi in Eh3a (seed disruption), Eh3b (ingestion from seeds), as well as for NWEi in Eh3b.

The FTLP varied significantly among treatments. Insects in the Control group exhibited the highest average final probing time (about 44 hours), whereas those treated with *C. javanica* or *M. anisopliae* displayed markedly shorter final probing times (around 31 hours) and the Chemical treatment (about 39 hours). These findings indicate that entomopathogenic fungi reduced the overall duration of feeding.

Table 1. Comparisons of means (± standard errors) of experimental groups for the variables Number of Waveform Events per Insect (NWEi), Waveform Duration per Event per Insect (WDEi in minutes), and Final Time of the Last Probe (FTLP in hours) of *Euschistus heros* recorded by EPG.

	Waveforms			
	Z		**Eh1**	
Treatments	**NWEi - events/ insect**	**WDEi - min**	**NWEi - events/ insect**	**WDEi - min**
Control (untreated)	17.95 ± 3.17 b	121.00 ± 23.05 a	75.55 ± 55.52 b	6.00 ± 1.10 a
Cordyceps javanica	9.90 ± 1.75 ab	234.30 ± 44.50 b	47.21 ± 35.37 ab	4.40 ± 0.70 a
Metarhizium anisopliae	9.50 ± 1.68 ab	246.80 ± 46.90 b	38.44 ± 27.23 ab	5.00 ± 0.80 a
Chemical	6.15 ± 1.09 a	387.06 ± 73.70 b	23.54 ± 18.30 a	65.60 ± 1.30 a
Distribution	Gamma	Inverse Gamma	Pareto	Zero-adjusted Inverse Gaussian
AIC	530	1883	548	1013
p-value	0.0005	0.0002	0.1493	0.4083
	Eh2		**Eh3a**	
Treatments	**NWEi - events/ insect**	**WDEi - min**	**NWEi - events/ insect**	**WDEi - min**
Control (untreated)	3.93 ± 0.88 ab	155.40 ± 93.80 b	45.92 ± 34.05 a	46.30 ± 30.80 a
Cordyceps javanica	8.15 ± 2.83 a	28.50 ± 7.90 a	21.40 ± 16.93 a	57.65 ± 40.80 a
Metarhizium anisopliae	3.23 ± 0.71 ab	52.10 ± 19.50 ab	29.76 ± 21.94 a	53.20 ± 36.50 a
Chemical	2.91 ± 0.66 b	43.10 ± 16.00 ab	16.85 ± 13.70 a	38.20 ± 27.50 a
Distribution	Zero-adjusted Inverse Gaussian	Zero-adjusted Inverse Gaussian	Pareto	Pareto
AIC	353	1064	507	1051
p-value	0.0286	0.0263	0.8901	0.9967
	Eh3b		**Eh4**	
Treatments	**NWEi - events/ insect**	**WDEi - min**	**NWEi - events/ insect**	**WDEi - min**
Control (untreated)	6.38 ± 1.53 a	0.56 ± 0.18 b	3.33 ± 1.01 a	0.93 ± 0.07 a
Cordyceps javanica	3.78 ± 1.10 a	0.15 ± 0.03 a	7.25 ± 3.43 a	1.40 ± 0.46 a
Metarhizium anisopliae	5.11 ± 1.34 a	0.31 ± 0.08 ab	2.43 ± 0.71 a	2.16 ± 0.88 a
Chemical	3.06 ± 0.98 a	0.34 ± 0.16 ab	4.00 ± 1.99 a	3.16 ± 2.06 a
Distribution	Negative binomial	Zero-adjusted inverse Gaussian	Zero-adjusted inverse Gaussian	Zero-adjusted inverse Gaussian
AIC	411	513	236	415
p-value	0.1875	0.0150	0.2127	0.1447

Treatments	**FTLP - h**
Control (untreated)	44.09 ± 4.13 b
Cordyceps javanica	31.53 ± 5.27 a
Metarhizium anisopliae	30.80 ± 6.18 a
Chemical	39.52 ± 4.46 ab
Distribution	Gamma
AIC	185
p-value	0.1402

Values are presented as mean ± standard error. Means followed by the same letter within a column are not significantly different, according to Tukey's test at 5% significance. Waveforms: Z (non-probing), Eh1 (stylet penetration), Eh2 (xylem sap ingestion), Eh3a (seed disruption), Eh3b (ingestion from seeds), Eh4 (ingestion from unknown location – probably phloem), FTLP (The final time of the last probe).

The overall proportions of time spent probing versus non-probing waveforms are shown in Figure 1. In the Control group, insects spent 63% of the recording time probing. However, the fungal treatments shifted this balance considerably: *C. javanica* promoted 72% of non-probing time, and *M. anisopliae* 69% of non-probing. Chemical treatment induced the most pronounced shift, with non-probing behavior accounting for 81% of the recording time. These alterations suggest that while the treated groups reduced active feeding, the entomopathogenic fungi distinctly modified the probing pattern, potentially lowering feeding damage.

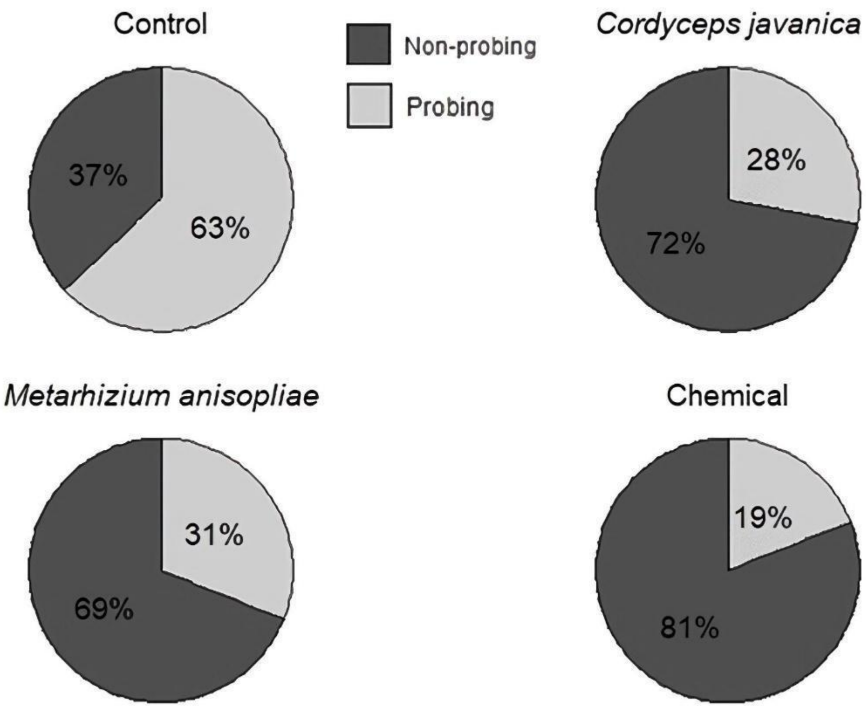

Figure 1. Percentage of time spent in probing vs. non-probing by *E. heros* feeding on soybean pods under four treatments (Control, *C. javanica*, *M. anisopliae*, Chemical). Values are descriptive proportions computed from the total recorded time per treatment (aggregated data); therefore, error bars/boxplots are not shown. The number of insects (n) and total recording time (T) for each treatment: Control (n = 20; T = 72 hours), *C. javanica* (n = 20; T = 72 hours), *M. anisopliae* (n = 20; T = 72 hours), Chemical (n = 20; T = 72 hours). *Probing* denotes periods with stylet penetration/feeding waveforms, *non-probing* accounts for all remaining time. Statistical analyses based on per-insect data are presented in Table 1.

4.4 DISCUSSION

The present study elucidates the effects of two entomopathogenic fungi, *Cordyceps javanica* and *Metarhizium anisopliae*, on the probing behavior of *Euschistus heros*. Our 72 hours observations capture the early infection window of entomopathogenic fungi, when conidial adhesion/germination is followed by cuticle penetration and onset of hemocoel colonization, during which sublethal, behavior-modifying effects can already emerge (toxins and enzymes, such as destruxins, proteases, and chitinases, interacting with host immunity)[27].

Within this period, it was detected reduced probing and delayed feeding initiation, consistent with comparative reports of feeding suppression in insects exposed to entomopathogenic fungi and with the recognized suitability of EPG for resolving stylet activities and linking waveform metrics to injury and management endpoints in *E. hero*[2,21].

The main finding was the significant reduction by fungal treatments in both the Number of Waveform Events per Insect (NWEi) and the Waveform Duration per Event per Insect (WDEi) for critical feeding behaviors, particularly stylet penetration (Eh1), xylem sap ingestion (Eh2), and seed ingestion (Eh3b). These behavioral disruptions suggest that entomopathogenic fungi impair the capacity of *E. heros* to initiate and maintain effective feeding. One hypothesis for these findings is that fungal infection compromises neuromuscular coordination, possibly through the production of mycotoxins such as destruxins (associated with *Metarhizium* spp.) or cordycepin (produced by *Cordyceps* spp.), which have been shown to interfere with insect physiological functions[28]. These compounds may reduce responsiveness or cause partial paralysis, leading to reduced probing frequency and duration[29], which limits plant damage during the pod-filling (R5) stage of soybean.

Another plausible explanation is that the energy costs associated with mounting an immune response to fungal infection divert resources away from feeding activities[30]. Hemipteran insects, including *E. heros*, exhibit innate immune responses, such as melanization and antimicrobial peptide production[31]. The activation of these defenses is energetically costly and may lead to behavioral trade-offs, including reduced locomotion and feeding[32]. This phenomenon may account for the increased non-probing (Z waveform) durations observed in fungi-treated insects, as they may remain stationary or inactive to conserve energy during systemic fungal infection[33].

Interestingly, while the sub-dose of chemical treatment (Thiamethoxam + Lambda-cyhalothrin) also resulted in significant increases in non-probing time, its profile was distinct. Insects exposed to the sub-dose of the chemical insecticide exhibited the highest average WDEi for the Z waveform, suggesting that while the chemical effectively induced feeding cessation, the effect could be immediate but transient. Conversely, the fungal treatments appeared to exert more gradual but sustained sublethal effects, which were reflected in the probing behavior throughout recording time (72 hours). This temporal differentiation in mode of action highlights the potential complementary roles of chemical and biological control agents within Integrated Pest Management (IPM)[34,35].

The reduction in the final time of the last probe duration among the experimental groups indicates a tangible decrease in potential plant damage during the R5.5 soybean growth stage, a critical period for yield formation. Previous studies have demonstrated that feeding by *E. heros* during this stage leads to significant reductions in seed quality and weight[36,37]. The Final Time of the Last Probe (FTLP) for fungal treatments was ~31 h, which is biologically consistent with the infection timeline of entomopathogenic fungi. After conidial adhesion and germination (within hours), *Metarhizium* and *Cordyceps* typically penetrate the cuticle within ~24–48 hours and begin hemocoel colonization[32]. During this early window, sublethal effects, e.g., destruxin-mediated immune and neuromuscular disruption as well as infection-induced metabolic stress, can reduce feeding propensity and delay the first successful stylet insertion detected by EPG[38].

A reduction of 45% in FTLP of *E. heros* treated with *M. anisopliae* compared to untreated insects is reported by some authors[15], who state that this information is valuable for managing NBSB in the field. It shows that, even if the insect is still alive, its feeding is compromised and, consequently, the damage caused to soybean seeds is reduced. By shortening the active feeding period, entomopathogenic fungi may mitigate yield losses even in the absence of high mortality rates[38]. Thus, EPG data support the hypothesis that sublethal effects can substantially contribute to pest suppression, a concept increasingly recognized in biocontrol research[39].

Moreover, the altered proportion of probing versus non-probing behavior in fungi-treated insects suggests a shift in behavioral priorities, possibly due to discomfort or physiological impairment. The increase in non-probing time could also reflect aversive learning or behavioral avoidance if the insects associate the feeding process with adverse physiological effects post-infection[19]. Though traditionally considered non-cognitive, some Hemiptera exhibit behavioral plasticity, including changes in host preference or feeding site selection following sublethal exposure to stressors[40]. Whether *E. heros* possesses such behavioral adaptability warrants further investigation, but it may be relevant in understanding long-term pest population dynamics under sustained fungal pressure.

Due to their specificity and environmental compatibility, entomopathogenic fungi offers a valuable alternative or supplement to chemical control, especially in the face of rising resistance issues and regulatory constraints on synthetic pesticides[41,42]. Furthermore, the compatibility of fungal treatments with other biocontrol agents, such as parasitoids or predators, presents opportunities for synergistic interactions that enhance overall pest management[43].

Finally, this study highlights the EPG values as a diagnostic tool in entomological research. By capturing real-time, fine-scale feeding behaviors, EPG allows the detection of subtle but meaningful impacts of pest management interventions. EPG data can refine our understanding of pest–host interactions and enlighten the development of more effective, targeted control strategies[44].

In conclusion, the application of *C. javanica* and *M. anisopliae* significantly disrupts the probing behavior of *E. heros* feeding on soybean pods, reducing its potential for crop damage. The present findings support the incorporation of entomopathogenic fungi into IPM programs, with the added benefit of mitigating the drawbacks associated with exclusive chemical control. EPG may be a promising tool to evaluate the feeding behavior of NBSB treated with entomopathogenic fungi, helping in the selection of different species or isolates of fungi, showing whether certain isolates inhibit insect feeding, verifying whether different concentrations of fungi affect the insect probing behavior, and strengthening biological control programs.

However, sublethal infected insects may resume feeding depending on the fungal isolate, dose, and abiotic conditions (notably temperature and humidity). Thus, the persistence of feeding suppression is context-dependent and warrants longer observation (≥ 7–10 d) under field-realistic conditions to determine whether effects are transient or sustained[45]. Within this framework, the short-term attenuation document during reproductive stages (e.g., soybean R5.5) is agronomically meaningful because reduced stylet activity and can lower injury to productive structures even before mortality occurs.

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4.7 DATA AVAILABILITY STATEMENT

The raw datasets generated during the current study are available in the Zenodo repository at: https://doi.org/10.5281/zenodo.16572536.

4.8 ADDITIONAL INFORMATION

The authors declare no competing interests.

4.9 REFERENCES

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5. CAPÍTULO III

(Normas de acordo com a revista Contribuciones a Las Ciencias Sociales)

Integrating thermal imaging and X-ray analysis to assess the effects of entomopathogenic fungi on *Euschistus heros* and soybean seed damage

Integração de imagens térmicas e análise por raios X para avaliar os efeitos de fungos entomopatogênicos sobre Euschistus heros e o dano em sementes de soja

Integración de imágenes térmicas y análisis por rayos X para evaluar los efectos de hongos entomopatógenos sobre *Euschistus heros* y el daño en semillas de soja

ABSTRACT

Entomopathogenic fungi are important biological control agents with the potential to affect insect performance beyond mortality. Here, we present an integrative approach combining thermal imaging and X-ray analysis to assess the effects of fungal exposure on *Euschistus heros* and associated seed damage in soybeans. Insects were exposed to *Metarhizium anisopliae*, *Cordyceps javanica*, a synthetic insecticide, or a control, and monitored under controlled conditions during electropenetrography (EPG) recordings. Thermal imaging revealed temporal variations in insect dorsal temperature ($p < 0.05$), with higher values in the chemical treatment and intermediate responses in fungal treatments. X-ray imaging enabled non-destructive detection of internal seed damage and showed that fungal treatments significantly reduced damage incidence compared to the control (65.5%), with values of 27.8% for *M. anisopliae* and 34.4% for *C. javanica* ($p < 0.05$), being comparable to or more effective than the synthetic insecticide (37.3%). These findings indicate that fungal exposure can reduce feeding damage prior to insect mortality, supporting the potential of biological control in soybean production systems.

Keywords: *Cordyceps javanica*, *Metarhizium anisopliae*, neotropical brown stink bug.

RESUMO

Fungos entomopatogênicos são importantes agentes de controle biológico com potencial para afetar o desempenho de insetos além da mortalidade. Neste estudo, apresentamos uma abordagem integrativa combinando imagens térmicas e análise por raios X para avaliar os efeitos da exposição fúngica em *Euschistus heros* e o dano associado em sementes de soja. Os insetos foram expostos a *Metarhizium anisopliae*, *Cordyceps javanica*, um inseticida sintético ou controle, sendo monitorados durante registros de eletropenetrografia (EPG). A análise térmica revelou variações temporais na temperatura dorsal dos insetos ($p < 0{,}05$), com valores mais elevados no tratamento químico e respostas intermediárias nos tratamentos com fungos. A análise por raios X permitiu a detecção não destrutiva de danos internos nas sementes, demonstrando que os tratamentos com fungos reduziram significativamente a incidência de danos em comparação ao controle (65,5%), com valores de 27,8% para *M. anisopliae* e 34,4% para *C. javanica* ($p < 0{,}05$), sendo comparáveis ou superiores ao inseticida sintético (37,3%). Os resultados indicam que a exposição aos fungos pode reduzir o dano por alimentação antes da mortalidade, reforçando o potencial do controle biológico em sistemas de produção de soja.

Palavras-chave: *Cordyceps javanica*; Eletropenetrografia; *Metarhizium anisopliae*; percevejo-marrom.

RESUMEN

Los hongos entomopatógenos son importantes agentes de control biológico con el potencial de afectar el desempeño de los insectos más allá de la mortalidad. En este estudio, se presenta un enfoque integrador que combina imágenes térmicas y análisis por rayos X para evaluar los efectos de la exposición fúngica en *Euschistus heros* y el daño asociado en semillas de soja. Los insectos fueron expuestos a *Metarhizium anisopliae*, *Cordyceps javanica*, un insecticida sintético o un control, y monitoreados bajo condiciones controladas durante registros de electropenetración (EPG). El análisis térmico reveló variaciones temporales en la temperatura dorsal de los insectos ($p < 0,05$), con valores más altos en el tratamiento químico y respuestas intermedias en los tratamientos con hongos. El análisis por rayos X permitió la detección no destructiva de daños internos en las semillas y demostró que los tratamientos con hongos redujeron significativamente la incidencia de daño en comparación con el control (65,5%), con valores de 27,8% para *M. anisopliae* y 34,4% para *C. javanica* ($p < 0,05$), siendo comparables o superiores al insecticida sintético (37,3%). Estos resultados indican que la exposición a los hongos puede reducir el daño por alimentación antes de la mortalidad, destacando su potencial en sistemas de producción de soja.

Palabras clave: *Cordyceps javanica*, chinche marrón, *Metarhizium anisopliae*.

5.1 INTRODUCTION

The neotropical brown stink bug, *Euschistus heros* (Fabricius, 1798) (Hemiptera: Pentatomidae), is a major pest in soybean crops, causing substantial damage and economic losses. *E. heros* is known for directly feeding soybean pods, leading to reduced seed quality and yield (Betinelli et al., 2023). The pest's impact on soybean production has prompted extensive research into its population dynamics, host preferences, and control strategies (Hickmann et al., 2023; Souza et al., 2020; Tibola et al., 2021). Understanding the behavior, biology, and ecological interactions of *E. heros* is crucial for developing sustainable integrated pest management strategies to mitigate its detrimental effects on agricultural production (Filho et al., 2022b; Santos, 2023; Tognon et al., 2020).

Traditional control methods, particularly the repeated use of synthetic insecticides, have imposed strong selection pressure on *E. heros* populations, resulting in an increased frequency of resistant phenotypes and highlighting the need for alternative management strategies (Lira et al., 2023). One promising and environmentally friendly approach is the utilization of entomopathogenic fungi for biological control (Nanzer et al., 2021). Studies have demonstrated the insecticidal potential of *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorok. against *E. heros*, highlighting their virulence and potential as biocontrol agents (Battisti et al., 2022; Resquín-Romero et al., 2020, Sousa et al., 2023).

Entomopathogenic fungi not only act as lethal agents against insect pests but can also influence their feeding behavior, reducing their capacity to cause crop damage. Studies have demonstrated that fungal infections can lead to decreased food intake and altered feeding patterns in pests such as *E. heros* and the corn leafhopper (*Dalbulus maidis*) (Maluta et al., 2023; Almeida et al.,2025). These behavioral changes may be associated with physiological stress or the production of fungal secondary metabolites that interfere with insect feeding mechanisms (Lacey et al., 2015). One effective method for assessing the feeding behavior of sucking insects is the Electropenetrography (EPG) technique (Backus et al., 2019). The EPG technique showed that neotropical brown stink bug treated with *M. anisopliae* spent significantly less time on probing activities (reduced by 86% at day four and ceased at day five) than untreated insects, and consequently, its damage to soybean seeds was reduced (Almeida et al., 2025).

Entomopathogenic fungi typically require several days to kill their host after application, usually ranging from 2 to 10 days, which is considerably slower than synthetic insecticides. This delayed mortality is an adaptive trait, as it allows the pathogen to maximize nutrient acquisition from the host (De Sousa et al., 2023).

Sublethal responses provide key insights into host defense mechanisms, such as behavioral fever. This response, defined as an increase in body temperature induced by the insect, can impair pathogen development and replication (Clancy et al., 2018). Therefore, it may increase host survival and modulate the infection process. These physiological and behavioral adjustments can directly influence the overall efficacy of biological control strategies (Ouedraogo et al., 2003).

In this context, advanced phenotyping approaches are essential to capture these dynamic interactions. Thermal imaging has emerged as a powerful and non-invasive tool to detect real-time changes in insect body temperature and behavior following exposure to entomopathogenic fungi (Al-doski; Bin Mansor; Zulhai Bin Mohd Shafri, 2016). This technique enables the detection of subtle temperature variations associated with stress and immune responses, providing deeper insights into pathogen-host interactions, mode of action, and control efficiency (Amendt et al., 2017; Hoffmann et al., 2013)

In parallel, imaging techniques have also advanced the ability to quantify pest-induced damage in plant structures. X-ray imaging offers a non-destructive and highly sensitive method to detect internal damage in soybean seeds caused by insect feeding (Oliveira et al., 2021; Pinto et al., 2009). This approach allows the identification of subtle morphological changes, including tissue degradation, cracks, and hidden infestations (Raju Ahmed et al., 2020). Moreover, it enables the assessment of the impact of insect injury on seed viability and germination, providing a comprehensive evaluation of damage beyond visual symptoms (Rohr et al., 2023).

Here, we present an integrated and innovative approach combining thermal imaging and X-ray analysis to evaluate the effects of entomopathogenic fungi exposure on *Euschistus heros*. Specifically, this study aimed to assess changes in insect body temperature following fungal exposure using thermal imaging, during EPG recordings, as well as to evaluate seed damage through X-ray analysis. This integrative approach provides a novel perspective on the functional efficacy of biological control agents by linking temperature dynamics and seed damage, allowing a more comprehensive assessment of control outcomes.

5.2 THEORETICAL FRAMEWORK

5.2.1 BIOLOGICAL BASIS OF *Euschistus heros* DAMAGE AND THE NEED FOR OBJECTIVE ASSESSMENT

The neotropical brown stink bug, *Euschistus heros* (Hemiptera: Pentatomidae), exhibits ecological traits that support persistence and infestation build-up in soybean areas. Population dynamics may sustain rapid increases in field abundance depending on environmental and cropping conditions (Ecco et al., 2020). Host–pest interactions may further influence fitness-related responses, affecting insect performance and continuity in agroecosystems (Santos, 2023)

A key challenge in stink bug injury interpretation is that feeding damage may be underestimated when assessment relies only on external pod symptoms. Internal lesions caused by stylet penetration and tissue disruption can compromise seed integrity and physiological quality. X-ray images demonstrate that stink bug feeding produces internal seed damage associated with impaired seed quality, reinforcing the importance of objective diagnostic methods for accurate damage assessment (Pinto et al., 2009).

5.2.2 CHEMICAL CONTROL CONSTRAINTS AND ENTOMOPATHOGENIC FUNGI AS BIOLOGICAL ALTERNATIVES

Evidence indicates that *E. heros* populations have undergone shifts in susceptibility to insecticides due to selection pressure, which constrains the long-term sustainability and efficiency of chemical control programs (Tibola et al., 2021). This reinforces the importance of complementary tactics within Integrated Pest Management (IPM), including biological control tools that reduce reliance on synthetic insecticides.

Entomopathogenic fungi are key biological control agents in agroecosystems, playing an important role in regulating insect populations and contributing to sustainable pest management (De Oliveira et al., 2025). Several fungal species have been investigated for the management of stink bugs, including *Metarhizium anisopliae*, *Beauveria bassiana*, and *Cordyceps* spp (Resquín-Romero et al., 2020). These fungal-based approaches have demonstrated the capacity to infect *E. heros* and affect key biological traits such as survival and reproduction, indicating their potential for population suppression (Battisti et al., 2022).

The entomopathogenic fungi performance is strongly influenced by environmental conditions. Temperature and relative humidity affect key stages of the infection process, including conidial germination, host penetration, and fungal development within the insect (Athanassiou et al., 2017). In parallel, infected insects may exhibit physiological and behavioral responses to infection, such as behavioral fever, characterized by an increase in body temperature. This response can impair fungal development and modulate the infection outcome by increasing host survival (De Oliveira et al., 2025).

Under field-relevant conditions, the agronomic value of fungal infection is not limited to mortality. Sublethal effects may play a critical role, particularly through the suppression of feeding activity. Evidence indicates that fungal exposure can reduce feeding intensity and, consequently, decrease soybean injury, suggesting that damage mitigation may occur prior to insect death (De Oliveira et al., 2025).. This perspective supports a functional interpretation of biological control, in which efficacy is associated with the damage reduction rather than mortality alone. However, the mechanisms linking infection-induced stress responses, such as temperature changes, to feeding suppression remain poorly understood and require integrated investigation.

5.2.3 INTEGRATED BEHAVIORAL AND PHYSIOLOGICAL ASSESSMENT: EPG AND THERMAL IMAGING

Electropenetrography (EPG) enables detailed measurement of probing and feeding patterns in piercing–sucking insects, capturing feeding duration, interruption, and probing frequency (Figure 1).

Figure 1. Monitoring the feeding behavior of *Euschistus heros* on soybean pods using Electropenetrography (EPG).

Source: Prepared by the authors. The image shows an experiment using Electropenetrography (EPG) to assess the feeding behavior of the brown stink bug (*Euschistus heros*) on soybean pods (*Glycine max*). The insect is connected to a gold wire, allowing the recording of stylet penetration into plant tissues. The insect highlights a stink bug feeding on the plant pods, providing insights into feeding patterns and the damage caused to the crop.

Evidence indicates that fungal infection disrupts feeding behavior, reducing probing activity and reinforcing feeding suppression as a key sublethal mechanism (Maluta; Castro; Spotti Lopes, 2023). Combined with injury-related outcomes, such behavioral metrics strengthen causal interpretation of damage reduction (Almeida et al., 2025). In parallel, thermal imaging provides a non-invasive method to detect physiological changes through temperature dynamics and has been explored in insect-related agricultural contexts(Al-doski; Bin Mansor; Zulhai Bin Mohd Shafri, 2016). Infection-driven physiological responses may alter tolerance patterns and thermal-associated parameters (Porras et al., 2021).

Additionally, elevated host body temperature may constrain fungal development, indicating that temperature modulation can act as a defensive response during infection (Ouedraogo et al., 2003). Nevertheless, body temperature changes are still rarely assessed together with feeding behavior, limiting a more integrated understanding of how infection-induced stress contributes to feeding suppression and damage reduction.

5.2.4 LINKING TEMPERATURE DYNAMICS DURING EPG RECORDINGS AND SEED DAMAGE

Seed injury caused by stink bug feeding is often internal and not externally visible, requiring objective approaches for damage assessment. X-ray imaging enables non-destructive evaluation of internal seed structures and allows the detection of hidden lesions associated with reduced seed quality (Pinto et al., 2009). Thus, imaging-based methods reinforce the importance of internal seed assessment for accurate damage quantification in soybean systems (Raju Ahmed et al., 2020).

Overall, the literature indicates that *Euschistus heros* causes internal damage that is frequently underestimated by external evaluation (Pinto et al., 2009), that chemical control is constrained by reduced susceptibility due to selection pressure (Tibola et al., 2021), and that entomopathogenic fungi represent promising tools for sustainable pest management (Battisti et al., 2022; Resquín-Romero et al., 2020). However, a key limitation is the lack of integrative approaches, as studies typically assess fungal effects, temperature responses, feeding behavior, and seed injury separately.

Fungal infection may induce changes in insect body temperature that can be detected using thermal imaging (Porras et al., 2021). These temperature changes may be associated with alterations in insect activity and feeding patterns, potentially contributing to reduced plant injury (Maluta; Castro; Spotti Lopes, 2023). At the same time, seed damage can be objectively quantified through X-ray imaging (Pinto et al., 2009; Raju Ahmed et al., 2020). Integrating these approaches provides a more comprehensive understanding of how fungal exposure affects insect responses and damage outcomes, supporting a more robust evaluation of biological control performance against *E. heros* in soybean.

5.3 METHODOLOGY

5.3.1 REARING OF *E*uschistus *heros* UNDER CONTROLLED CONDITIONS

Euschistus heros was reared in the Integrated Pest Management Laboratory (LabMIP) at Instituto Federal Goiano, Urutaí, Goiás, Brazil. Specimens were obtained and housed in plastic containers (25×20×20 cm) lined with filter paper. They were provided with green bean pods (*Phaseolus vulgaris* L.), soybean seeds (*Glycine max* L.), and raw peanut kernels (*Arachis hypogaea* L.) as food sources. The containers were cleaned, and the food was replenished every two days. Voile fabric served as an oviposition substrate, and the collected eggs were transferred to Gerbox containers (11×11×3 cm) containing moistened soybean pods and filter paper.

During the first and second instars, nymphs were fed exclusively with soybean pods. From the third instar onward, they were given the same diet as adults. Upon reaching the fifth instar, nymphs were transferred to plastic containers for continued rearing. Throughout development the insects were maintained under controlled conditions (25 ± 2 ºC, 60 ± 10% RH, 14h photoperiod).

5.3.2 SOYBEAN CULTIVATION AND PREPARATION FOR EPG MONITORING

Soybean seeds were sown in 18 liters plastic pots containing a mixture (2:1) of soil and sand, and kept in a greenhouse to prevent pest infestations, as no insecticide applications were made. NPK fertilization was provided at sowing and as a top dressing using monoammonium phosphate (MAP). Irrigation was applied as needed to support plant growth. At the R5.5 growth stage, stem sections containing pods were excised and transferred to 0.2-liter pots with moistened soil for EPG recording.

5.3.3 ELECTROPENETROGRAPHY (EPG) EXPERIMENT

The experiment was conducted under controlled conditions (25 ± 2 ºC, 70 ± 10% RH, and a 14h photoperiod). Insects were topically treated with suspensions of commercial entomopathogenic fungi (*Cordyceps javanica* BRM 27666 and *Metarhizium anisopliae* IBCB 425), the synthetic insecticide (Thiamethoxam + Lambda-cyhalothrin), or an aqueous solution of Polysorbate 80 (0.01% v/v). The control group received only Polysorbate 80. EPG recordings started four hours after contamination. The experiment followed a randomized block design (RBD) with four treatments (the control, two fungi and a synthetic insecticide) and twenty replicates per treatment, in which each replicate consisted of a single insect.

Fungal suspensions were prepared by weighing 0.65g of *C. javanica* and 8.13g of *M. anisopliae*, which were then mixed with 900 mL of water and 100 mL of 0.01% v/v aqueous Polysorbate 80 in 1-liter beakers to ensure homogeneous distribution. The final concentration of the suspension was 5×10^6 conidia/mL for *C. javanica* and 6.15×10^6 conidia/mL for *M. anisopliae*. The synthetic insecticide was prepared by diluting 0.025mL of the product in 100 mL of water, using a 100mL volumetric flask for precise measurement. Subsequently, 2 µL of each treatment suspension was applied to the pronotum of insects.

Adults' female of similar age were selected and fasted for four hours before recording. After this period, they were immobilized to attach the gold wire. A strip of tape was used to hold the insects in place, and their pronotum was gently abraded for the application of silver glue (Lucini; Panizzi, 2016). The gold wire was then placed on the pronotum, followed by another layer of glue. Once the glue dried, the insects were connected to the EPG system. Each insect was monitored for 72 hours, with recordings conducted inside a Faraday cage to minimize external electrical noise.

5.3.4 THERMAL IMAGES

During the EPG experiment, thermal images of insects were captured. The images were obtained using a Flir C2 thermal camera, and image processing was performed using the softwares Flir Tools and the R (R Development Core Team, 2024) package Thermimage (Tattersall, 2022).

Photographs were taken before the experiment began, then every 5 minutes for 2 hours. After this initial period, images were captured every 30 minutes for the next 4 hours. Next, images were taken every 2 hours until the 6-hour mark. Finally, images were taken at 4-hour intervals, extending the total observation period to 36 hours. Thermal images were used to monitor and analyze temperature changes in the dorsal region of the insects over time. This evaluation provided insights into temperature variation throughout the experiment, potentially revealing physiological responses induced by the treatments.

5.3.5 X-RAY IMAGES

After the EPG experiment, soybean pods were carefully collected, placed in labeled paper bags, and stored under controlled conditions (25 ± 2 ºC, 40 ± 10% RH) for 12 weeks to maintain their integrity. This storage period ensured that all pods were analyzed simultaneously. Subsequently, pods from each treatment plot underwent X-ray analysis using a Faxitron HP, model 43855A.

During imaging, pods were carefully positioned to optimize visualization of internal structures. Seeds were exposed to radiation for 10 seconds at 31 kV, allowing for clear detection of potential damage or structural alterations. Based on the X-ray images, seeds damage incidence was assessed by identifying structural modifications and other indicators of seed impairment. This analysis provided valuable insights about the impact of different treatments on seeds integrity.

5.3.6 DATA ANALYSIS

Temperature measurements obtained from thermal images were analyzed by fitting a linear mixed-effects model with the R (R Core Team, 2025) package *nlme* (Pinheiro; Bates, 2002) under the effects of treatment, time, and their interaction as fixed effects, while individual insects were treated as random effects to account for repeated measures. Post-hoc analysis consisted of pairwise comparisons of estimated marginal means of treatments (groups) using Tukey's test with 5% significance, with the R package *emmeans* (Lenth, 2024).

The incidence of stink bug-induced pod damage was analyzed by fitting a generalized linear model with binomial response and logit link. Estimated marginal means of treatments (groups) were used in pairwise comparisons based on Tukey's test with 5% significance. All models were validated through residual graphical analysis. The data obtained directly from the EPG experiment are not included in this study, as they are part of another work already published (De Oliveira et al., 2025) .

5.4 RESULTS AND DISCUSSIONS

Thermal imaging analysis revealed significant temporal variations in the dorsal temperature of *Euschistus heros*, with clear differences among treatments (Table 1). From the onset of the EPG experiment (1 min) up to 840 min (14 h), insects treated with the synthetic insecticide exhibited higher temperatures (27.3 °C) compared to the control group (26.6 °C) ($p < 0.05$), indicating a pronounced physiological disturbance, likely associated with acute metabolic stress or neurotoxic effects(Cervantes et al., 2017). Similarly, *Metarhizium anisopliae* induced a significant increase in temperature relative to the control after 360 min ($p < 0.05$), suggesting activation of host-pathogen interactions, potentially related to early immune responses or fungal establishment within the host(Sikorski; Poltronieri, 2022b). However, after 14 h, no significant differences were observed among treatments ($p > 0.05$).

Table 1. Temperature (ºC) on insects dorse over time and incidence of damage to soybean seeds (%).

Treatments	**Time (min)**							
	1		**5**		**10**		**15**	
Control	26.6	b	26.5	b	25.5	b	26.5	b
Cordyceps javanica	27.0	ab	27.0	ab	26.9	ab	26.9	ab
Metarhizium anisopliae	27.0	ab	27.0	ab	27.0	ab	27.0	ab
Chemical	27.3	a	27.3	a	27.3	a	27.3	a

Treatments	**Time (min)**							
	20		**25**		**30**		**60**	
Control	26.5	b	26.5	b	26.5	b	26.5	b
Cordyceps javanica	26.9	ab	26.9	ab	26.9	ab	26.9	ab
Metarhizium anisopliae	27.0	ab	27.0	ab	27.0	ab	27.0	ab
Chemical	27.3	a	27.3	a	27.3	a	27.3	a

Treatments	**Time (min)**							
	90		**120**		**150**		**180**	
Control	26.5	b	26.5	b	26.5	b	26.5	b
Cordyceps javanica	26.9	ab	26.9	ab	26.9	ab	26.9	ab
Metarhizium anisopliae	26.9	ab	26.9	ab	26.9	ab	26.9	ab
Chemical	27.2	a	27.2	a	27.2	a	27.2	a

Treatments	**Time (min)**							
	210		**240**		**330**		**360**	
Control	26.5	b	26.5	b	26.5	b	26.4	b
Cordyceps javanica	26.9	ab	26.8	ab	26.8	ab	26.8	ab
Metarhizium anisopliae	26.9	ab	26.9	ab	26.9	ab	26.9	a
Chemical	27.2	a	27.2	a	27.1	a	27.1	a

Treatments	**Time (min)**							
	390		**420**		**450**		**480**	
Control	26.4	b	26.4	b	26.4	b	26.4	b
Cordyceps javanica	26.8	ab	26.8	ab	26.7	ab	26.7	ab
Metarhizium anisopliae	26.8	a	26.8	a	26.9	a	26.8	a
Chemical	27.1	a	27.1	a	27.1	a	27.1	a

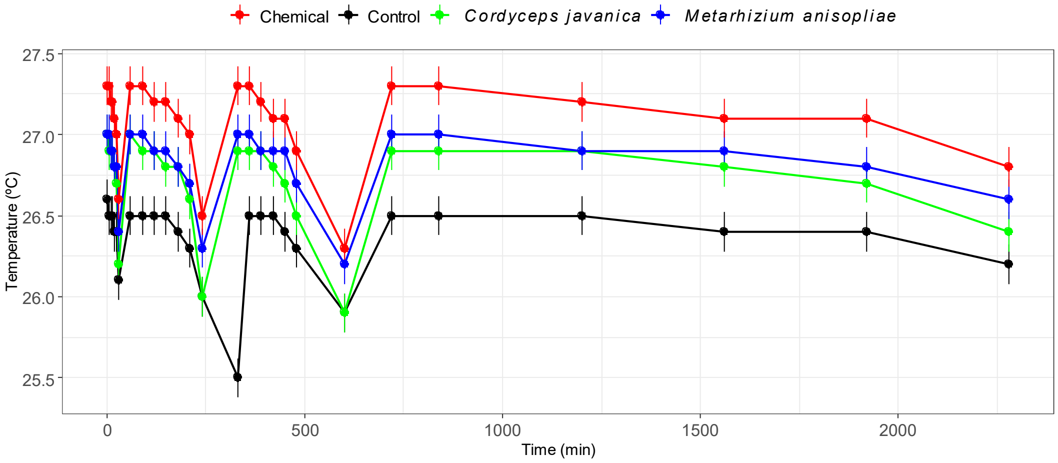
Treatments	**Time (min)**							
	600		**720**		**840**		**1200**	
Control	26.4	b	26.3	b	26.3	b	26.2	a
Cordyceps javanica	26.7	ab	26.6	ab	26.5	ab	26.4	a
Metarhizium anisopliae	26.8	ab	26.7	ab	26.7	ab	26.6	a
Chemical	27.0	a	27.0	a	26.9	a	26.8	a

Treatments	**Time (min)**						**Damage (%)**	
	1560		**1920**		**2280**			
Control	26.1	a	26.0	a	25.9	a	65.5	c
Cordyceps javanica	26.2	a	26.0	a	25.9	a	34.4	b
Metarhizium anisopliae	26.4	a	26.3	a	26.2	a	27.8	a
Chemical	26.6	a	26.5	a	26.3	a	37.3	b

Source: Prepared by the authors. Means followed by the same letter within a column are not significantly different, according to Tukey's test at a 5% significance level ($p \leq 0.05$).

Over time, especially after 600 min, a gradual temperature decline was observed across all treatments (Figure 2). The fungal treatments (*C. javanica* and *M. anisopliae*) exhibited intermediate temperatures, higher than the control and lower than the synthetic insecticide. Previous studies have demonstrated that entomopathogenic fungi can alter the metabolic processes of insect hosts, leading to reduced activity and impaired homeostasis, which can be detected through temperature fluctuations (Porras et al., 2021; Zhang; Qi; Zhang, 2025).

Figure 2. Temperature (ºC) variation on the dorsal side of *E. heros* over time for different treatments.



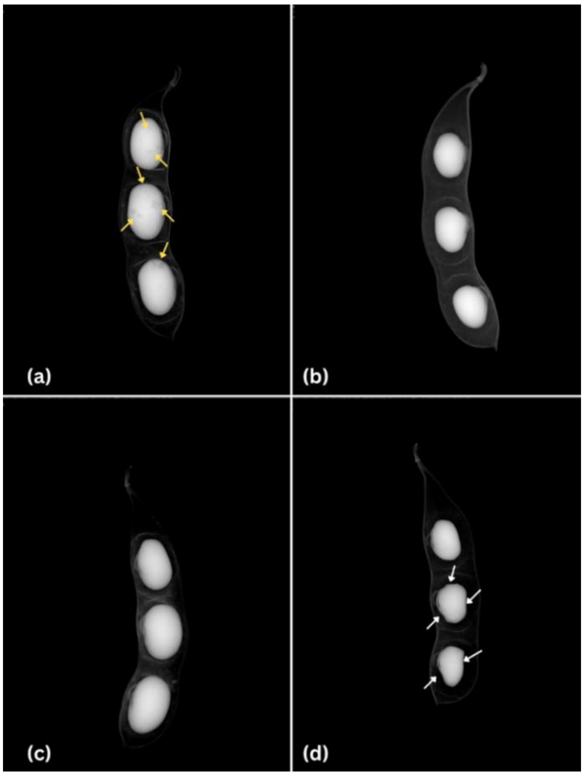
Source: Prepared by the authors. The figure shows the dorsal temperature (°C) of *E. heros* over time (minutes) under different treatments. Vertical lines represent the standard error (0.12 ºC). The chemical treatment is tiametoxam/lambda-cialotrina.

The observed decline in dorsal temperature in stink bugs treated with fungal pathogens is consistent with previous research indicating that fungal infections disrupt normal metabolic functions leading to a reduced ability to generate heat through muscular activity, resulting in detectable temperature fluctuations (Athanassiou et al., 2017). For instance, a study about the effects of fungal infections on insect physiology found that such pathogens can constrain the physiology and behavior of insects, leading to altered metabolic pathways and increased ATP synthesis as a response to infection-induced stress (Porras et al., 2021)

This disruption is mainly associated with the secretion of hydrolytic enzymes by entomopathogenic fungi, including proteases, chitinases, and lipases, which degrade the insect cuticle and underlying tissues, thereby facilitating fungal penetration and colonization (Sánchez-Pérez et al., 2014). In addition, these fungi produce secondary metabolites, such as destruxins, oosporein, and cytochalasins, which are reported to interfere with neural signaling and muscle function, potentially impairing insect mobility and feeding behavior (Liu; Smagghe; Liu, 2023).

Although physiological mechanisms were not directly assessed in the present study, these processes may help explain the reduced feeding activity observed in treated insects. This interpretation is supported by X-ray imaging of soybean seeds, which enabled a non-destructive assessment of internal structures and revealed a lower incidence of internal damage in treatments with entomopathogenic fungi (Figure 3). The use of X-ray analysis is particularly relevant, as stink bug feeding often causes concealed internal injuries that are not detectable by external inspection, providing a more accurate estimation of damage (Rohr et al., 2023).

Figure 3. X-ray images of soybean pods from a feeding experiment with *E. heros*.



Source: Prepared by the authors. The figure presents X-ray images of soybean pods from different treatments, highlighting damage caused by *E. heros* feeding and other mechanical injuries. (a) Yellow arrows indicate seed damage caused by *E. heros* feeding in pods without treatment, showing significant internal deterioration. (b) Pod from the *C. javanica* treatment, with seeds exhibiting no visible damage. (c) Pod from the *M. anisopliae* treatment, also showing intact seeds, without visible feeding damage. (d) Pod from the chemical treatment, illustrating mechanical damage to the seeds (white arrows), i.e., injuries unrelated to *E. heros* feeding and likely resulted from handling or external physical stress.

X-ray imaging (Figure 3) and the damage incidence (%) (Table 1) provided clear evidence that entomopathogenic fungi significantly reduced the feeding damage caused by *E. heros*. The control group exhibited the highest incidence of seed damage, with 65.5% of seeds affected, reinforcing the destructive potential of stink bug in soybean. By contrast, fungal treatments presented significantly lower damage levels, with *M. anisopliae* (27.8%) and *C. javanica* (34.4%) outperforming the synthetic insecticide (37.3%) in mitigating feeding damage. X-ray images provide visual confirmation of these findings. Figure 3a shows soybean pods from control treatment, where extensive internal seed damage is visible, marked by multiple lesions. In contrast, seeds from the *M. anisopliae* treatment (Figure 3b) appear structurally intact, with no significant signs of insect damage.

Similarly, *C. javanica* (Figure 3c) demonstrated minimal damage, whereas seeds from the Chemical treatment (Figure 3d) exhibited no feeding damage.

Studies have highlighted the importance of imaging techniques, such as radiography, for the accurate detection of damage caused by stink bugs in soybean seeds. According to Pinto et al. (2009) and Zacharias et al. (2024), X-ray analysis enables precise identification of internal structural changes and mechanical injuries associated with stink bug feeding, which directly impact the physiological quality of the seeds. Zacharias et al. (2024) demonstrated that such damage significantly compromises soybean seed germination and vigor, reinforcing the radiography as an essential tool for screening high-quality seed lots.

In this context, Rohr et al. (2023) emphasized that the occurrence of internal damage is correlated with reduced germination potential and uneven seedling emergence in the field. These factors may jeopardize the initial establishment of the crop and, consequently, the final yield. Additionally, radiographic imaging allows for non-destructive screening of seed lots, supporting seed certification programs and improving decision-making about the use and commercialization of seed material. The adoption of this technology therefore represents a significant advancement in traceability and quality control of soybean seeds (França-Silva et al., 2023; Raju Ahmed et al., 2020; Zacharias et al., 2024).

Moreover, data from the present study suggest that the treatments applied, particularly those involving entomopathogenic fungi, have the potential to positively influence seed quality. The lower incidence of internal damage observed in seeds from these treatments may support the formation of lots with higher vigor, uniformity, and germination potential, which are critical attributes for standardization and commercial value (Oliveira et al., 2021). Thus, a direct relationship can be established between the application of the tested products and the improvement of qualitative parameters in soybean seed lots.

According to Almeida et al. (2025), the number of feeding punctures by *E. heros* females treated with *M. anisopliae* was significantly reduced compared to untreated females. Insects exposed to the fungus showed a 45% reduction in the final time of the last probe (FTLP), from 55.1 h in the control group to 31.1 h in the treated group, indicating that feeding activity was interrupted earlier. Additionally, the same study reported a drastic decrease in probing waveform duration: 86% at 48 hours and 98% at

72 hours post-treatment. These behavioral disruptions directly correlate with the reduction in physical seed damage, reinforcing the biocontrol potential of *M. anisopliae*.

The reduction in seed damage observed in fungal treatments can also be attributed to other factors, including direct pathogenic effects on the insect and indirect behavioral modifications induced by fungal infection. Another critical aspect to consider is the action mode of *M. anisopliae* and *C. javanica*, both of which have been documented to produce secondary metabolites that interfere with insect neural and muscular function (Jia et al., 2016). These metabolites can act as mycotoxins, impairing coordination and suppressing feeding behavior even before mortality occurs. Such effects provide additional support for the superior efficacy of fungal treatments in reducing seed damage, as infected stink bugs are likely to experience physiological impairments that deter their ability to sustain prolonged feeding bouts (Qu et al., 2022; Wang et al., 2021).

The infection process begins with fungal spores adhering to the insect cuticle, followed by germination and penetration into the host's body (Zhang; Qi; Zhang, 2025). This initial phase is facilitated by the secretion of cuticle-degrading enzymes, allowing the fungus to invade the hemocoel and proliferate systemically. Once inside, the fungus colonizes host tissues, consuming essential nutrients and triggering physiological stress responses (Wang et al., 2021). The host's immune system activates defense mechanisms, including antimicrobial peptides and melanization pathways, but these responses often fail to halt fungal progression, further exacerbating energy depletion (Sánchez-Pérez et al., 2014).

As fungal growth advances, the increasing production of secondary metabolites intensifies physiological impairment. Destruxins, for example, disrupt ion channel function, impairing neural signaling and muscular coordination, while oosporein induces oxidative stress, compounding cellular damage (Zhang; Qi; Zhang, 2025). Furthermore, the progression of infection over time correlates with increasingly pronounced physiological impairments. As fungal biomass within the host increases, the demand on host resources intensifies, exacerbating energy depletion and further diminishing the insect's capacity for thermogenesis (Wang et al., 2021).

5.5 CONCLUSION

This study provides an integrative evaluation of the effects of entomopathogenic fungi on *Euschistus heros*, combining thermal imaging and X-ray analysis to assess insect thermal responses and seed damage. Fungal treatments, particularly *Metarhizium anisopliae*, were associated with temporal changes in insect temperature and, more importantly, with a significant reduction in feeding damage to soybean seeds. X-ray imaging proved to be an effective non-destructive method for detecting internal seed damage, revealing that fungal treatments reduced concealed injuries more efficiently than the synthetic insecticide.

Although physiological mechanisms were not directly assessed, the results indicate that fungal exposure may impair insect performance prior to mortality, contributing to damage reduction. These findings support a functional perspective of biological control, in which the efficacy is linked not only to mortality but also to the suppression of feeding activity. Overall, this integrative approach enhances the evaluation of biological control strategies and highlights the potential of entomopathogenic fungi as sustainable tools in soybean pest management.

5.6 ACKNOWLEDGEMENTS

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6. CONCLUSÃO GERAL

1. Dados de eletropenetrografia (EPG) apresentam alta complexidade, caracterizados por contagens correlacionadas e sobredispersas. O modelo multinomial mostrou melhor desempenho para análise desses dados, com controle adequado da taxa de erro tipo I ($\approx$ 0,05) e maior poder estatístico, enquanto modelos como o Poisson apresentaram erro tipo I inflado (> 0,40);
2. Fungos entomopatogênicos alteram significativamente ($p < 0{,}05$) o comportamento alimentar *de Euschistus heros* em soja. A exposição a *Cordyceps javanica* e *Metarhizium anisopliae* reduz a frequência e a duração dos eventos de alimentação (Eh1, Eh2 e Eh3b). Os tratamentos com fungos aumentam o tempo em não alimentação (Z), indicando menor atividade alimentar dos insetos;
3. O tempo final da última prova (FTLP) é reduzido de aproximadamente 44 h no controle para a cerca de 31 h nos tratamentos com fungos;
4. A análise por imagens térmicas revelou variações temporais significativas na temperatura dorsal dos insetos ($p < 0{,}05$);
5. As alterações no comportamento alimentar estão diretamente associadas à redução do dano interno em sementes de soja. A análise por raios X demonstrou redução significativa do dano ($p < 0{,}05$), de 65,5% no controle para 27,8% (*Metarhizium anisopliae*) e 34,4% (*Cordyceps javanica*);
6. De forma integrada, os resultados demonstram que fungos entomopatogênicos reduzem a frequência, duração e continuidade dos eventos de alimentação, resultando em menor dano à cultura.