Revista CADERNO PEDAGÓGICO

REVISTA CADERNO PEDAGÓGICO – Studies Publicações Ltda.
ISSN: 1983-0882

Impact of *Euschistus heros* (F.) (Heteroptera: Pentatomidae) feeding on soybean seeds chemical compounds

Impacto da alimentação de *Euschistus heros* (F.) (Heteroptera: Pentatomidae) em compostos químicos de sementes de soja

Efectos de la alimentación por *Euschistus heros* (F.) (Heteroptera: Pentatomidae) en los compuestos químicos de las semillas de soja

DOI: 10.54033/cadpedv22n8-272

Originals received: 5/26/2025
Acceptance for publication: 6/19/2025

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ABSTRACT
The aim of this study was to evaluate the impact of feeding by the stink bug *Euschistus heros* (F.) on the dry matter of seeds and on the protein, oil and fatty acid contents. Soybean plants at developmental stage R6 (full pod filling) were exposed in protected cages to *E. heros* adults for 15 days. After this period, insects were removed and the plants kept in cages until the seeds were physiologically mature. Mature seeds damaged by insect feeding were characterized by examining the damage under a stereoscopic microscope. Protein, oil and fatty acid contents were analyzed from the damaged seeds. Results indicated that *E. heros* feeding altered the chemical components of seeds, reducing the percentage protein content and increasing the percentage oil content. The percentage content of palmitic acid was reduced, while that of linoleic and arachidic acids increased. The dry matter contents and stearic, oleic and linolenic acids of the seeds were not altered due to the feeding of *E. heros*.

Keywords: Soybean Seed Damage. Fatty Acids. Oil. Protein. Stink Bug.

RESUMO
O objetivo desse estudo foi avaliar o impacto que a alimentação do percevejo *Euschistus heros* (F.) causa na matéria seca das sementes de soja e nos teores de proteína, óleo e ácidos graxos. Plantas de soja, em estádio de desenvolvimento R6 (enchimento completo da semente), foram protegidas por gaiolas para confinamento e alimentação de adultos de *E. heros*, por 15 dias. Após esse período, os insetos foram retirados e as plantas mantidas em gaiola até a maturação fisiológica das sementes. A partir das sementes maduras, caracterizou-se as danificadas devido à alimentação dos insetos, por meio de visualização dos danos sob microscópio estereoscópico. Na sequência, foram obtidos os teores de proteína, óleo e ácidos graxos. A alimentação de *E. heros* alterou os componentes químicos das sementes, ocasionando redução percentual do teor de proteína e aumento percentual no teor de óleo. O conteúdo percentual do ácido palmítico foi reduzido, enquanto dos ácidos linoleico e araquídico aumentaram. Os teores de matéria seca e os ácidos esteárico, oleico e linolênico das sementes não foram alterados devido a alimentação de *E. heros*.

Palavras-chave: Danos em Semente de Soja. Ácidos Graxos. Óleo. Proteína. Percevejo Fitófago.

RESUMEN
El objetivo de este estudio fue evaluar el impacto de la alimentación por el chinche *Euschistus heros* (F.) en la materia seca de las semillas de soja y en el contenido de proteína, aceite y ácidos grasos. Las plantas de soja en la fase de

desarrollo R6 (llenado completo de la semilla) se protegieron con jaulas para confinar y alimentar a los adultos de *E. heros* durante 15 días. Tras este periodo, se retiraron los insectos y las plantas se mantuvieron en jaulas hasta que las semillas maduraron fisiológicamente. De las semillas maduras, las dañadas por la alimentación de los insectos se caracterizaron observando los daños con un microscopio estereoscópico. A continuación se obtuvo el contenido en proteínas, aceite y ácidos grasos. La alimentación de *E. heros* alteró los componentes químicos de las semillas, provocando una reducción porcentual del contenido en proteínas y un aumento porcentual del contenido en aceite. El contenido porcentual de ácido palmítico se redujo, mientras que los ácidos linoleico y araquídico aumentaron. El contenido de materia seca y de ácidos esteárico, oleico y linolénico de las semillas no se vio alterado por la alimentación con *E. heros*.

Palabras-clave: Daños a la Semilla de Soja. Ácidos Grasos. Aceite. Proteínas. Chinche Fitófaga.

1 INTRODUCTION

Soybean seeds contain about 20% oil and 40% protein (Delarmelino-Ferraresi *et al*., 2014). It is an important source of fatty acids, mainly omega 3 (linolenic acid), omega 6 (linoleic acid) and omega 9 (oleic acid), which are beneficial for human health, as they are related to reductions in heart disease, diabetes, cancer processes, body lipid mass, allergies and infections (e.g., Tinoco *et al*., 2007; Carrillo *et al*., 2012; Lucatto *et al*., 2014; Marangoni *et al*., 2020).

Stink bugs are considered major pests of economically important crops across all continents, including soybean, particularly in tropical regions (Schaefer and Panizzi, 2000). In the Neotropical Region, stink bugs are frequently found damaging plants from families such as Asteraceae, Brassicaceae, Fabaceae, Poaceae, and Solanaceae (Smaniotto and Panizzi, 2015).

Among the various species of stink bugs, *Euschistus heros* (F.), commonly known as the Neotropical brown stink bug, was relatively rare in the 1970s and is now considered the primary and most abundant pest of the soybean crop in Brazil (Panizzi *et al*., 2012).

The damage caused by stink bugs to infested plants is associated with two main strategies that these insects use during their feeding, namely the salivary sheath and cell rupture. In this second strategy, the bug employs two tactics: laceration (the insertion of mandibular stylets into and out of the plant tissue, tearing this tissue and causing mechanical cell destruction) and maceration (stylets spread aqueous saliva rich in digestive enzymes to degrade the cells). In both tactics, the insect ingests the “broth” produced by mechanical and/or chemical degradation (Backus *et al*., 2005). The action of enzymes, especially salivary enzymes, when stink bugs feed on the endosperm of seeds, causes cell rupture and dissolution of protein bodies (Depieri and Panizzi, 2011).

The feeding of stink bugs on soybean pods can lead to an increase in the production of inviable/dead seeds and a reduction in their vigor, germination potential and yield (Panizzi *et al*. 1979; Nunes and Corrêa-Ferreira, 2002; Scopel *et al*., 2016).

In addition to the damage caused by stink bugs related to the physical aspects of the seeds (e.g., mass and visual appearance), several studies have shown that these insects can also cause damage related to chemical aspects (e.g., protein and oil contents) (Todd and Turnipseed, 1974; Panizzi *et al*., 1979; Crancianinov *et al*., 2005; Bae *et al*., 2014; Vergara *et al*., 2019). Studies have also shown that the feeding of stink bugs on soybean seeds can cause changes in the composition of fatty acids (Todd *et al*., 1973).

Considering that the feeding of stink bugs on soybean plants can affect seed quality parameters, this study aimed to evaluate the impact on the chemical composition of soybean seeds caused by the damage of *E*. *heros* feeding activity.

2 METHODOLOGY

2.1 STINK BUG COLONY AND SOYBEAN PLANTS

Adult *E. heros* specimens were collected from soybean plants (*Glycine max* (L.) Merrill) at the National Wheat Research Center (Embrapa Wheat), located in Passo Fundo, RS, Brazil (28°15′S, 52°24′W). The collected bugs were transported to the Laboratory of Entomology, where they were housed in rearing plastic cages (25 × 20 × 20 cm) lined with paper to establish a colony. A cotton ball and a piece of toilet paper were placed in the cages as substrates for oviposition. The cages were maintained in a controlled walk-in chamber set at 25 ± 1°C, 65 ± 10% relative humidity, and a photoperiod of 14 hours of light.

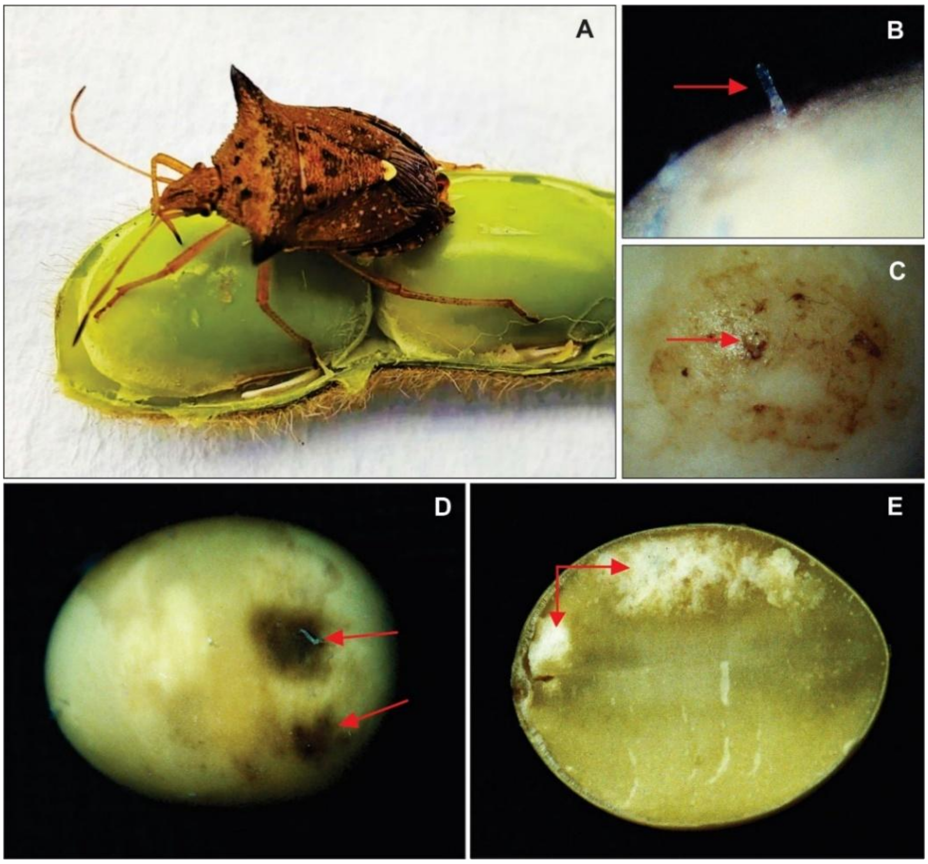
The insects were fed with a natural diet consisting of fresh green bean pods (*Phaseolus vulgaris* L.), soybean seeds (*G. max*), and raw peanuts (*Arachis hypogaea* L.). The food was replaced twice per week. Egg masses from the rearing cages were transferred to smaller plastic cages (11 × 11 × 3.5 cm) lined with filter paper and supplied with the same diet. These cages also included a wet cotton ball placed on a plastic lid (2 cm in diameter) to provide water and maintain humidity. Fifth-instar nymphs were subsequently moved to larger rearing cages (25 × 20 × 20 cm) to obtain adults for use in experiments. The rearing cages were maintained in a BOD climate chamber under the same climatic conditions as the adults, at 25 ± 1°C, 65 ± 10% relative humidity, and a 14-hour photoperiod. Soybean plants were cultivated in pots (2 liters) in a greenhouse by sowing seeds of the cultivar BRS 5601RR at biweekly intervals between October and November 2023. Only one plant was kept in each pot to use in the experiments.

2.2 INFESTATION OF SOYBEAN PLANTS

The experiment was conducted in a greenhouse under semi-controlled conditions. After reaching the R6 developmental stage (full pod filling), each potted plant was fully caged using a fine mesh net supported by a metal frame. The experiment employed a completely randomized design with two treatments (cages with infested plants and cages with non-infested plants) and 15 replicates, involving a total of 30 potted plants. In the infested treatment, plants were infested with 10 adult stink bugs (5 females + 5 males with ca. 20-day-old) from the laboratory colony. The stink bugs remained confined to the plants for 15 days for feeding and producing damaged seeds (Figure 1A). Daily inspections were conducted to remove any egg masses laid by females to prevent nymphal emergence and replace any dead insect. At the end of the infestation period, the stink bugs and all cages were removed, and the plants (infested and non-infested) were allowed to mature. Once mature, the soybean plants were harvested manually to obtain the seeds.

In the laboratory, seeds from cages infested with stink bugs were evaluated under a stereomicroscope to visualize the damage, aiming to select only the attacked seeds for use in chemical analyses. Seeds that showed signs of stink bug bites (punctures), with or without flanges (Figures 1B-C), and dark spots on the seed coat/cotyledons (Figure 1D) were considered damaged. Additionally, to ensure that the seeds had been damaged by the insects, a cut was made in each seed transversely to the cotyledons to visualize the internal whitish areas (indicative of the insect's stylet activity) (Figure 1E).

Figure 1. Soybean seeds damaged by *Euschistus heros*. A) Adult feeding on seed at stage R6 (full pod filling); B) Puncture with flange (salivary sheath outside seed tissue); C) Puncture without flange; D) Dark spots on coat/cotyledons; E) Internal whitish areas on the cotyledons (cross-section). Photos: Alberto L. Marsaro Júnior.



Source: Elaborated by the authors.

2.3 SEED QUALITY ANALYSIS

Damaged seeds were obtained and combined with undamaged ones to produce seed batches with increasing levels of damage: 0, 1, 2, 4, 8, 16, 32, and 64%, based on weight proportion. Each batch is composed of a sample of 20 grams of seeds (experimental unit), measured using an electronic balance (Sartorius model BP210S). For each level of damage, four repetitions were conducted to perform the chemical analyses.

Seed analyses were conducted at the Physical-Chemical and Chromatographic Analysis Laboratory of Embrapa Soybean in Londrina, PR. Each seed sample was ground using an experimental water-cooled mill (Tecnal multi-use mill, model TE 631/4) and sieved through a 35-mesh sieve to produce flour. Aliquots of the resulting flour were taken in duplicate for quality parameter assessments, including crude protein, dry matter, total oil, and fatty acid profile (palmitic, stearic, oleic, linoleic, linolenic, and arachidic acids).

Crude protein levels were determined by measuring total nitrogen content using the micro Kjeldahl method, as described in the Instituto Adolfo Lutz (2008) physical-chemical methods for food analysis. Dry matter content was calculated based on humidity levels measured with an infrared heating balance (Ohaus, model MB-45). Total oil levels were determined following the Soxhlet extraction method (AOCS, 2009). The fatty acid profile of the oil was analyzed through methylation with 1% sodium methoxide, followed by separation via gas chromatography (Christie, 1989; Rayford *et al*., 1994; Abidi *et al*., 1999), using a Thermo Trace GC Ultra system.

To assess whether bugs feeding on seeds altered seed mass, 140 damaged seeds and 140 undamaged seeds were individually weighed using an electronic scale (Sartorius model BP210S). Next, seed moisture was determined according to the methodology proposed by Brasil (2009). Finally, the values obtained were corrected for the dry mass of the seeds.

2.4 STATISTICAL ANALYSIS

In this study, the effects of damage caused by *E. heros* on soybean seed quality were analyzed using the statistical program R version 4.4.1 (R Core Team, 2024). Before model fitting, we conducted an exploratory data analysis to verify model assumptions, ensuring a suitable framework for our analysis. The data were fitted to a nonlinear model to effectively capture the relationship between damage levels and quality indicators. Visualizations created with the R package ggplot2 (Wickham, 2016) included the adjusted model line and 95% confidence intervals, calculated through bootstrapping (1000 replicates) to provide robust estimates for potential data non-normality. The nonlinear model fitting was as follows:

$$y = a \cdot exp(bx),$$

where:

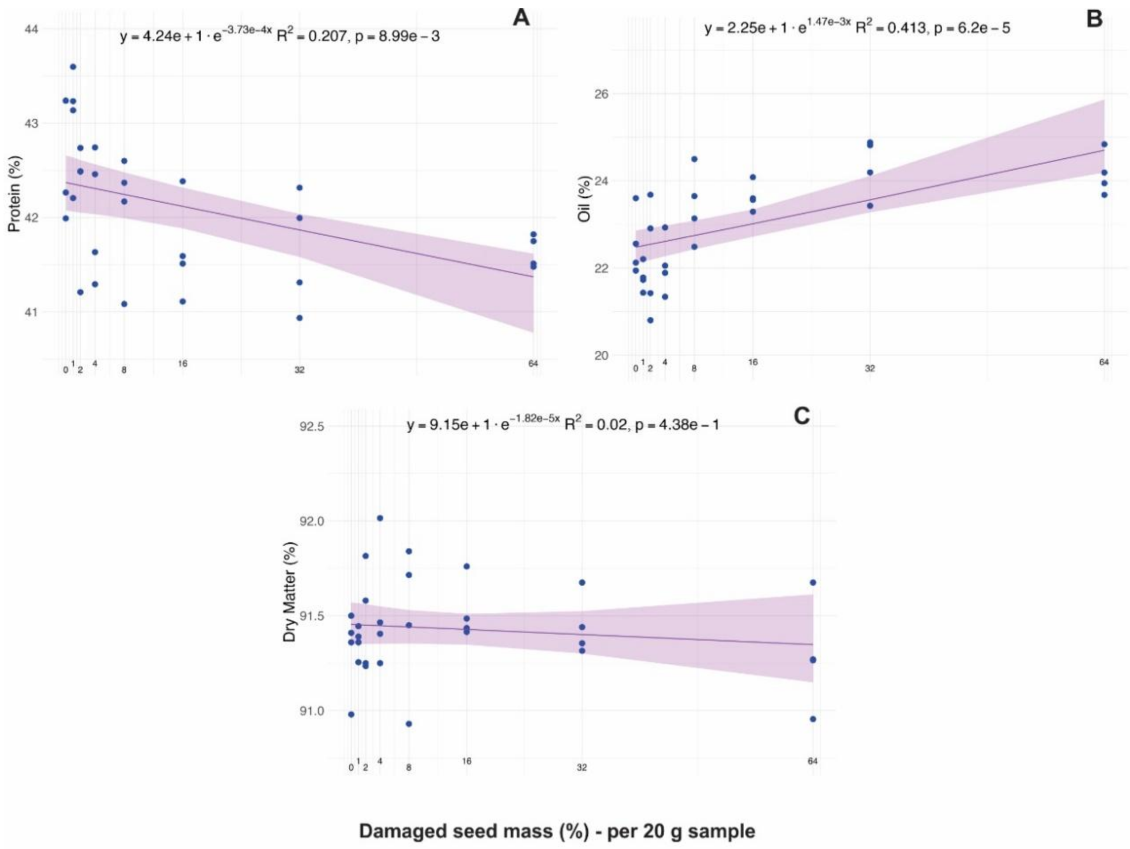
- y is the dependent variable,
- a is a constant that scales the function,
- exp represents the exponential function,
- b is the rate of growth or decay, and
- x is the independent variable.

Following model fitting, we conducted diagnostics to validate the model's performance and reliability, ensuring sound conclusions. The masses of damaged and undamaged seeds were compared using a two-sample independent t-test.

3 RESULTS AND DISCUSSION

The non-linear relationship between seed damage caused by *Euschistus heros* feeding and its effects on protein, oil, and dry matter composition is illustrated in Figures 2 A-C; its effects on the contents of palmitic, linoleic, arachidic, stearic, oleic, and linolenic fatty acids are depicted in Figures 3 A-F; and the impact of stink bug feeding on seed mass is presented in Figure 4. The analyses showed a significant negative association between damaged seeds and protein content (p≤8.99e-3), indicating that this component percentage decreases as damage levels increase (Figure 2A). Conversely, there was a significant positive association between damaged seeds and oil content (p≤6.20e-5), suggesting that this component percentage increases with higher damage levels (Figure 2B).

Figure 2. Nonlinear regression model fit for the relationship between soybean seed damaged by *Euschistus heros* and protein (A), oil (B), and dry matter (C). The model y = ae^{bx} was fitted to the data, where *a* and *b* are estimated parameters. The solid line represents the fitted curve, and the shaded ribbon indicates the 95% confidence interval (CI) around the predicted values.

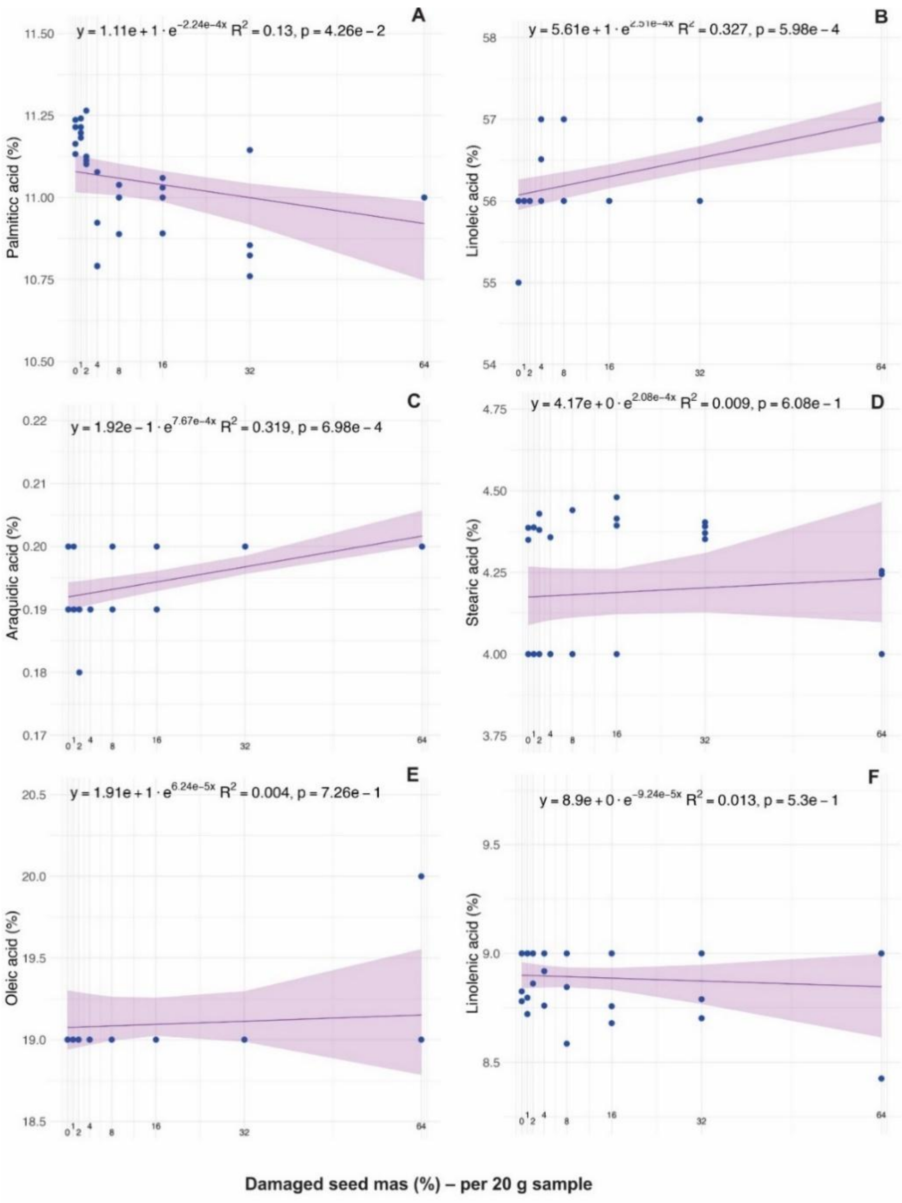


Source: Elaborated by the authors.

During feeding, stink bugs insert their mandibular stylets into plant tissue to cause mechanical destruction of cells and to inject saliva rich in digestive enzymes for cell degradation (Backus *et al*., 2005). These enzymes, mainly amylases, lipases, and proteases, are present in the salivary glands and also in the intestine of these sucking insects (Silva *et al*., 2012; Lomate and Bonning, 2016; Ferreira, 2017; Campos *et al*., 2021). Soybean seeds have a protein content approximately twice that of their oil content. Therefore, due to this greater availability of protein, which the insect presents proteases in the salivary glands and intestine, and aiming to meet its nutritional needs, it is likely that these digestive enzymes presented greater enzymatic activity than the other enzymes, consequently causing the reduction in the protein percentage content of the seeds observed in the present study. Vergara *et al*. (2019) also found reductions in protein content with increasing levels of damage to soybean seeds caused by stink bugs. Other authors, however, report a slight increase in protein content in damaged soybean seeds by stink bugs compared to undamaged seeds (e.g., Todd and Turnipseed, 1974; Bae *et al*., 2014; Rolando *et al*., 2025). These contrasting results may be due to differences in the methodologies used and the interpretation of the results. The increase in protein reported may be, in fact, its greater percentage value related to other chemicals of the seed in damaged seeds, and not an absolute increase in protein content *per se*. The same can be said with relation to the tendency of increase in oil content as the levels of seeds damaged by *E. heros* increased (Figure 2B). The observed inverse relationship between the protein and oil contents (i.e., increase in protein content corresponds to the reduction in oil content and vice versa) has been reported by other authors (e.g., Brennan and Bolland, 2009; Delarmelino-Ferraresi *et al*., 2014).

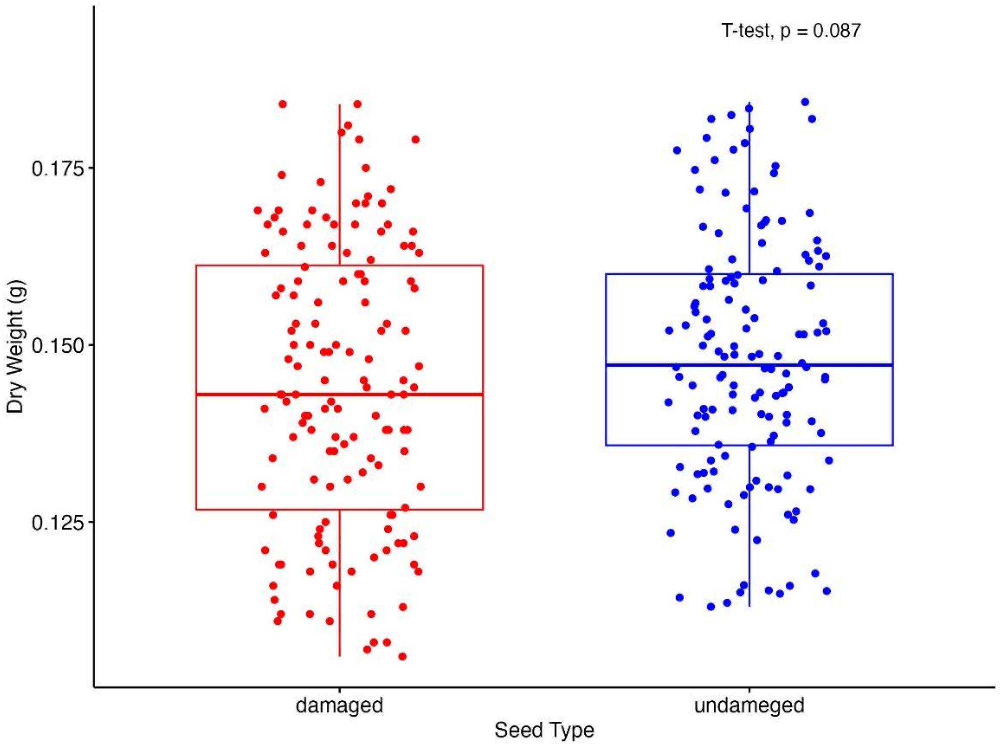
In undamaged seeds, the levels of fatty acids found in soybean oil were: linoleic (55.8%), palmitic (11.2%), oleic (19.7%), linolenic (8.8%), stearic (4.4%), and arachidic (0.2%) (Figures 3 A-F), which are similar to those found in soybean seeds in the studies by Martin *et al*. (2008) and Ivanov *et al*. (2010). There was a significant negative association between damaged seeds and palmitic acid content ($p \leq 4.26e\text{-}2$), indicating that this component percentage decreases as damage levels increase (Figure 3A). Conversely, there was a significant positive association between damaged seeds and linoleic acid ($p \leq 5.98e\text{-}4$) and arachidic acid ($p \leq 6.98e\text{-}4$) content, with their percentage levels increasing alongside damage levels (Figures 3B and 3C). Although the levels of the other acids did not change significantly as damage levels increased, stearic acid ($p \leq 6.08e-1$), oleic acid ($p \leq 7.26e-1$), and linolenic acid ($p \leq 5.30e-1$) remained consistent (Figures 3D, 3E, and 3F), respectively. Todd *et al*. (1973) observed that as the levels of damage by *N. viridula* to soybean seeds increased, there was a tendency for the levels of oleic, palmitic, stearic, and arachidic acids to rise. Conversely, there was a tendency for the levels of linoleic and linolenic acids to decrease. The trend for arachidic acid in the present study aligns with the findings of Todd *et al*. (1973), while the trends for the other acids diverged. These divergences in results between studies can likely be attributed to the different methodologies and species of stink bugs examined. *E. heros* obtains fatty acids from seeds to fulfill its nutritional requirements. Furthermore, studies have demonstrated that in addition to their nutritional role, fatty acids, along with waxes and hydrocarbons found in the insect's body, are linked to defense mechanisms against infections by entomopathogenic fungi. They can also inhibit the attachment of fungi to the insect's cuticle and exhibit antifungal activity (Wrońska *et al*., 2023).

Figure 3. Nonlinear regression model fit for the relationship between soybean seed damaged by *Euschistus heros* and palmitic acid (A), linoleic acid (B), arachidic acid (C), stearic acid (D), oleic acid (E), and linolenic acid (F). The model y = ae^{bx} was fitted to the data, where *a* and *b* are estimated parameters. The solid line represents the fitted curve, and the shaded ribbon indicates the 95% confidence interval (CI) around the predicted values.



Source: Elaborated by the authors.

Figure 4. Boxplot comparing dry weight (g) between damaged and undamaged seeds. The dots represent the observed values and the T-test p-value is shown in the top-right corner.



Source: Elaborated by the authors.

Interestingly, there was no significant change in the dry matter content of the seeds as the levels of damage increased (p≤4.38e-1) (Figure 2C). Additionally, when comparing the mass of damaged seeds (mean = 0.144 g) with undamaged seeds (mean = 0.148 g), there was no statistically significant difference (independent t-test, p>0.05) (Figure 4). This indicates that the feeding damage caused by *E. heros* does not significantly affect the mass of the seeds.

4 CONCLUSION

In summary, results of this study show that the feeding activity of *E. heros* on soybean seeds alters their chemical components, causing a reduction in protein content percentage and an increase in oil content percentage. In addition, the palmitic acid content was reduced percentage while the linoleic and arachidic acid contents were increased percentage; however, the stearic, oleic, and linolenic acid contents, as well as the dry matter of the seeds, were not altered.

The results of this study are relevant to academy as they show that stink bugs feeding alters physical and chemical components relevant to the products from seed processing. Results are also important for society, especially farmers, who might improve the management of stink bugs to minimize their overlooked chemical damage.

Despite the relevance of the results obtained, one limitation was the lack of oligosaccharide analysis and the inclusion of only one species of stink bug. Therefore, future studies should fill this gap.

ACKNOWLEDGMENTS

We thank the anonymous reviewers for their comments and improvements to the manuscript.

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