

ORIGINAL ARTICLE OPEN ACCESS

Leaf Extracts of *Coffea racemosa* Inhibit Trypsin Activity and Affect Larval Development in *Spodoptera frugiperda* and *Diatraea saccharalis*

Nicole de Paula Souza¹ | Diandro Ricardo Barilli² | Sandy Souza Fonsêca¹ | Ana Letícia Zero dos Santos¹

¹Universidade Estadual Paulista, Campus de Jaboticabal, Jaboticabal, São Paulo, Brazil | ²Universidade Tecnológica Federal do Paraná, Campus Francisco Beltrão, Francisco Beltrão, Paraná, Brazil

Correspondence: Nicole de Paula Souza (npsouza@outlook.com.br)

Received: 24 July 2025 | **Revised:** 9 March 2026 | **Accepted:** 17 March 2026

Keywords: *Diatraea saccharalis* | digestive enzyme inhibition | insect resistance | plant metabolites | *Spodoptera frugiperda*

ABSTRACT

Lepidopteran pests, such as *Spodoptera frugiperda* and *Diatraea saccharalis*, cause significant economic losses in agriculture, especially in tropical regions. Traditional control methods face challenges due to the development of insect resistance to chemical and protein-based insecticides, necessitating the search for new management strategies. This study evaluated the insecticidal potential of *Coffea racemosa* leaf extracts against *S. frugiperda* and *D. saccharalis*, providing the first evidence of this plant species' activity against these pests. Enzymatic extracts from larval midguts were used to assess trypsin-like digestive enzyme activity, while leaf extracts and chromatographic fractions were tested for inhibitory effects and biological parameters. Significant inhibition of digestive trypsins was observed in both species, with stronger inhibition and higher mortality recorded in *D. saccharalis*. Molecular filtration revealed that both protein and non-protein fractions possessed inhibitory activity, indicating that low-molecular-weight non-protein compounds are likely the main contributors to the observed insecticidal effects. While *S. frugiperda* exhibited reduced larval and pupal weights and prolonged development without significant mortality, *D. saccharalis* showed marked mortality in addition to impaired development. These findings highlight *C. racemosa* metabolites as a promising source of insecticidal molecules for the management of lepidopteran pests.

1 | Introduction

Brazil is one of the world's largest food producers and exporters, with agribusiness accounting for 29.4% of the Brazilian GDP (CEPEA 2025). Despite its magnitude, the sector faces several phytosanitary challenges, including attacks by arthropod pests. These pests annually reduce global agricultural production by approximately 18%–20%, resulting in economic losses exceeding 470 billion dollars (Sharma et al. 2017; Savary et al. 2019).

Lepidopteran pests cause significant agricultural damage through defoliation, stem boring, and by facilitating pathogen entry into plant tissues. Among the most economically important species are the fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith,

1797) (Lepidoptera: Noctuidae), and the sugarcane borer (SCB), *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae).

S. frugiperda is a highly polyphagous species native to the Americas that has currently spread across Africa, Asia, and Oceania (Goergen et al. 2016; Chormule et al. 2019; Qi et al. 2021). It attacks a wide range of crops, including corn, cotton, rice, soybean, and sugarcane, and is considered one of the main pests of corn, where yield losses may reach 25% under conventional management (Barros et al. 2010; Montezano et al. 2018; Overton et al. 2021). In Brazil, FAW occurs in both first and second crop seasons, with larval stages damaging plants throughout vegetative and reproductive phases, particularly by feeding on young leaf whorls (Sarmiento et al. 2002; Farinelli and Fornasieri Filho 2006).

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2026 The Author(s). *Entomologia Experimentalis et Applicata* published by John Wiley & Sons Ltd on behalf of Netherlands Entomological Society.

D. saccharalis is an oligophagous species that feeds primarily on Poaceae crops, including sugarcane, corn, rice, and sorghum (Cruz 2007; Vilela et al. 2017; Pelosi et al. 2023). It is widely distributed in the sugarcane-growing regions of Brazil, where it is regarded as a key pest (Macedo and Macedo 2004; Sandoval and Senô 2010). Larvae bore into plant stems, forming galleries that compromise plant structure, reduce yield, and facilitate the entry of secondary pests and pathogens, negatively affecting both productivity and raw material quality (Ferreira et al. 2018; Peñaflor and Bento 2019; Gallan et al. 2022). In Brazilian sugarcane fields, infestation levels can result in internode perforation rates exceeding 20% (Carbognin 2016; Francischini et al. 2019).

The control of herbivores in agricultural systems is essential to ensure the economic viability of production. However, available control methods have limitations, mainly due to the development of resistance to insecticidal molecules and proteins (Amaral et al. 2023; do Nascimento et al. 2023; Tabashnik et al. 2023). In this context, plant species that exhibit natural resistance to insect herbivory represent a promising and still underexplored source of insecticidal compounds for the management of major agricultural pests, including *S. frugiperda* and *D. saccharalis*.

Therefore, the search for new insecticidal molecules has become crucial for establishing pest management strategies. Among the most promising sources of such compounds are plants, which, due to their long history of co-evolution with insects, have resistance mechanisms involving chemical compounds known as secondary metabolites. The main classes of these compounds include terpenes, nitrogen-containing compounds, phenolic compounds, and defense-related proteins (Boiça Junior et al. 2016; Ahmed et al. 2017; De Souza and Peñaflor 2024).

An important class of defense proteins comprises the protease inhibitors, which disrupt insect digestion by inhibiting the activity of digestive enzymes. In lepidopterans, serine proteases—primarily trypsin and chymotrypsin—are the predominant digestive enzymes in the midgut. Inhibiting these enzymes impairs nutrient absorption, thereby affecting insect development (Pinto et al. 2018; Singh et al. 2020; Divekar et al. 2023).

A wild coffee species, *Coffea racemosa* Lour., has been studied as a source of insecticidal molecules due to its resistance to the coffee leaf miner, *Leucoptera coffeella* (Guérin-Mèneville and Perrottet 1842) (Lepidoptera: Lyonetiidae). In addition to its potential for breeding resistant hybrids, *C. racemosa* is a promising source of secondary metabolites with insecticidal activity, which can be utilized in the management of lepidopteran pests (Guerreiro Filho et al. 1991; Ramiro et al. 2004; Alves et al. 2011; Souza 2019; Costa et al. 2025).

Given the economic impact caused by *S. frugiperda* and *D. saccharalis* and the increasing limitations of conventional control strategies, this study evaluates the insecticidal potential of compounds present in *Coffea racemosa* leaves against these pests, focusing on their effects on digestive enzyme activity and key biological parameters.

2 | Materials and Methods

2.1 | Enzymatic Extracts From Larval Midguts

Larvae of *S. frugiperda* and *D. saccharalis*, obtained from laboratory rearing colonies, were anesthetized on ice for 10 min. The head and posterior extremities were removed, and larvae were dissected in a saline solution (125 mM NaCl). Midguts were extracted by making a longitudinal incision along the ventral region. The midgut was separated from the posterior intestine by a transverse cut at the point of insertion of the Malpighian tubules. Midguts including their peritrophic membranes and contents were homogenized in a Potter-Elvehjem homogenizer at a ratio of 3 midguts per 1 mL of water (4°C) for *S. frugiperda* and 5 midguts per 1 mL of water (4°C) for *D. saccharalis*. The homogenates were centrifuged (10000g, 10 min, 4°C), and the supernatants were collected as enzymatic extracts for subsequent activity assays. Enzymatic extracts were stored at −20°C until use.

2.2 | Preparation of *Coffea racemosa* Leaf Extract

Leaves of *C. racemosa* with uniform age and appearance were flash-frozen in liquid nitrogen and ground to a fine powder. The material was extracted with cold distilled water (1:5, w/v) under agitation (150 rpm) for 30 min at 4°C. After centrifugation (10000g, 10 min, 4°C), the supernatant was collected as the inhibitor source and stored at −20°C until use.

2.3 | Trypsin Activity Assay

Trypsin activity of the larval midguts was determined using the enzymatic extracts and the substrate BApNA at 1.0875 mM, prepared in 0.1 mM glycine-NaOH buffer at pH 9.0 (Erlanger et al. 1961). The assay was conducted at 30°C with absorbance recorded every minute at 410 nm using a Multiskan Sky microplate spectrophotometer (Thermo Scientific). The enzymatic extract was diluted to obtain proportional results. Each reaction contained 40 µL of 40×-diluted enzymatic extract and 160 µL of substrate.

One trypsin unit (U) was defined as the amount of enzyme required to produce 1 nmol of p-nitroaniline per minute, calculated using a p-nitroaniline standard curve (Erlanger et al. 1961). Trypsin activity was expressed as trypsin units per gram of midgut tissue (U/g of midgut).

2.4 | Inhibition of Trypsin Activity by Leaf Extract

Trypsin inhibition in the midguts of *S. frugiperda* and *D. saccharalis* caused by *C. racemosa* leaf extract was assessed by comparing the trypsin activity in standard enzyme assays with those containing the leaf extract. Inhibition data were expressed as a percentage (%) relative to the activity observed in control reactions (100% activity). The inhibition assays contained 40 µL of leaf extract, 40 µL of 40×-diluted enzymatic extract, and 160 µL of BApNA substrate. Control reaction contained 40 µL of distilled water instead of the leaf extract.

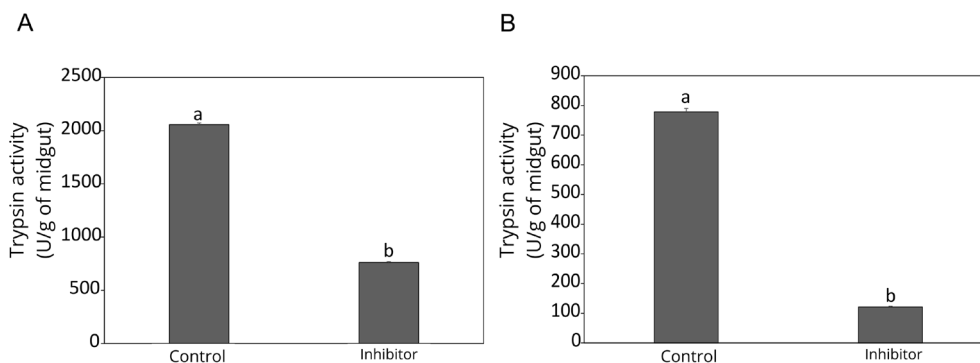


FIGURE 1 | Digestive trypsin activity of *Spodoptera frugiperda* (A) and *Diatraea saccharalis* (B) in trypsin units per gram of tissue (U/g of midgut) in control treatments and treatments containing *Coffea racemosa* leaf extract ($n = 5$). Means followed by different letters differ by ANOVA ($p \leq 0.05$).

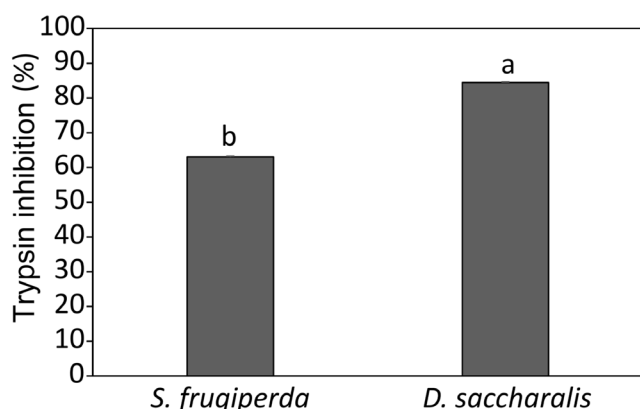


FIGURE 2 | Inhibition of digestive trypsin (%) of *Spodoptera frugiperda* and *Diatraea saccharalis* larvae by *Coffea racemosa* leaf extract ($n = 5$). Means followed by different letters differ by ANOVA ($p \leq 0.05$).

2.5 | Molecular Filtration of Leaf Extract

The *C. racemosa* leaf extract was subjected to molecular filtration, a purification technique that separates molecules by size exclusion in a chromatographic column. The resulting fractions were used for biochemical and biological evaluations to determine their effects on the target larvae. The leaf extract was lyophilized and concentrated 10 $\times$. The concentrated sample was applied to a molecular filtration column (39 cm height, 2.5 cm diameter) containing Sephadex G-25 resin (GE HealthCare, Uppsala, Sweden) occupying 27 cm of column height. The flow rate was adjusted to 1.5 mL/min, and eluted fractions were analyzed at 280 nm to detect the presence of proteins and aromatic groups, such as phenolic molecules, by placing 200 μ L of each fraction in 96-well plates compatible with UV reading (Corning, Kennebunk, ME, USA). Fractions were grouped based on similar molecular weight elution profiles, and biochemical tests were performed to determine their inhibitory effects on larval digestive enzymes.

2.6 | Effects of Fractionated Leaf Extract on Larval Development

Fraction groups obtained by molecular filtration of *C. racemosa* leaf extract (hereafter referred to as groups of fractions, GF) were quantified for total protein content using the Bradford method

(Bradford 1976) and adjusted to a concentration of 23 μ g/mL. Concentrated fractions were applied (2000 μ L) to the surface of artificial diets for *S. frugiperda* and *D. saccharalis*, following the protocols of Kasten et al. (1978) and Hensley and Hammond (1968), respectively.

Larvae were individually placed in plastic containers and maintained at 25°C with a 12 h photoperiod. The parameters evaluated included larval weight at 7 and 14 days, pupal weight 24 h after pupation, duration of larval and larval–adult stages, and viability of immature stages. These were compared to insects reared on untreated diet (control). The experiment followed a completely randomized design with seven treatments: five fraction groups (GF), crude foliar extract (CE), and control, with approximately 50 replicates per treatment.

2.7 | Statistical Analysis

Data were tested for normality using the Cramer–von Mises test ($\alpha = 0.05$) and for homogeneity of variances using the Bartlett test. Variables with normal distribution and homoscedasticity were analyzed by ANOVA, followed by Tukey's post hoc test ($\alpha = 0.05$). For non-normally distributed data, the Kruskal–Wallis test with Bonferroni correction was applied. Statistical analyses were performed using R software 4.0 (R Core Team 2020).

3 | Results

3.1 | Trypsin Activity and Inhibition by Coffea racemosa Leaf Extract

Trypsin activity in *S. frugiperda* midguts was approximately 2057 U/g of midgut tissue (Figure 1A), while *D. saccharalis* exhibited lower activity, around 778 U/g (Figure 1B). Significant inhibition of trypsin activity was observed in the presence of *C. racemosa* leaf extract for both species (*S. frugiperda*: $F_{1,4} = 986.52$, $p < 0.001$; *D. saccharalis*: $F_{1,4} = 1560.85$, $p < 0.001$).

Comparing the two lepidopteran species, it was observed that the *C. racemosa* leaf extract resulted in a significantly higher inhibition (~84%) of the digestive trypsin activity of *D. saccharalis* compared to *S. frugiperda* (~64%) ($F_{1,4} = 1204.15$; $p < 0.001$) (Figure 2).

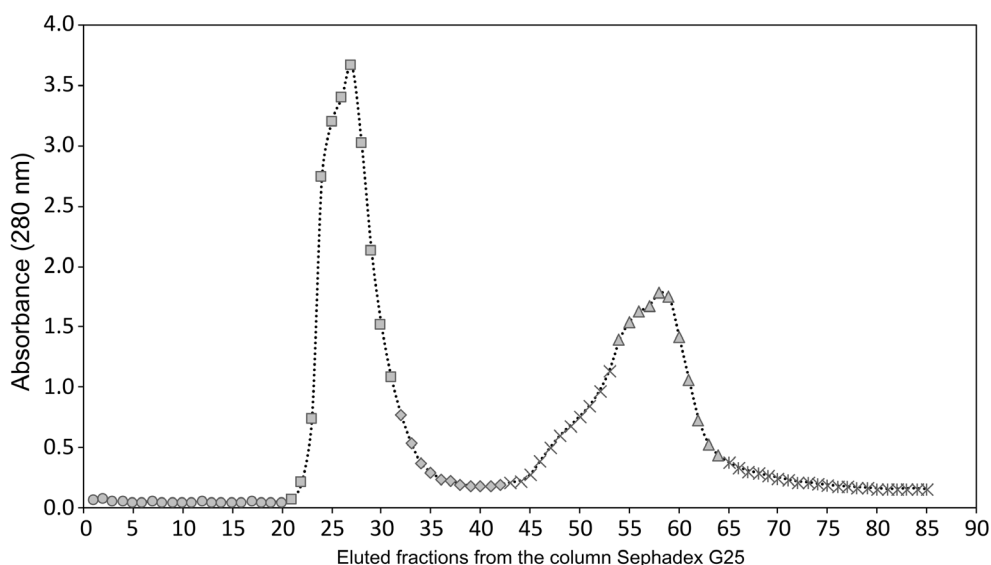


FIGURE 3 | Elution profile generated by molecular filtration of *Coffea racemosa* leaf extract using Sephadex G-25 observed in a spectrophotometer set at a wavelength of 280 nm. Fractions 1 to 20 make up G1 (○); fractions 21 to 31 make up G2 (□); fractions 32 to 42 make up G3 (◇); fractions 43 to 53 make up G4 (×); fractions 54 to 64 make up G5 (△); fractions 65 to 80 make up G6 (*).

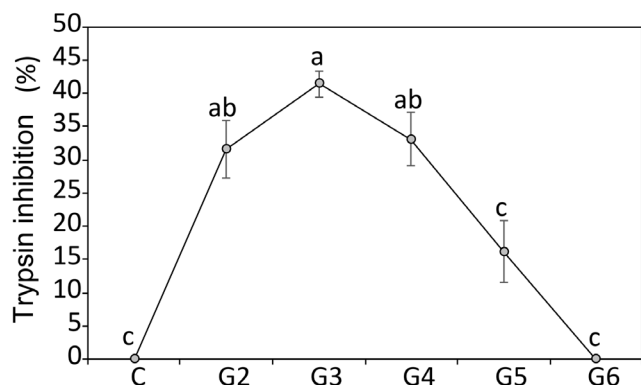


FIGURE 4 | Trypsin inhibition (%) of *Spodoptera frugiperda* in the presence of the content of the fraction groups of *Coffea racemosa* leaf extract (G2–G6) obtained after molecular filtration on Sephadex G-25. C = trypsin inhibition in the absence of *C. racemosa* extract. Means followed by the same letter do not differ by Tukey's test ($p > 0.05$).

3.2 | Molecular Filtration of *Coffea racemosa* Leaf Extract

The molecular filtration of leaf extract produced a chromatographic elution profile with two main peaks at 280 nm. Based on this profile, fractions were grouped according to their elution order and molecular weights. Fractions 1 to 20 (belonging to Group 1) were discarded as they represented the column's dead volume. Fractions 21 to 31 (first peak) made up Group 2, fractions 32 to 42 (first region between peaks) made up Group 3, fractions 43 to 53 (second region between peaks) made up Group 4, fractions 54 to 64 (second peak) made up Group 5, and fractions 65 to 80 (end of the run) made up Group 6 (Figure 3).

The highest concentration of total proteins quantified by the Bradford method was observed in G2 (0.66 µg/mL), which corroborates what is expected for this type of chromatography. No

proteins were detected in fractions G3 and G5, and only 0.11 µg/mL in G4 and 0.31 µg/mL in G6. These results suggest that the protein content was concentrated in G2 and only non-protein molecules that interfere with the Bradford assay were detected in groups G4 and G6.

3.3 | Effects of *Coffea racemosa* Leaf Extract on Trypsin Activity and the Development of *Spodoptera frugiperda*

The fractionation of the *C. racemosa* leaf extract resulted in 5 groups of fractions (G2–G6). When analyzed for their ability to inhibit *S. frugiperda* digestive trypsins, it was observed that only G2, G3, and G4 inhibited digestive trypsins ($F_{5,12} = 20.90$; $p < 0.0001$) (Figure 4).

As trypsin inhibition was observed in the G2 group, it is suggested that this inhibition is due to a protein inhibitor. The inhibitions observed for groups G3 and G4, conversely, are suggested to be due to non-protein inhibitors, as fractions G3 and G4 showed no protein (null or low value by Bradford quantification and elution after the first peak in chromatography).

In terms of the biological development of *S. frugiperda*, the extract of *C. racemosa* leaves did not result in larval mortality either in the form of the crude extract (CE) or in the groups of fractions (GF) produced ($F_{6,28} = 1.60$; $p = 0.1829$). However, developmental parameters were affected by consumption of the CE or the GF.

The weight of the FAW larvae was assessed 7 days after the start of feeding and was reduced by the consumption of groups G2 and G4 (with trypsin inhibition) and G5 (without trypsin inhibition) ($F_{6,245} = 9.45$; $p < 0.05$) (Figure 5A). After 14 days, both the CE and groups G2, G3, and G4 (with trypsin inhibition) and groups G5 and G6 (without trypsin inhibition) resulted in significantly

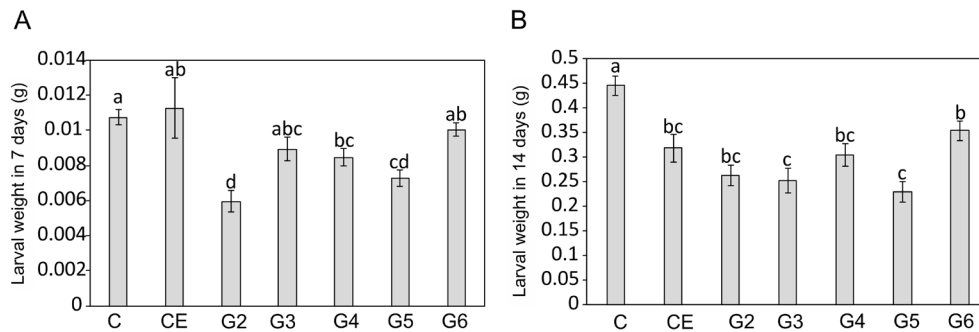


FIGURE 5 | Weight (g) of *Spodoptera frugiperda* larvae at 7 (A) and 14 (B) days after starting to feed on *Coffea racemosa* leaf extract. C = diet without *C. racemosa* extract. CE = diet containing crude foliar extract of *C. racemosa*. G = diet containing groups of *C. racemosa* extract fractions obtained by molecular filtration. Means followed by the same letter do not differ according to the Tukey test ($p > 0.05$).

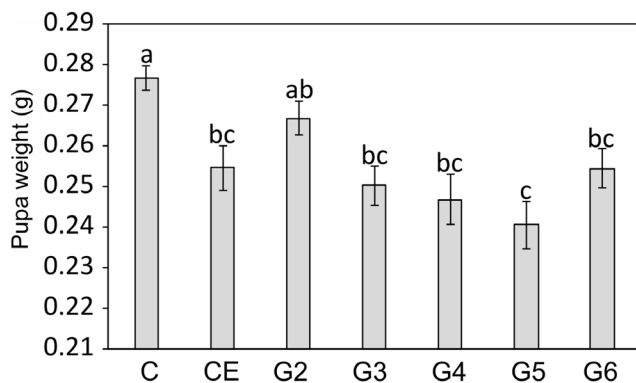


FIGURE 6 | Pupa weight (g) of *S. frugiperda* 24 h after being formed from larvae fed *Coffea racemosa* leaf extract. C = diet without *C. racemosa* extract. CE = diet containing crude foliar extract of *C. racemosa*. G = diet containing groups of fractions of the *C. racemosa* extract obtained by molecular filtration. Means followed by the same letter do not differ according to the Tukey test ($p > 0.05$).

lower weights of *S. frugiperda* larvae ($F_{6,246} = 11.44$; $p < 0.05$) (Figure 5B).

The weight of *S. frugiperda* pupae was measured 24 h after ecdysis and a reduction in pupae weight was observed for the treatments in which CE was consumed, G3, G4, G5, and G6 ($F_{6,236} = 6.38$; $p < 0.05$) (Figure 6).

The larval period was prolonged by approximately 3 days in all treatments except G6 ($H_6 = 58.13$; $p < 0.05$) (Figure 7A). A similar pattern was observed for the total development time from neonate to adult, with all treatments, except G6, leading to significant prolongation of the development period of *S. frugiperda* ($H_6 = 37.17$; $p < 0.05$) (Figure 7B).

3.4 | Effects of *Coffea racemosa* Leaf Extract on Trypsin Activity and the Development of *Diatraea saccharalis*

Similar to *S. frugiperda* trypsins, groups G2, G3 and G4 significantly inhibited the activity of *D. saccharalis* digestive trypsins ($F_{5,12} = 63.26$; $p < 0.05$) (Figure 8). However, unlike FAW, the inhibition of SCB trypsins promoted by group G2 (~35%) was

significantly higher than the inhibition observed in group G3 (~20%) followed by G4 (~12.5%).

In contrast to *S. frugiperda*, consumption of the CE of *C. racemosa* as well as fractions G3, G4, and G5 resulted in larval mortality of *D. saccharalis* (Figure 9).

Among surviving *D. saccharalis* larvae, no significant differences were observed in relation to the control in terms of larval weight at 7 days after the start of feeding ($F_{6,107} = 4.43$; $p < 0.05$), larval weight at 14 days after the start of feeding ($F_{6,107} = 4.43$; $p < 0.05$), weight gain between 7 and 14 days after the start of feeding ($F_{6,107} = 4.43$; $p < 0.05$), pupal weight ($F_{6,104} = 2.18$; $p = 0.051$ ns), period of larval development ($H_6 = 7.27$; $p = 0.30$ ns) and period of development from neonate to adults ($H_6 = 1.02$; $p = 0.99$ ns).

4 | Discussion

Among the evaluated Lepidoptera, FAW (*S. frugiperda*) exhibits a broader host range than SCB (*D. saccharalis*), a difference that may be associated with physiological traits related to digestion (Pinto 2020). In this study, digestive trypsin activity (U/g of intestine) was significantly higher in FAW larvae than in SCB larvae, suggesting that enhanced proteolytic capacity may contribute to FAW's ability to utilize a wider range of host plants.

The residual trypsin activity in FAW larvae after exposure to a *C. racemosa* crude leaf extract was also higher than in SCB, potentially explaining the lower developmental impact and absence of mortality in FAW, in contrast to the high mortality observed in SCB after exposure to both the crude extract and fractionated groups.

Furthermore, FAW larvae may exhibit greater tolerance to the presence of trypsin inhibitors compared to SCB larvae due to their ability to modulate gene expression in response to protease inhibitor ingestion (Brioschi et al. 2007; Paulillo et al. 2012; Oliveira et al. 2013; Souza et al. 2016).

In addition to its broad host utilization capacity, FAW's adaptive potential to insecticidal molecules and proteins is widely recognized. This adaptability is attributed to the presence of detoxification mechanisms, which enable this species to metabolize a

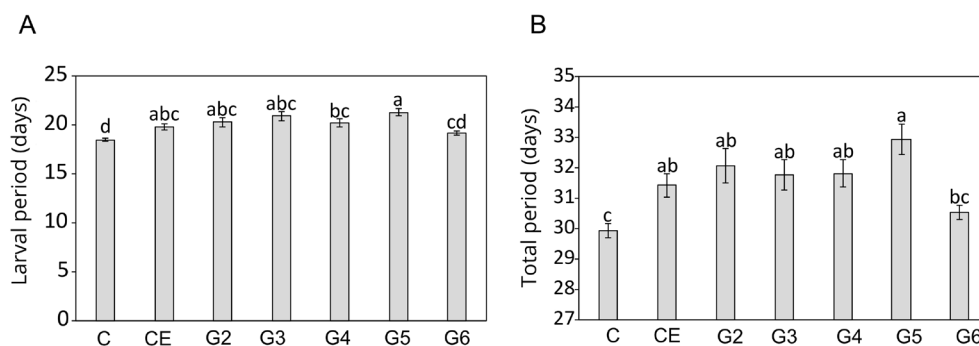


FIGURE 7 | Duration of the larval period (A) and duration of the development period from neonate to adults (B) (days) of *Spodoptera frugiperda* fed on *Coffea racemosa* leaf extract. C = diet without *C. racemosa* extract. CE = diet containing crude foliar extract of *C. racemosa*. G = diet containing groups of *C. racemosa* extract fractions obtained by molecular filtration. Means followed by the same letter do not differ by the Kruskal Wallis test ($p > 0.05$).

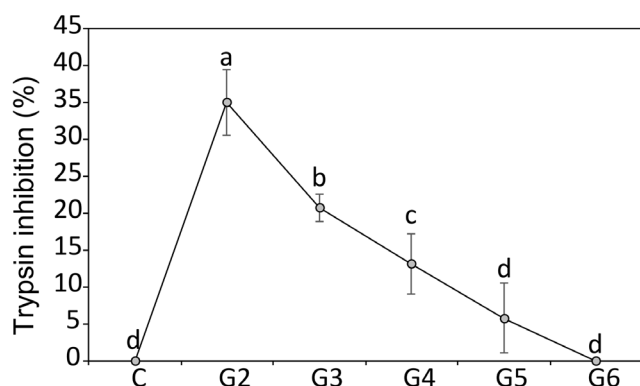


FIGURE 8 | Inhibition of digestive trypsins (%) of *Diatraea saccharalis* in the presence of the content of the fraction groups of *Coffea racemosa* leaf extract (G2–G6) obtained after molecular filtration on Sephadex G-25. C = trypsin inhibition in the absence of *C. racemosa* extract. Means followed by the same letter do not differ according to Tukey's test ($p > 0.05$).

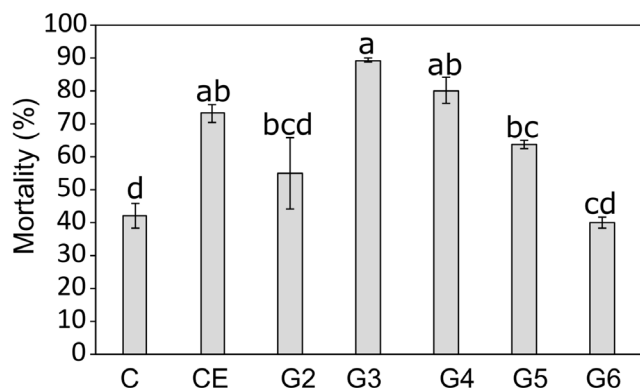


FIGURE 9 | Mortality (%) of *Diatraea saccharalis* after feeding on diet containing *Coffea racemosa* leaf extract. C = diet without *C. racemosa* extract (control); CE = diet containing crude foliar extract of *C. racemosa*; G = diet containing groups of *C. racemosa* extract fractions obtained by molecular filtration. The experiment was conducted in a completely randomized design with seven treatments, each consisting of approximately 50 replicates, with one larva per replicate. Means followed by the same letter do not differ according to Tukey's test ($p > 0.05$).

variety of plant allelochemicals (Fatoretto et al. 2017; Amezian et al. 2021; Hilliou et al. 2021).

On the other hand, protease inhibitors exert a stronger antinutritional effect on SCB neonate larvae, leading to mortality, reduced larval weight, and deformities in adults surviving from larvae that ingested protease inhibitors (Pompermayer et al. 2001, 2003; Medeiros et al. 2016; Noriega et al. 2020). Therefore, the evaluated species exhibit different tolerances to the consumption of protease inhibitors.

The identity of the compounds responsible for the insecticidal activity remains unknown. In the present study, a partial purification of *C. racemosa* leaf compounds through molecular filtration was performed, with focus on digestive trypsin inhibition. In molecular filtration, low molecular weight molecules penetrate the resin pores (Sephadex G-25), move slowly through the column, and are consequently eluted only at the end of the process. In contrast, high-molecular-weight molecules, such as proteins, do not penetrate the resin pores, move more quickly, and are eluted in the first fractions (Gelotte 1960).

The G2 fraction group consisted of a mixture of high-molecular-weight protein molecules, including proteins associated with protease inhibition in the evaluated insects. The second peak, specifically G4, is composed of non-protein, low molecular weight molecules, most likely phenolic compounds, as they absorbed light at 280 nm and are known as secondary metabolites with insecticidal activity.

A key and novel finding of this study is that SCB mortality was primarily driven by non-protein molecules, rather than the protein fraction (G2, which exhibited the strongest trypsin inhibition in SCB). Higher SCB mortality values were observed for the non-protein fractions (G3, G4, and G5, with G3 and G4 also showing trypsin inhibition potential in SCB). This clearly indicates that the main insecticidal activity of *C. racemosa* leaf extract against SCB is attributable to low-molecular-weight, non-protein secondary metabolites, independent of their direct effects on digestive trypsin inhibition. This dissociation between protease inhibition and mortality highlights a potentially novel mode of action for these compounds, distinguishing them from typical protein-based plant defenses.

Historically, plants have been important sources of insecticidal molecules, which can be used both as natural products and in the development of synthetic products. The applicability of secondary metabolites in pest insect management is highly variable. Plant-derived molecules such as nicotine and pyrethrin, for example, served as templates for the development of neonicotinoid and pyrethroid insecticides.

To further identify the protein and non-protein molecules responsible for the biological activity of *C. racemosa* leaf extract against the evaluated Lepidoptera, future studies should focus on bioassay-guided fractionation combined with advanced analytical techniques. Sequential purification using high-performance liquid chromatography (HPLC) would allow further separation of the active low-molecular-weight fractions identified in this study. Structural characterization of these compounds could then be achieved using liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS), providing molecular mass and fragmentation profiles, followed by nuclear magnetic resonance (NMR) spectroscopy for definitive structural elucidation. The application of these approaches would enable the identification of bioactive secondary metabolites and contribute to a better understanding of the differential responses observed between *S. frugiperda* and *D. saccharalis*.

5 | Conclusion

The results indicate that non-protein compounds in *C. racemosa* leaves significantly inhibit trypsin activity in both *S. frugiperda* and *D. saccharalis*, with stronger effects observed in the latter. The reduction in larval and pupal weights, as well as the prolonged developmental periods, highlight the potential of *C. racemosa* metabolites for pest control strategies. These findings support the prospect of using *C. racemosa* as a sustainable source of bioactive molecules for the management of resistant pest populations.

Author Contributions

Nicole de Paula Souza: investigation, methodology, conceptualization, writing – original draft, formal analysis, writing – review and editing, data curation, funding acquisition. **Sandy Souza Fonsêca:** investigation, writing – review and editing, methodology. **Ana Letícia Zero dos Santos:** methodology, supervision. **Diandro Ricardo Barilli:** supervision, data curation, writing – review and editing.

Acknowledgements

The Article Processing Charge for the publication of this research was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) (ROR identifier: 00x0ma614).

Funding

This research was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Finance Code 001. The funders had no role in the design of the study, data collection, analysis, or interpretation.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in Zenodo at <https://doi.org/10.5281/zenodo.18196168>. Additional information (raw bioassay data) can be found in the Supporting Information of this article.

References

- Ahmed, E., M. Arshad, M. Z. Khan, et al. 2017. “Secondary Metabolites and Their Multidimensional Prospective in Plant Life.” *Journal of Pharmacognosy and Phytochemistry* 6: 205–214.
- Alves, D. R., D. F. Oliveira, G. A. Carvalho, and M. A. I. Santos. 2011. “Extrato de *Coffea racemosa* Como Alternativa no Controle do Bicho Mineiro do Cafeeiro.” *Ciência e Agrotecnologia* 35: 250–258. <https://doi.org/10.1590/S1413-70542011000200004>.
- Amaral, F. S. A., R. H. Kanno, A. R. B. Do Nascimento, A. S. Guidolin, and C. Omoto. 2023. “Trends Towards Lower Susceptibility of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to Teflubenzuron in Brazil: An Evidence for Field-Evolved Resistance.” *Insects* 14: 129. <https://doi.org/10.3390/insects14020129>.
- Amezian, D., R. Nauen, and G. Le Goff. 2021. “Comparative Analysis of the Detoxification Gene Inventory of Four Major Spodoptera Pest Species in Response to Xenobiotics.” *Insect Biochemistry and Molecular Biology* 138: 103646. <https://doi.org/10.1016/j.ibmb.2021.103646>.
- Barros, E. M., J. B. Torres, and A. F. Bueno. 2010. “Oviposição, Desenvolvimento e Reprodução de *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) em Diferentes Hospedeiros de Importância Econômica.” *Neotropical Entomology* 39: 996–1001. <https://doi.org/10.1590/S1519-566X2010000600023>.
- Boiça Junior, A. L., B. H. S. Souza, Z. A. Ribeiro, W. I. Eduardo, L. Nogueira, and M. M. Di Bello. 2016. “Resistência de Plantas: Explorando o Potencial de Defesa aos Insetos.” In *Tópicos em Entomologia Agrícola—IX*, edited by R. C. Castilho, A. C. Busoli, D. J. Andrade, G. D. Rossi, M. M. Di Bello, and J. A. M. Brenha, 73–94. Gráfica Multipress.
- Bradford, M. M. 1976. “A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding.” *Analytical Biochemistry* 72: 248–254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3).
- Brioschi, D., L. D. Nadalini, M. H. Bengtson, M. C. Sogayar, D. S. Moura, and M. C. Silva-Filho. 2007. “General Upregulation of *Spodoptera frugiperda* Trypsins and Chymotrypsins Allows Its Adaptation to Soybean Proteinase Inhibitor.” *Insect Biochemistry and Molecular Biology* 37: 1283–1290. <https://doi.org/10.1016/j.ibmb.2007.07.016>.
- Carbognin, E. R. 2016. “Modelos de Previsão de Ocorrência de Adultos de *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae) em Cana-de-Açúcar (*Saccharum* spp.).” Dissertação (Mestrado em Agronomia—Entomologia Agrícola), Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista (UNESP), Câmpus de Jaboticabal, Jaboticabal, Brazil. 68. <http://hdl.handle.net/11449/138060>.
- Centro de Estudos Avançados em Economia Aplicada (CEPEA), Confederação Nacional da Agricultura e Pecuária (CNA). 2025. *PIB do Agronegócio Brasileiro: Série Histórica 1996–2025*. CEPEA. <https://www.cepea.esalq.usp.br/br/pib-do-agronegocio-brasileiro.aspx>.
- Chormule, A., N. Shejawal, S. S. Deshmukh, C. M. Kalleshwaraswamy, R. Asokan, and H. M. Mahadeva Swamy. 2019. “First Report of the Fall Armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera, Noctuidae) on Sugarcane and Other Crops From Maharashtra, India.” *Journal of Entomology and Zoology Studies* 7: 114–117.
- Costa, D. C. M., B. H. S. Souza, C. H. S. Carvalho, and O. Guerreiro Filho. 2025. “Characterization and Levels of Resistance in *Coffea arabica* × *Coffea racemosa* Hybrids to *Leucoptera coffeella*.” *Journal of Pest Science* 98: 1075–1084. <https://doi.org/10.1007/s10340-024-01844-4>.

- Cruz, I. 2007. *A Broca da Cana-de-Açúcar, Diatraea saccharalis, em Milho, no Brasil*. Vol. 90, 12. Embrapa Milho e Sorgo.
- Divekar, P. A., V. Rani, S. Majumder, et al. 2023. "Protease Inhibitors: An Induced Plant Defense Mechanism Against Herbivores." *Journal of Plant Growth Regulation* 42: 6057–6073. <https://doi.org/10.1007/s0034-022-10767-2>.
- do Nascimento, A. R. B., J. G. Rodrigues, R. H. Kanno, et al. 2023. "Susceptibility Monitoring and Comparative Gene Expression of Susceptible and Resistant Strains of *Spodoptera frugiperda* to Lambda-Cyhalothrin and Chlorpyrifos." *Pest Management Science* 79: 2206–2219. <https://doi.org/10.1002/ps.7399>.
- Erlanger, B. F., W. Cohen, and N. Kokowsky. 1961. "The Preparation and Properties of Two New Chromogenic Substrates of Trypsin." *Archives of Biochemistry and Biophysics* 95: 271–278. [https://doi.org/10.1016/0003-9861\(61\)90145-X](https://doi.org/10.1016/0003-9861(61)90145-X).
- Farinelli, R., and D. Fornasieri Filho. 2006. "Avaliação de Dano de *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae) em Cultivares de Milho." *Científica* 34: 197. <https://doi.org/10.15361/1984-5529.2006v34n2p197-202>.
- Fatoretto, J. C., A. P. Michel, M. C. Silva-Filho, and N. Silva. 2017. "Adaptive Potential of Fall Armyworm (*Spodoptera frugiperda*) Limits Bt Trait Durability in Brazil." *Journal of Integrated Pest Management* 8: 1–10. <https://doi.org/10.1093/jipm/pmx011>.
- Ferreira, C. A. S., M. V. Santana, J. B. Santos, T. T. M. Santos, L. M. Lôbo, and P. M. Fernandes. 2018. "Yield and Technological Quality of Sugarcane Cultivars Under Infestation of *Diatraea saccharalis* (Fabr., 1794)." *Arquivos do Instituto Biológico* 85: e0042017. <https://doi.org/10.1590/1808-1657000042017>.
- Francischini, F. J. B., E. M. G. Cordeiro, J. B. Campos, et al. 2019. "*Diatraea saccharalis* History of Colonization in the Americas. The Case for Human-Mediated Dispersal." *PLoS One* 14: e0220031. <https://doi.org/10.1371/journal.pone.0220031>.
- Gallan, D. Z., A. B. Penteriche, M. O. Henrique, and M. C. Silva-Filho. 2022. "Sugarcane Multitrophic Interactions: Integrating Belowground and Aboveground Organisms." *Genetics and Molecular Biology* 46: e20220163. <https://doi.org/10.1590/1678-4685-GMB-2022-0163>.
- Gelotte, B. 1960. "Studies on Gel Filtration Sorption Properties of the Bed Material Sephadex." *Journal of Chromatography* 3: 330–342.
- Goergen, G., P. L. Kumar, S. B. Sankung, A. Togola, and M. Tamò. 2016. "First Report of Outbreaks of the Fall Armyworm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera, Noctuidae), a New Alien Invasive Pest in West and Central Africa." *PLoS One* 11: e0165632. <https://doi.org/10.1371/journal.pone.0165632>.
- Guerreiro Filho, O., H. P. Medina Filho, and A. Carvalho. 1991. "Fontes de Resistência ao Bicho Mineiro, *Perileuoptera coffeella*, em *Coffea* spp." *Bragantia* 50: 45–55.
- Hensley, S. D., and A. M. Hammond Jr. 1968. "Diet and Methods for Mass Rearing the Sugarcane Borer in the Laboratory." *Journal of Economic Entomology* 61, no. 6: 1742–1743. <https://doi.org/10.1093/jee/61.6.1742>.
- Hilliou, F., T. Chertemps, M. Maïbèche, and G. Le Goff. 2021. "Resistance in the Genus *Spodoptera*: Key Insect Detoxification Genes." *Insects* 12: 544. <https://doi.org/10.3390/insects12060544>.
- Kasten, P., Jr., A. A. C. M. Precetti, and J. R. P. Parra. 1978. "Dados biológicos comparativos de *Spodoptera frugiperda* (J. E. Smith, 1797) em duas dietas artificiais e substrato natural." *Revista de Agricultura* 53, no. 1-2: 69–78.
- Macedo, N., and D. Macedo. 2004. "As Pragas de Maior Incidência nos Canaviais e Seus Controles." *Visão Agrícola* 1: 38–46.
- Medeiros, A. H., F. B. Mingossi, R. O. Dias, et al. 2016. "Sugarcane Serine Peptidase Inhibitors, Serine Peptidases, and Clp Protease System Subunits Associated With Sugarcane Borer (*Diatraea saccharalis*) Herbivory and Wounding." *International Journal of Molecular Sciences* 17: 1444. <https://doi.org/10.3390/ijms17091444>.
- Montezano, D., A. Specht, D. R. Sosa-Gómez, et al. 2018. "Host Plants of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Americas." *African Entomology* 26: 286–300. <https://hdl.handle.net/10520/EJC-112bc16060>.
- Noriega, D. D., F. B. M. Arraes, J. D. Antonino, et al. 2020. "Comparative Gut Transcriptome Analysis of *Diatraea saccharalis* in Response to the Dietary Source." *PLoS One* 15: e0235575. <https://doi.org/10.1371/journal.pone.0235575>.
- Oliveira, C. F. R., T. P. Souza, J. R. P. Parra, S. Marangoni, M. C. Silva-Filho, and M. L. R. Macedo. 2013. "Insensitive Trypsins Are Differentially Transcribed During *Spodoptera frugiperda* Adaptation Against Plant Protease Inhibitors." *Comparative Biochemistry and Physiology* 165: 19–25. <https://doi.org/10.1016/j.cbpb.2013.02.008>.
- Overton, K., J. L. Maino, R. Day, et al. 2021. "Global Crop Impacts, Yield Losses and Action Thresholds for Fall Armyworm (*Spodoptera frugiperda*): A Review." *Crop Protection* 145: 105641. <https://doi.org/10.1016/j.cropro.2021.105641>.
- Paulillo, L. C. M. S., A. M. Sebben, M. T. V. C. Derbyshire, A. Góes-Neto, M. A. P. Brotto, and A. Figueira. 2012. "Evaluation of in Vitro and in Vivo Effects of Semipurified Proteinase Inhibitors From *Theobroma* Seeds on Midgut Protease Activity of Lepidopteran Pest Insects." *Archives of Biochemistry and Biophysics* 81: 34–53. <https://doi.org/10.1002/arch.21038>.
- Pelosi, A. P., F. C. Silva, A. G. Vaz, et al. 2023. "Tolerance in Rice Genotypes to the Sugarcane Borer *Diatraea saccharalis* (Lepidoptera: Crambidae)." *Biologia (Bratislava)* 78: 2157–2165. <https://doi.org/10.1007/s11756-023-01358-x>.
- Peñaflor, M. F. G. V., and J. M. S. Bento. 2019. "Red-Rot Infection in Sugarcane Attenuates the Attractiveness of Sugarcane Borer-Induced Plant Volatiles to Parasitoid." *Arthropod-Plant Interactions* 13: 117–125. <https://doi.org/10.1007/s11829-018-9629-6>.
- Pinto, C. P. G. 2020. "A Digestão dos Insetos Mastigadores: Fisiologia e Potencial uso no Manejo Integrado de Pragas (MIP)." *Instituto Biológico (São Paulo)* 81: 1–13. <https://doi.org/10.31368/1980-6221v81a10005>.
- Pinto, C. P. G., D. R. Barilli, J. B. Silva, et al. 2018. "Fontes Potenciais de Moléculas Inseticidas." In *Tópicos em Entomologia Agrícola—XI*, edited by R. C. Castilho, C. C. Truzzi, and C. P. G. Pinto, 103–123. Gráfica Multipress.
- Pompermayer, P., M. C. Falco, J. R. P. Parra, and M. C. Silva-Filho. 2003. "Coupling Diet Quality and Bowman-Birk and Kunitz-Type Soybean Proteinase Inhibitor Effectiveness to *Diatraea saccharalis* Development and Mortality." *Entomologia Experimentalis et Applicata* 109: 217–224. <https://doi.org/10.1046/j.0013-8703.2003.00107.x>.
- Pompermayer, P., A. R. Lopes, W. R. Terra, J. R. P. Parra, M. C. Falco, and M. C. Silva-Filho. 2001. "Effects of Soybean Proteinase Inhibitor on Development, Survival and Reproductive Potential of the Sugarcane Borer, *Diatraea saccharalis*." *Entomologia Experimentalis et Applicata* 99: 79–85. <https://doi.org/10.1046/j.1570-7458.2001.00804.x>.
- Qi, G. J., J. Ma, J. Wan, et al. 2021. "Source Regions of the First Immigration of Fall Armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) Invading Australia." *Insects* 12: 1104. <https://doi.org/10.3390/insects12121104>.
- R Core Team. 2020. "R: A Language and Environment for Statistical Computing." R Foundation for Statistical Computing. <https://www.R-project.org/>.
- Ramiro, D. A., O. Guerreiro Filho, R. B. Queiroz-Voltan, and S. C. Matthiesen. 2004. "Caracterização Anatômica de Folhas de Cafeeiros Resistentes e Suscetíveis ao Bicho Mineiro." *Bragantia* 63: 363–372. <https://doi.org/10.1590/S0006-87052004000300006>.

- Sandoval, S. S., and K. C. A. Senô. 2010. "Comportamento e Controle de *D. saccharalis* na Cultura Cana-de-Açúcar." *Nucleus (Calcutta)* 7: 243–257. <https://doi.org/10.3738/1982.2278-311>.
- Sarmiento, R. A., R. W. S. Aguiar, R. A. S. S. Aguiar, S. M. J. Vieira, H. G. Oliveira, and A. M. Holtz. 2002. "Revisão da Biologia, Ocorrência e Controle de *Spodoptera frugiperda* (Lepidoptera, Noctuidae) em Milho no Brasil." *Bioscience Journal* 18: 41–48.
- Savary, S., L. Willocquet, S. J. Pethybridge, P. Esker, N. McRoberts, and A. Nelson. 2019. "The Global Burden of Pathogens and Pests on Major Food Crops." *Nature Ecology & Evolution* 3: 430–439. <https://doi.org/10.1038/s41559-018-0793-y>.
- Sharma, S., R. Kooner, and R. Arora. 2017. "Insect Pests and Crop Losses." In *Breeding Insect Resistant Crops for Sustainable Agriculture*, 45–66. Springer Singapore.
- Singh, S., A. Singh, S. Kumar, P. Mittal, and I. K. Singh. 2020. "Protease Inhibitors: Recent Advancement in Its Usage as a Potential Biocontrol Agent for Insect Pest Management." *Insect Science* 27: 186–201. <https://doi.org/10.1111/1744-7917.12641>.
- Souza, L. A., and M. F. G. V. Peñaflor. 2024. "Small but Strong: Herbivory by Sap-Feeding Insect Reduces Plant Progeny Growth but Enhances Direct and Indirect Anti-Herbivore Defenses." *Oecologia* 205: 191–201. <https://doi.org/10.1007/s00442-024-05567-2>.
- Souza, N. P. 2019. "Bioatividade de um Inibidor de Tripsinas Digestivas Presente em Folhas de *Coffea racemosa* Lour. Sobre Imaturos de *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae)." Undergraduate thesis, Universidade Estadual Paulista (Unesp), Jaboticabal, Brazil. <http://hdl.handle.net/11449/239736>.
- Souza, T. P., R. O. Dias, E. C. Castelhana, M. M. Brandão, D. S. Moura, and M. C. Silva-Filho. 2016. "Comparative Analysis of Expression Profiling of the Trypsin and Chymotrypsin Genes From Lepidoptera Species With Different Levels of Sensitivity to Soybean Peptidase Inhibitors." *Comparative Biochemistry and Physiology* 196: 67–73. <https://doi.org/10.1016/j.cbpb.2016.02.007>.
- Tabashnik, B. C., J. A. Fabrick, and Y. Carrière. 2023. "Global Patterns of Insect Resistance to Transgenic Bt Crops: The First 25 Years." *Journal of Economic Entomology* 116: 297–309. <https://doi.org/10.1093/jee/toac183>.
- Vilela, M., A. J. N. Santos, M. L. F. Simeone, et al. 2017. "Influence of *Diatraea saccharalis* (Lepidoptera: Crambidae) Infestation on Sweet Sorghum Productivity and Juice Quality." *African Journal of Agricultural Research* 12: 2877–2885.