Bioprocesses and Sustainability

Revista Brasileira de Ciências Ambientais
Brazilian Journal of Environmental Sciences

Pathogenicity of bacteria and viruses to *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

Patogenicidade de bactérias e vírus no controle de *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

Elisangela de Souza Loureiro[1], *Lidiane Arissa Yokota*[1], *Gabriella Silva de Gregori*[1], *Andressa Silva Rodrigues*[1], *Luis Gustavo Amorim Pessoa*[1], *Pamella Mingotti Dias*[2]

ABSTRACT

Combining integrated pest management and biological control has been an effective, economical, and sustainable strategy for controlling agricultural pests. The larvae of *Spodoptera frugiperda,* a pest of several crops, cause financial losses to the agribusiness sector, and entomopathogens have been widely used for the biological control of this species. Therefore, the objective of this study was to evaluate the pathogenicity of bacteria and viruses for the control of *S. frugiperda* under laboratory conditions. Two bioassays were conducted in a completely randomized design, one using second-instar and other using third-instar larvae, with six treatments and five replications. The entomopathogens used were *S. frugiperda Multiple nucleopolyhedrovirus* (*SfMNPV*), *Autographa californica multiple nucleopolyhedrovirus* (*AcMNPV*), *Bacillus subtilis*, *B. thuringiensis* subsp. *thoworthy*, and *B. thuringiensis* subsp. *kurstaki*. Mortality was assessed daily until the end of the larval cycle. Analysis of variance (F-test) was applied, followed by mean comparison through the Scott-Knott test at a 5% significance level. The efficacy of the entomopathogens as bioinsecticides was evaluated using Abbott's formula. Overall, the most effective entomopathogens for controlling second-instar *S. frugiperda* larvae were *SfMNPV* and *B. subtilis*, resulting in 100% control, whereas *AcMNPV* was the most effective against third-instar larvae, showing maximum lethality in 76.67% of samples.

Keywords: fall armyworm; integrated pest management; sustainable agriculture.

RESUMO

A utilização do manejo integrado de pragas, associado ao controle biológico, tem sido uma estratégia eficiente, econômica e mais sustentável no controle de agentes danosos à agricultura. A fase larval de *Spodoptera frugiperda,* uma praga agrícola presente em diversas culturas, é causadora de prejuízos financeiros para o agronegócio, e os entomopatógenos têm sido amplamente utilizados no controle biológico dessa espécie. Assim, o objetivo do trabalho foi avaliar a patogenicidade de bactérias e vírus no manejo de *S. frugiperda*, em condições de laboratório. Para os bioensaios, utilizamos seis tratamentos por cinco repetições contendo seis lagartas (com segundo e terceiro instares) em cada. Os entomopatógenos utilizados foram *S. frugiperda Multiple nucleopolyhedrovirus* (*SfMNPV*), *Autographa californica multiple nucleopolyhedrovirus* (AcMNPV), *Bacillus subtilis*, *B. thuringiensis* subsp. *thoworthy*, e *B. thuringiensis* subsp. *kurstaki*. A avaliação da mortalidade ocorreu diariamente até a finalização do ciclo larval. Para a avaliação da mortalidade, utilizou-se a análise de variância (teste F), comparada posteriormente ao teste Scott-Knott a 5% de probabilidade e à eficiência dos bioinseticidas através da fórmula de Abbott. De modo geral, os entomopatógenos mais eficientes foram *SfMNPV* e *B. subtilis*, obtendo 100% de mortalidade nas lagartas de segundo instar, enquanto para as de terceiro instar, o *AcMNPV* mostrou letalidade máxima em 76,67% das amostras.

Palavras-chave: lagarta-do-cartucho; manejo integrado de pragas; agricultura sustentável.

[1]Federal University of Mato Grosso do Sul – Chapadão do Sul (MS), Brazil.

[2]Federal University of Grande Dourados – Dourados (MS), Brazil.

Correspondence author: Elisangela de Souza Loureiro – MS-306 Road, km 105 – Rural Area – CEP: 79560-000 – Chapadão do Sul (MS), Brazil.
E-mail: elisangela.loureiro@ufms.br

Conflicts of interest: the authors declare no conflicts of interest.

Funding: this study was partly funded by the Brazilian Coordination for the Improvement of Higher Education Personnel (CAPES) financial code 001, the Women in Science Program 2023 of the Federal University of Mato Grosso do Sul (UFMS); the Foundation for Support of Education, Science, and Technology Development of the State of Mato Grosso do Sul (FUNDECT), which granted PhD scholarship (No. 59/300.513/2016), and the project funding agency (No. 224/2022, No. 71/027.188/2022, and SIAFEM No. 31970).

Received on: 01/31/2024. Accepted on: 05/06/2024.

https://doi.org/10.5327/Z2176-94781952

Introduction

The presence of pests in monoculture areas in Brazil, mainly favored by regional microclimates and abundant food sources, has been among the main factors causing economic losses to the agribusiness sector (Freire et al., 2024). *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae), commonly known as the fall armyworm, is an agricultural pest and is one of the most damaging to maize, soybean, and rice crops (Nascimento et al., 2022). It is a polyphagous species native to the Americas but it has recently invaded several countries in Africa, Asia, and Europe (Hussain et al., 2021). The larval stage of this species lasts approximately 15 days, which can vary with environmental temperature, reaching the adult stage in approximately 30 days, with a rapid reproduction (Sosa-Gómez et al., 2020).

The excessive and inappropriate use of synthetic insecticides in agricultural pest control has been a critical issue, as it has resulted in the resurgence of previously controlled pests, selection of resistant individuals, reduction in natural predator populations, proliferation of secondary pests, and environmental damage (Arakere et al., 2022; De Jesus et al., 2022). Therefore, the integrated pest management emerged as a more sustainable strategy (Paiva et al., 2024), contributing to minimizing the impacts of conventional chemical pesticides (Araújo et al., 2019; Staback et al. 2020). Biological control is one of the practices used in integrated pest management for managing *S. frugiperda*, involving the use of entomopathogenic bacteria and viruses, which act through ingestion, and entomopathogenic fungi, which act through contact (Rao and Jurat-Fuentes, 2020; Arakere et al., 2022).

Baculoviruses (Baculoviridae) are the most studied viruses for the biological control of insects, particularly lepidopterans, due to their high pathogenicity to this group; the species *Spodoptera frugiperda multiple nucleopolyhedrovirus* (*SfMNPV*) is the most used because of its high pathogenicity to *S. frugiperda* (Hussain et al., 2021). Baculoviruses act through ingestion; once ingested, the polyhedral reach the host's midgut, where they dissolve, releasing viral particles that penetrate the intestinal cells and replicate within their nuclei (Maciel et al., 2024).

Pathogenic bacteria species such as *Bacillus thuringiensis* (Berliner, 1911) (Eubacteriales: Bacillaceae) (Araújo et al., 2019) and *B. subtilis* (Karshanal and Kalia, 2023) are among the most commonly used for biological control of lepidopterans. The virulence of *B. thuringiensis* is mainly due to its high production of Cry and Vip3 proteins and spores, which have insecticidal activity (Crickmore, 2021; Nascimento et al., 2022).

Combining biological control agents has been a strategy to extend the scope of action in terms of time, space, and the number of target pathogens (Arakere et al., 2022). However, effective pest control requires integrative approaches to agricultural management, as various species, including plant pathogens, plant-parasitic nematodes, and insect pests, should be managed simultaneously, requiring the integration of different biocontrol agents (Parra, 2023). These integrated management systems are more economically and ecologically sustainable (Liordos, 2024) when combined with other management practices.

Thus, focusing on contributing to the resilience and sustainability of production systems, the objective of this study was to evaluate the pathogenicity of entomopathogenic bacteria and viruses in controlling second- and third-instar larvae of *S. frugiperda*.

Material and methods

Study location and sample acquisition and rearing

The study was conducted at the Laboratory of Entomology of the Federal University of Mato Grosso do Sul (UFMS), Chapadão do Sul campus (CPCS), MS, Brazil. Bioassays were based on second-generation larval stages of *S. frugiperda* from a laboratory-reared colony established at the UFMS/CPCS Entomology Laboratory. *Spodoptera frugiperda* larvae at all stages were maintained in a climate-controlled chamber at 25°C, standard deviation (±) 1°C, 70±10% relative humidity (RH), and a 12-hour photoperiod. Parents were kept in 100×200 mm polyvinyl chloride (PVC) cages lined with sulfite paper; the top opening was covered with a voile fabric secured with an elastic band, and the bottom end was sealed with a Styrofoam plate lined with a paper towel. The larvae were fed a paste made of equal parts (v v^{-1}) of honey and brewer's yeast, and placed on absorbent cotton wool in a glass container attached to the top. The paste was replaced every two days. The eggs obtained were used for bioassays and colony maintenance (Figure 1A). Newly hatched larvae were kept in 145-mL cylindrical plastic containers with 5 cm diameter (Figures 1B and 1C). The colony was maintained on an artificial diet adapted from Greene et al. (1976).

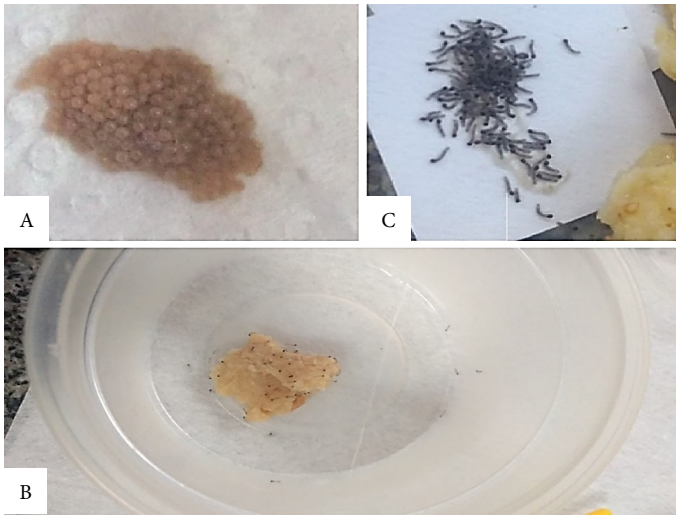

Figure 1 – Egg mass of Spodoptera frugiperda (A); plastic container with larvae feeding on the diet (B); and newly hatched larvae (C).

Bioassays and evaluation of results

Two bioassays were conducted in a completely randomized experimental design with five replications, consisting of six treatments (including a control without entomopathogen). Second-instar larvae were used in the first experiment, whereas third-instar larvae were used in the second experiment. Each plot consisted of six larvae (Table 1). Each larva from the mass-rearing colony was placed individually in a Petri dish of 9 cm diameter for the bioassays. The commercial entomopathogens and their respective rates used in each treatment are shown in Table 1. Entomopathogens at the full recommended rate were sprayed onto the larvae and a 2 cm^2 diet using a Potter tower with a pressure of 15 psi. This diet was replaced with fresh, untreated food after 48 hours (Loureiro et al., 2024). The Petri dishes were sealed with plastic wrap and then incubated in a climate-controlled room (26±1°C, 70±10% RH, and 12-hour photoperiod). Mortality was assessed daily until the completion of the larval stage. Confirmation of pathogen-induced mortality was based on the methodology of Loureiro and Moino Junior (2007). Confirmed cumulative mortality represents the pathogen's ability to colonize and surpass all competing agents within the insect body. The data obtained for confirmed cumulative mortality were subjected to analysis of variance (F-test), the means were grouped using the Scott-Knott test at a 5% significance level, and the efficacy of the entomopathogens as bioinsecticides was evaluated ten days after application of the treatments using Abbott's formula (Abbott, 1925).

Results and discussion

The results denoted the viability of sustainably managing *S. frugiperda* using the tested biological agents (entomopathogens). Overall, the mortality of second- and third-instar larvae subjected to the entomopathogens significantly differed from the control (Table 2). The mortality rate for second-instar larvae was 100% in treatments with *SfMNPV* (Figure 2A) and *B. subtilis*, but did not differ significantly from treatments with *AcMNPV* (Figure 2B) and *B. thuringiensis* subsp. *thoworthy* (Figure 2C). However, the treatment containing *B. thuringiensis* subsp. *kurstaki* resulted in considerably lower larval mortality as compared to the other treatments (Table 2).

S. frugiperda is susceptible to viral infection only during its larval stage, with this susceptibility decreasing as the larva develops. Viral infection occurs orally when larvae feed on parts of the plant contaminated with the virus. Host death typically occurs within 6–8 days after ingestion (Pinto et al., 2020). Thus, the results of the present study confirmed the susceptibility of young larvae (second instar) (Table 2).

Table 2 – Confirmed cumulative mortality (mean±standard deviation) of second- and third-instar larvae of Spodoptera frugiperda.

Treatments	Second-instar larvae mortality (%)	Third-instar larvae mortality (%)
T1- Control (without entomopathogen)	0.0±0.00 c	0.0±0.00 b
T2- *SfMNPV*	100.0±0.00 a	70.0±0.14 a
T3- *AcMNPV*	96.7±0.07 a	90.0±0.15 a
T4- *Bacillus subtilis*	100.0±0.10 a	80.0±0.18 a
T5- *B. thuringiensis* subsp. *thoworthy*	83.3±0.23 a	70.0±0.24 a
T6- *B. thuringiensis* subsp. *kurstaki*	60.0±0.19 b	60.0±0.36 a
CV%	17.36	14.36

SfMNPV: *Spodoptera frugiperda multiple nucleopolyhedrovirus*; *AcMNPV*: *Autographa californica multiple nucleopolyhedrovirus*; CV: coefficient of variation. Means followed by the same letter in the column are not significantly different from each other by the Scott-Knott test at a 5% significance level.

Figure 2 – Confirmation of mortality under a stereoscopic microscope at 40x magnification. (A) Spodoptera frugiperda larva killed by Spodoptera frugiperda multiple nucleopolyhedrovirus (SfMNPV); (B) S. frugiperda larva killed by Autographa californica multiple nucleopolyhedrovirus (AcMNPV); (C) S. frugiperda larva killed by Bacillus thuringiensis subsp. thoworthy; D. Healthy S. frugiperda larva in the control (without entomopathogen).

Table 1 – Treatments and their respective entomopathogen rates used for controlling second- and third-instar larvae of Spodoptera frugiperda.

	Commercial product	Entomopathogen	Rate
T1	Control	Without entomopathogen	-
T2	Cartugen®	*Spodoptera frugiperda multiple nucleopolyhedrovirus - SfMNPV*	200 mL ha^{-1}
T3	Lepigen®	*Autographa californica multiple nucleopolyhedrovirus - AcMNPV*	150 ml ha^{-1}
T4	Bio-Immune®	*Bacillus subtilis* strain BV-02	8 L ha^{-1}
T5	Crystal®	*Bacillus thuringiensis* subsp. *thoworthy* isolate 344	1 L ha^{-1}
T6	BT-Turbo Max®	*Bacillus thuringiensis* subsp. *kurstaki* strain HD-1	1 L ha^{-1}

Source: Agrofit (2023).

Considering the treatments with bacteria of the genus *Bacillus*, those with *B. subtilis* resulted in higher larval mortality (100 and 80% for second- and third-instar larvae, respectively) and control efficacy (Table 2 and Figure 3). Treatment with *B. thuringiensis* subsp. *thoworthy* resulted in mortality rates of 83.3 and 70%, respectively (Table 2), with an efficacy of 66.67% control for both larval instars (Figure 3). Treatments with *B. thuringiensis* subsp. *kurstaki* resulted in 60% mortality for both larval instars, with better efficacy in controlling second-instar larvae (Figure 3). Recent studies have shown that mutations in specific genes (mainly in ABC transporters) are significantly involved in the resistance of *S. frugiperda* to *B. thuringiensis* (Jurat-Fuentes et al., 2021). This may explain the lower efficacy and mortality in treatments with *B. thuringiensis* compared to those with *B. subtilis*. According to Heckel (2020), the genes involved in resistance to *B. thuringiensis* can have different combinations, resulting in varying susceptibility to the biological agent. The use of microorganisms emerged as an important strategy in agricultural production, particularly for sustainable and environmentally sound pest control, as these microorganisms offer a natural and effective solution for this purpose (Parra, 2023).

The mortality rates of second-instar larvae did not differ significantly between treatments with entomopathogenic viruses: 96.7% *AcMNPV* and 100% *SfMNPV*. Regarding third-instar larvae, *AcMNPV* resulted in higher mortality (90%) than *SfMNPV* (70%) (Table 2). Both viruses showed control efficacy consistent with the mortality results. *SfMNPV* had 100 and 60% efficacy in controlling second- and third-instar larvae, respectively, whereas *AcMNPV* was more effective in controlling second-instar larvae (76.7%) (Figure 3).

Baculoviruses are commonly used in the biological control of *S. frugiperda* (Hussain et al., 2021). The application of bioinsecticides containing *SfMNPV* has yielded high mortality rates of fall armyworms, mainly during the early larval stages, and can increase the lethal and sublethal effects on neonates and surviving adults when applied to transgenic crops containing the Cry1Ac gene (Zakseski et al., 2021). *AcMNPV* has pathogenic potential against a wider variety of insect species, resulting in a broader host range compared to *SfMNPV*, which specifically infects *S. frugiperda* (Hussain et al., 2021). This may explain the lower mortality found when using *AcMNPV* compared to *SfMNPV* in this study.

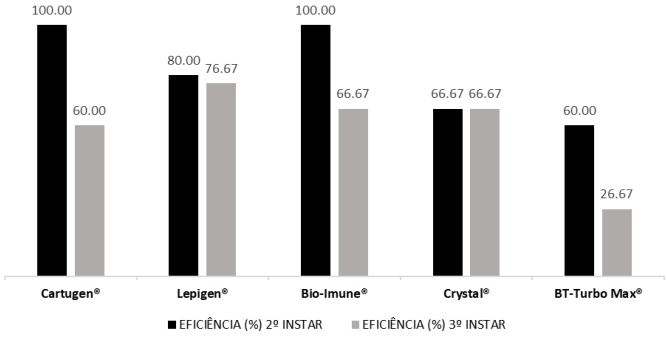

Figure 3 – Efficacy of entomopathogens as bioinsecticides for the control of second- and third-instar larvae of Spodoptera frugiperda, ten days after application. Spodoptera frugiperda multiple nucleopolyhedrovirus – SfMNPV (Cartugen®); Autographa californica multiple nucleopolyhedrovirus – AcMNPV (Lepigen®); Bacillus subtilis (Bio-Immune®); B. thuringiensis subsp. thoworthy (Crystal®); and B. thuringiensis subsp. kurstaki (BT-Turbo Max®).

According to Tomquelski and Martins (2007), a pesticide is effective when it achieves more than 80% control. Therefore, treatments using *SfMNPV*, *AcMNPV*, and *B. subtilis* as bioinsecticides were effective in controlling second-instar larvae. However, none of the treatments were effective against third-instar larvae; only the treatment with *AcMNPV* almost reached the efficacy of control (76.67%) (Figure 3). The lower efficacy of bioinsecticides on more developed larvae is a common limiting factor for biological pest control due to anatomical changes in body structure and chemical changes in the midgut of these insects (Farder-Gomes et al., 2022), which hinder contamination by entomopathogens used as biological control agents.

Sustainability is achieved when the goals of its three core components — society, environment, and economy — are respected (Liordos, 2024). Thus, bioproducts are an innovation in the agribusiness sector, providing sustainable solutions that balance crop yields and environmental respect. They are ecologically sustainable methods with lower environmental costs than conventional chemical products (Parra, 2023).

Conclusions

All treatments with entomopathogenic bacteria and viruses resulted in at least 60% mortality of second- and third-instar larvae of *Spodoptera frugiperda*. Considering the bioinsecticides containing entomopathogenic viruses, the application of *Spodoptera frugiperda multiple nucleopolyhedrovirus* (*SfMNPV*) resulted in higher mortality of second-instar larvae, whereas *Autographa californica multiple nucleopolyhedrovirus (AcMNPV*) resulted in higher mortality of third-instar larvae. *SfMNPV* and *Bacillus subtilis* yielded 100% control efficacy against second-instar larvae.

Thus, using these microorganisms for controlling *S. frugiperda* results in less environmental damage, maintaining agricultural sustainability for future crops by conserving natural resources and increasing biodiversity in various production systems.

Acknowledgements

The authors thank the Graduate Program in Agronomy of the Federal University of Mato Grosso do Sul (UFMS) in Chapadão do Sul (MS), the Graduate Program in Entomology and Biodiversity Conservation of the Federal University of Grande Dourados (UFGD), Dourados (MS), the Brazilian National Council for Scientific and Technological Development (CNPq), and the members of the Integrated Pest Management Laboratory (LAMIP) research group of the Federal University of Mato Grosso do Sul (UFMS).

Authors' contributions

LOUREIRO, E.S.: conceptualization, funding, data acquisition, methodology, project administration, resources, supervision, writing – original draft. YOKOTA, L.A.: conceptualization, data curation, formal analysis, investigation, methodology, software, supervision, validation, visualization, writing – original draft, writing – review & editing. PESSOA, L.G.A.: data curation, formal analysis, project administration, supervision, validation. DIAS, P.M.: methodology, software. RODRIGUES, A.S.: visualization, writing – original draft. GREGORI, G.S.: writing – review & editing.

References

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, v. 18, (2), 265-267. https://doi.org/10.1093/jee/18.2.265a

Agrofit, 2023. Sistema de registro de agrotóxicos do Ministério da Agricultura (Accessed January 11, 2024) at:. https://extranet. agricultura.gov.br/agrofit_cons/principal_agrofit_cons

Arakere, U.C.; Jagannath, S.; Krishnamurthy, S.; Chowdappa, S.; Konappa, N., 2022. Microbial bio-pesticide as sustainable solution for management of pests: achievements and prospects. Biopesticides, v. 2, 183-200. https://doi.org/10.1016/B978-0-12-823355-9.00016-X

Araújo, I.S.; de Oliveira, G.M.; de Lacerda, L.B.; de Luna Batista, J.; Lopes, G.N., 2019. Perspectivas atuais da utilização de bioinseticidas em *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Revista Brasileira de Meio Ambiente, v. 7, (3), 1-8. https://doi.org/10.5281/zenodo.3575253

Crickmore, N.; Berry, C.; Panneerselvam, S.; Mishra, R.; Connor, T.R.; Bonning, B.C., 2021. A structure-based nomenclature for *Bacillus thuringiensis* and other bacteria-derived pesticidal proteins. Journal of Invertebrate Pathology, v. 186, e107438. https://doi.org/10.1016/j.jip.2020.107438

De Jesus, J.C.; Silva, B.D.; Andaló, V.; Vasconcelos, B.N.F.; de Assis, G.A.; Carvalho, F.J., 2022. Ocorrência de bicho-mineiro (*Leucoptera coffeella*) em cafeeiros cultivados em sistemas agroflorestal e convencional. Revista Brasileira de Agroecologia, v. 1, (2), 90-100. https://doi.org/10.33240/rba.v17i2.23560

Farder-Gomes, C.F.; Saravanan, M.; Martinez, L.C.; Plata-Rueda, A.; Zanuncio, J.C.; Serrao, J.E. 2022. Azadirachtin-based biopesticide affects the respiration and digestion in *Anticarsia gemmatalis* caterpillars. Toxin Reviews, v. 41, (2), 466-475. https://doi.org/10.1080/15569543.2021.1892764

Freire, A.; Silva, E.; Santos, T.; Figueira, F., 2024. Importance, challenges and perspectives of using fungi in the biological control of plant diseases for Brazilian agriculture. Concilium, v. 24, (1), 486-504. https://doi.org/10.53660/CLM-2744-24B11

Greene, G.L.; Leppla, N.C.; Dickerson, W.A., 1976. Velvetbean caterpillar: A rearing procedure and artificial medium. Journal of Economic Entomology, v. 69, (4), 487-488. https://doi.org/10.1093/jee/69.4.487

Heckel, D.G., 2020. How do toxins from *Bacillus thuringiensis* kill insects? An evolutionary perspective. Archives of Insect Biochemistry and Physiology, v. 104, (2), 1-12. https://doi.org/10.1002/arch.21673

Hussain, A.G.; Wennmann, J.T.; Goergen, G.; Bryon, A.; Ros, V.I., 2021. Viruses of the fall armyworm *Spodoptera frugiperda*: a review with prospects for biological control. Viruses, v. 13, (11), 1-21. https://doi.org/10.3390/v13112220

Jurat-Fuentes, J.L.; Heckel, D.G.; Ferré, J. 2021. Mechanisms of resistance to insecticidal proteins from *Bacillus thuringiensis*. Annual Review of Entomology (online), v. 66, 121-140. https://doi.org/10.1146/annurev-ento-052620073348

Karshanal, J.; Kalia, V.K., 2023. Efficacy of native *Bacillus* isolates against different larval instars of fall armyworm, *Spodoptera frugiperda* alone and in combination. Egyptian Journal of Biological Pest Control, v. 33, (1), 1-11. https://doi.org/10.1186/s41938-023-00743-7

Liordos, V., 2024. Conservation, sustainability, conflict and coexistence: key themes in wildlife management. Sustainability, v. 16, 3271. https://doi.org/10.3390/su16083271

Loureiro, E.S.; Dias, P.M.; Pessoa, L.G.A.; Amaral, T.S.; Pessoa, M.B.; Gregori, G.S.; Rodrigues, A.S.; Godoy, M.S., 2024. Virulence of entomopathogenic fungi in larvae of Lepidoptera: Noctuidae. Revista Caatinga, v. 37, e12375. http://dx.doi.org/10.1590/1983-21252024v3712375rc

Loureiro, E.S.; Moino Junior, A., 2007. Patogenicidade de Fungos Entomopatogênicos a *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), BioAssay, v. 2 (10) 1-8. https://doi.org/10.14295/BA.v2.0.15.

Maciel, R.M.A.; Luski, P.G.G.; Sutil, W.P.; Gonçalves, J.; Hayashida, R.; Queiroz, A.P.; Neves, P.M.O.J.; Bueno, A.F., 2024. The use of baculovirus *Spodoptera* SfMNPV alone and combined with herbicides and adjuvant to control *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae). Biological Control, v. 188, 105408. https://doi.org/10.1016/j.biocontrol.2023.105408

Nascimento, J.; Goncalves, K.C.; Dias, N.P.; De Oliveira, J.L.; Bravo, A.; Polanczyk, R.A., 2022. Adoption of *Bacillus thuringiensis*-based biopesticides in agricultural systems and new approaches to improve their use in Brazil. Biological Control, v. 165, 104792. https://doi.org/10.1016/j.biocontrol.2021.104792

Paiva, G.M.S.; Araujo, G.P.; Lins, I.X.; Cavalcanti, D.; Lima; Santos, L.B.; Benachour, M.; Santos, V.A., 2024. Sustainable reduction of sulfate contained in gypsum waste: perspectives and applications for agroforestry waste and sanitary sewage. Revista Brasileira de Ciências Ambientais, v. 59, e1752. https://doi.org/10.5327/Z2176-94781752

Parra, J.R.P., 2023. Biological Control in Brazil: state of art and perspectives. Scientia Agricola, v. 80, e20230080. http://doi.org/10.1590/1678-992X-2023-0080

Pinto, J.R.L.; Nascimento, J.; Polanczyk, R.A., 2020. Suscetibilidade de *Stegasta bosqueella* (Chambers, 1875) (Lepidoptera: Gelechiidae) a ACMNPV (Lepigen®). South American Sciences, v. 1, (2), e2024. https://doi.org/10.17648/sas.v1i2.24

Rao, T.; Jurat-Fuentes, J., 2020. Advances in the use of entomopathogenic bacteria/microbial control agents (MCAs) as biopesticides in suppressing crop insect pests. In: Birch, N.; Glare, T. (Eds.), Biopesticides for Sustainable Agriculture. Burleigh Dodds Science Publishing, Cambridge, pp. 1-37.

Sosa-Gómez, D.R.; Corrêa-Ferreira, B.S.; Kraemer, B.; Pasini, A.; Husch, P.E.; Delfino Vieira, C.E.; Negrão Lopes, I.O., 2020. Prevalence, damage, management and insecticide resistance of stink bug populations (Hemiptera: Pentatomidae) in commodity crops. Agricultural and Forest Entomology, v. 22, (2), 99-118. https://doi.org/10.1111/afe.12366

Staback, D.; Blanck, P.L.; Mariussi, V.; Galante, V.A., 2020. Uso do MIP como estratégia de redução de custos na produção de soja no estado do Paraná. Revista Americana de Empreendedorismo e Inovação, v. 2, (1), 187-200. https://doi.org/10.33871/26747170.2020.2.1.3297

Zakseski, M.R.; Da Silva Filho, J.G.; Rakes, M.; Pazini, J.D.B.; Da Rosa, A.P.S.; Marçon, P.; Popham, H.J.R.; Bernardi, O.; Bernardi, D., 2021. Pathogenic assessment of SfMNPV-based biopesticide on *Spodoptera frugiperda* (Lepidoptera: Noctuidae) developing on transgenic soybean expressing Cry1Ac insecticidal protein. Journal of Economic Entomology, v. 114, (6), 2264-2270. https://doi.org/10.1093/jee/toab170