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Dynamics of lepidopteran pests and a phylogeographic approach of three *Spodoptera* species (Lepidoptera: Noctuidae) on soybean fields in Brazil

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Thesis presented to obtain the degree of Doctor in Science. Area: Entomology

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RESUMO

Dinâmica de lepidópteros-praga e abordagem filogeográfica de três espécies de *Spodoptera* (Lepidoptera: Noctuidae) na cultura da soja no Brasil

A soja é uma das fontes mais importantes de óleo e proteína em todo o mundo. A área plantada com soja aumentou significativamente nos últimos 50 anos e, atualmente, o Brasil é o maior produtor mundial. Várias inovações agrícolas foram necessárias para aumentar a produção, tais como o sistema de plantio direto, correção da acidez do solo e adubação, novas variedades adaptadas a várias latitudes, inoculação de bactérias fixadoras de nitrogênio, sistema de multiculturas com pelo menos duas safras por ano e o uso de plantas geneticamente modificadas. A tecnologia de soja MON 87701 × MON 89788, que expressa a proteína Cry1Ac e confere a tolerância ao herbicida glifosato, vem sendo amplamente adotada no Brasil desde 2013. No entanto, mudanças na composição de pragas ou a evolução de resistência podem reduzir os benefícios desta tecnologia. Apesar da importância no Manejo Integrado de Pragas, o conhecimento sobre a dinâmica populacional, mudanças na composição e a estrutura genética das populações de pragas agrícolas no Brasil ainda são incipientes. Neste trabalho, são apresentadas evidências da redução regional no uso de inseticidas em áreas no Brasil onde a soja Cry1Ac é cultivada, com redução de até 50% no número de aplicações de inseticidas para o manejo de lepidópteros-praga em soja não-*Bt*, observado em locais específicos. Além disso, um número reduzido de adultos de *Chrysodeixis includens* foi capturado ao longo dos anos em um estudo de monitoramento de pragas em quatro mesorregiões. O número de adultos de *Helicoverpa* spp. capturados também foi reduzido em três mesorregiões. Em uma amostragem em larga escala de lagartas em lavouras comerciais de soja durante as safras de 2019 e 2020, *C. includens* foi o principal lepidóptero-praga presente em áreas não-*Bt*. Mais de 98% das lagartas encontradas na soja Cry1Ac foram representadas por *Spodoptera* spp., embora os números de *Spodoptera* spp. foram semelhantes entre os campos de soja Cry1Ac e não-*Bt*. A soja Cry1Ac resultou em um alto nível de proteção contra *Anticarsia gemmatalis*, *C. includens*, *Chloridea virescens* e *Helicoverpa* spp. Para investigar a diversidade genética, estrutura populacional e demografia de *Spodoptera eridania*, *Spodoptera cosmioides* e *Spodoptera frugiperda*, foram realizadas análises baseadas na sequência do COI mitocondrial. Alta diversidade genética foi observada para *S. eridania* amostrada em soja no Brasil. O índice de diversidade genética de *S. eridania* foi superior em relação aos valores observados para *S. cosmioides* e *S. frugiperda*. Baixa estrutura genética foram observadas para as três espécies de *Spodoptera*. O maior Φ_{ST} observado foi para *S. cosmioides* (0,058) seguido por *S. eridania* (0,058) e *S. frugiperda* (0,017). Há evidências de que as três espécies de *Spodoptera* avaliadas neste estudo estão em expansão demográfica e espacial. Os resultados do estudo também sugerem que a linhagem milho é a principal linhagem de *S. frugiperda* que ocorre na cultura da soja no Brasil. Esta pesquisa clarificou o conhecimento sobre as populações de insetos-praga que atacam a cultura da soja no Brasil. Foi observado uma supressão regional de lepidópteros-praga e redução do uso de inseticidas relacionado com a alta adoção da soja Cry1Ac, trazendo benefícios econômicos e ambientais. *Chrysodeixis includens* e *A. gemmatalis* continuam sendo os principais lepidópteros-praga da soja no Brasil e a soja Cry1Ac continua a oferecer proteção contra as pragas-alvo. O aumento da abundância relativa de *Spodoptera* spp. em soja não-*Bt* e Cry1Ac confirmou o aumento da

importância deste gênero na soja. Por fim, estas espécies de *Spodoptera* apresentam baixa estrutura genética e estão em expansão demográfica e espacial, provavelmente impulsionadas pela expansão da agricultura, principalmente com lavouras de soja.

Palavras-chave: Soja Cry1Ac, Planta *Bt*, MIP, Genética de população

ABSTRACT

Dynamics of lepidopteran pests and a phylogeographic approach of three *Spodoptera* species (Lepidoptera: Noctuidae) on soybean fields in Brazil

Soybean is one of the most important sources of oil and protein worldwide. The area planted with soybean significantly increased in the last 50 years, and currently, Brazil is the largest producer. Several agricultural innovations were needed to increase production such as no-tillage system cultivation, soil acid correction and fertilization, new crop varieties adapted to a range of latitudes, inoculation of nitrogen-fixing bacteria, multi-crop system with at least two cropping seasons per year and the use of genetically modified plants. The soybean technology MON 87701 × MON 89788, expressing Cry1Ac and conferring tolerance to glyphosate has been widely adopted in Brazil since 2013. However, pest shifts or resistance evolution could reduce the benefits of this technology. Despite the importance to Integrated Pest Management, knowledge on the population dynamics, shifts and genetic structure of agriculture pest populations in Brazil are still incipient. Here, is presented evidence of the regional reduction in insecticide use across areas in Brazil where Cry1Ac soybean is grown, with up to 50% reduction in the number of insecticide applications for managing lepidopteran pests on non-*Bt* soybean observed at specific locations. Furthermore, a reduced number of *Chrysodeixis includens* moths were captured across years in a pest-monitoring study in four mesoregions. The number of *Helicoverpa* spp. moths captured also were reduced at three mesoregions. In a large-scale sampling of larvae on commercial soybean fields during the 2019 and 2020 crop seasons, *C. includens* was the main lepidopteran pest in non-*Bt* fields. More than 98% of larvae found in Cry1Ac soybean were *Spodoptera* spp., although the numbers of *Spodoptera* spp. were similar between Cry1Ac soybean and non-*Bt* fields. Cry1Ac soybean provided a high level of protection against *Anticarsia gemmatalis*, *C. includens*, *Chloridea virescens* and *Helicoverpa* spp. To investigate the population genetic diversity, population structure, and demographic pattern of *Spodoptera eridania*, *Spodoptera cosmioides* and *Spodoptera frugiperda*, a mitochondrial COI sequence-based analyzes were performed. High genetic diversity was observed for *S. eridania* sampled on soybean in Brazil. The genetic diversity index of *S. eridania* was higher than that observed for *S. cosmioides* and *S. frugiperda*. Low genetic structure was observed for the three *Spodoptera* species. The highest Φ_{ST} observed was for *S. cosmioides* (0.058) followed by for *S. eridania* (0.058) and *S. frugiperda* (0.017). There is evidence that the three *Spodoptera* species evaluated in this study are in demographic and spatial expansion. The results of the study also suggest that corn strain is the major lineage of *S. frugiperda* occurring in soybean in Brazil. This research clarified and updated the knowledge of insect pest populations attacking soybean in Brazil. A regional suppression of lepidopteran pests and reduced insecticide use with the widespread adoption of Cry1Ac soybean has been observed, bringing economic and environmental benefits. *Chrysodeixis includens* and *A. gemmatalis* continue to be primary lepidopteran pests of soybean in Brazil and Cry1Ac soybean continues to effectively manage the target lepidopteran pests. Increase in the relative abundance of non-target *Spodoptera* spp. larvae in both non-*Bt* and Cry1Ac soybeans confirmed the emerging importance of this genera in soybean. Finally, these *Spodoptera* species show low genetic structure and are at demographic and spatial expansion, probably driven by agriculture expansion, especially soybean fields.

Keywords: Cry1Ac soybean, *Bt* plant, IPM, Population genetics

1. INTRODUCTION

Soybean is the main crop planted in Brazil and one of the most important sources of oil and protein worldwide (USDA 2022). Brazil is responsible for approximately 23% of global production, followed by the United States, China, and Argentina, with 20, 10 and 8%, respectively (USDA 2022). A turning point of the Brazilian agriculture was the expansion to the Cerrado, allowing the growth of soybean cultivated area from approximately 1.3 million hectares in 1970 to more than 38 million hectares in the 2020/21 season, with an estimated production of 135.9 million tons of soybean grain (CONAB 2022). The advance of agriculture in Cerrado was possible with innovations and, in a period of 50 years, several changes were observed: no-tillage system cultivation, soil correction and fertilization, new crop varieties adapted to a range of latitudes with increased yield, inoculation of nitrogen-fixing bacteria, the multi-crop system with at least two cropping season per year and the use of genetically modified plants are some examples (Fatoretto et al. 2017; Cattelan and Dall'Agnol 2018; Umburanas et al. 2022). As soybean production in Brazil transformed into a highly structured and organized large-scale business operation primarily targeting export markets, the need to reach high yield implies adopting good agricultural practices and consequently pest management.

Intensive and large use of agricultural land creates an environment conducive to the buildup of relatively large insect pest populations (Fatoretto et al. 2017; Silva et al. 2020). "Tropical agriculture" such as that practiced in Brazil is typically based on two or more crop seasons per year, allowing pest populations to go through multiple generations per year potentially under selection to control tactics pressure such as insecticides and *Bt* crops (Fatoretto et al. 2017). Insect populations in tropical agriculture might also present rapid plastic responses to environmental changes, as observed in *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae) (Silva-Brandão et al. 2017). Rapid responses, adaptations and high pest pressure are some of the reasons for the difficulty in managing pests in Brazil.

In this context, the soybean technology MON 87701 × MON 89788 (Intacta RR2 PRO®), expressing the Cry1Ac insecticidal protein (event MON 87701) and conferring tolerance to glyphosate (event MON 89788), was commercially launched and became available to farmers in Brazil in 2013. The adoption of Cry1Ac soybean by Brazilian farmers increased from 1.2 million hectares in the 2013/14 cropping season to 30 million hectares in the 2020/21 cropping season (CIB and AGROCONSULT 2018; SPARK 2021). The rapid adoption of MON 87701 × MON 89788 soybean has been driven by the significant yield advantage of varieties containing this technology and the high levels of protection against the primary soybean lepidopteran pests in Brazil. MON 87701 × MON 89788 soybean provides adequate protection against larval feeding by *Anticarsia gemmatalis* (Hübner, 1818) (Lepidoptera: Erebidae), *Chrysodeixis includens* (Walker, [1858]), *Chloridea virescens* (Fabricius, 1781) and *Helicoverpa armigera* (Hübner, 1808) (Lepidoptera: Noctuidae) (Bernardi et al. 2012, 2014; Dourado et al. 2016; Horikoshi et al. 2021).

Beyond the direct benefit of controlling target pests, *Bt* crops such as Cry1Ac soybean have the potential to provide additional benefits to insect management in agricultural systems, including a reduction in insecticide use (Brookes 2018), compatibility with biocontrol measures (Edgerton et al.

2012; Lu et al. 2012), and regional suppression of insect pest populations (Carrière et al. 2003; Hutchison et al. 2010; Wu et al. 2008; Dively et al. 2018). Regional pest suppression might occur when *Bt* plants account for a significant amount of available host plants for a pest species in the agricultural landscape, reducing the pest population (Tabashnik 2010). Suppression of target pests after a long period of use of highly efficacious *Bt* technologies has been documented in *Pectinophora gossypiella* (Saund., 1844) (Lepidoptera: Gelechiidae), *Ostrinia nubilalis* (Hübner, 1796) (Lepidoptera: Pyralidae) and *Helicoverpa zea* (Boddie, 1850) (Lepidoptera: Noctuidae) in the USA (Carrière et al. 2003; Hutchison et al. 2010; Dively et al. 2018) and *H. armigera* in China (Wu et al. 2008). Similarly, the high efficacy of Cry1Ac soybean against lepidopteran pests such as *C. includens* and *A. gemmatalis*, the leading soybean pests in Brazil (Bernardi et al. 2012), resulted in fewer insecticide sprays to manage lepidopteran larvae after five years of commercial use in Brazil (Brookes 2018).

In addition, resistance evolution by target pest populations can reduce the benefits of *Bt* crops (Gould 1998). Understanding the performance of a *Bt* crop against target pests at the field level is an important component to manage resistance and drive pest management strategies, as pest abundance might be directly affected. In some situations, where a *Bt* technology is ineffective against non-target secondary pest species and or broad-spectrum insecticide use has decreased due to highly effective control of the target species, secondary pests may increase in abundance over time (Lu et al. 2010; Zhao et al. 2011). Determining whether non-target pests are increasing in abundance can inform the need of adoption of appropriate Integrated Pest Management (IPM) practices in Brazil.

Among the non-target pest species of Cry1Ac soybean, the genus *Spodoptera* is highlighted. *Spodoptera* genus is along with *Helicoverpa* genus, a major problematic and widespread Lepidopteran pest genus worldwide (Kergoat et al. 2021). In Brazil, the most known species of this genus is the fall armyworm, *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae), the main corn pest (Blanco et al. 2016; Fatoretto et al. 2017) and the representative that is in the spotlight recently with the invasion of eastern hemisphere (Georgen et al. 2016, Kalleshwaraswamy et al. 2018; Jing et al. 2019). However, other pest species of this genera: *Spodoptera eridania* (Stoll, 1782) (Lepidoptera: Noctuidae) and *Spodoptera cosmioides* (Walker, 1898) (Lepidoptera: Noctuidae) are also economically important pests in Brazil (Montezano et al. 2014; Specht, Roque-Specht 2016; Horikoshi et al., 2021). Along with *S. frugiperda*, these three species are commonly referred to as *Spodoptera* complex and are the main *Spodoptera* species that attacks soybean in Brazil (Horikoshi et al., 2021). That complex was not considered a significant threat to the soybean crop until recently, and we can consider them as emerging pests of soybean crop (Panizzi, Corrêa-Ferreira 1997; Sosa-Gómez et al. 2014, Horikoshi et al. 2021). In addition, Cry1Ac soybean does not confer protection to these three *Spodoptera* species, which is why it needs to be monitored (Bernardi et al. 2014).

Phylogeography is the field of study to understand the principles and processes governing the geographic distribution of genealogical lineages (Freeland, 2011). Comprehend how historical events are influencing the shape of geographical dispersion of genes, population and species is the objective of phylogeography (Freeland, 2011). Changes in agricultural landscapes may affect the population dynamics. As agricultural practices modify the landscape and usually simplify the environment structure over large areas, it affects the gene flow among populations (Altieri 1999;

Gauffre et al. 2015; Alvarado-Serrano et al. 2019). Gene flow might result from dispersal, that is the movement of individuals and holds a central role in population dynamics and structure (Ronce 2007). Understanding population genetics is an essential piece of integrated pest management (IPM) as lineages or strains might be present in a country of continental proportions. *S. frugiperda* corn and rice strains are an example of the presence of host adapted lineages in the American continent (Pashley, Martin 1987; Nagoshi, Meagher 2008; Siva-Brandão et al. 2018). Recently, lineages were also found in *Euschistus heros* (Hemiptera: Pentatomidae) in Brazil, with an older and more diverse strain arising in the northern regions and a younger and less diverse strain occurring in the southern region (Soares et al. 2018). The presence of strains in the landscape might directly affect the response to control tactics, as different susceptibility is observed for insecticides and *Bt* proteins (Ríos-Díez, Saldamando-Benjumea 2011; Ingber et al. 2018).

The study of the dynamic of soybean lepidopteran pest species is essential to the implementation of effective pest management in Brazil. The expansion of agriculture in Cerrado along with changes in agricultural practices in the last 50 years, and more recently the introduction of *Bt* soybean in Brazil revolutionized pest management practices. Understanding the Cry1Ac soybean performance and its impacts on pest management is important to assess the benefits of technology in the production system. Also, clarifying the current scenario of pest abundance and distribution will aid in the refinement of pest management at a regional level, as large countries might have particularities regarding pest populations. Studies are necessary to evaluate the soybean pest dynamics to verify whether changes occurred after changes in agricultural practices in the last years. Moreover, the information on population diversity and structure is lacking for the emerging pests of soybean *S. eridania*, *S. cosmioides* and *S. frugiperda*. Therefore, the major goal in the current research were:

a) Assess whether the widespread adoption of Cry1Ac soybean in Brazil has promoted the regional suppression of natural populations of its target pests;

b) Evaluate Cry1Ac soybean performance and impacts on soybean pest management, assessing the relative abundance of lepidopteran pest species attacking soybean fields and comparing these results to data collected before the commercial introduction of Cry1Ac soybean;

c) Understand the genetic diversity and population dynamics of emerging pests of soybean *S. eridania*, *S. cosmioides* and *S. frugiperda* in Brazil.

References

Altieri, M. A. The ecological role of biodiversity in agroecosystems. In *Invertebrate biodiversity as bioindicators of sustainable landscapes* (pp. 19-31). Elsevier (1999).

Alvarado-Serrano, D. F., Van Etten, M. L., Chang, S. M., & Baucom, R. S. The relative contribution of natural landscapes and human-mediated factors on the connectivity of a noxious invasive weed. *Heredity*, **122,** 29-40 (2019).

Bernardi, O. *et al.* Assessment of the high-dose concept and level of control provided by MON 87701× MON 89788 soybean against *Anticarsia gemmatalis* and *Pseudoplusia includens* (Lepidoptera: Noctuidae) in Brazil. *Pest Manag. Sci.,* **68,** 7, 1083–1091 (2012).

Bernardi, O. *et al.* High levels of biological activity of Cry1Ac protein expressed on MON 87701× MON 89788 soybean against *Heliothis virescens* (Lepidoptera: Noctuidae). *Pest Manag. Sci.,* **70,** 4, 588–594 (2014).

Blanco, C. A. *et al.* Current situation of pests targeted by Bt crops in Latin America. *Curr. Opin. Insect Sci.*, **15,** 131-138, 2016.

Brookes, G. The farm level economic and environmental contribution of Intacta soybeans in South America: the first five years. *GM Crops Food,* **9,** 3, 140–151 (2018).

Carrière, Y. *et al.* Long-term regional suppression of pink bollworm by *Bacillus thuringiensis* cotton. *Proc. Natl. Acad. Sci.,* **100,** 4, 1519–1523 (2003).

Cattelan, A. J. & Dall'Agnol, A. The rapid soybean growth in Brazil. *Oilseeds Fats Crops Lipids,* **25,** D102 (2018).

CIB, AGROCONSULT. Impactos econômicos e sócio-ambientais da tecnologia de plantas resistentes a insetos no Brasil - Análise histórica, perspectivas e desafios futuros. p. 3-57. Disponível em: <http://apps.agr.br/wp-content/uploads/2018/12/Impactos-do-Milho-Bt-no-Brasil.pdf>. 2018.

CONAB—Companhia Nacional de Abastecimento. Acompanhamento da Safra Brasileira - Grãos. V.8 - SAFRA 2020/21 - N. 12 - Décimo segundo levantamento. https://www.conab.gov.br/info-agro/safras/graos/boletim-da-safra-de-graos (2021).

Dively, G.P. *et al.* Regional pest suppression associated with widespread Bt maize adoption benefits vegetable growers. *Proc. Natl. Acad. Sci.,* **115,** 13, 3320–3325 (2018).

Dourado, P.M. *et al.* High susceptibility to Cry1Ac and low resistance allele frequency reduce the risk of resistance of *Helicoverpa armigera* to Bt soybean in Brazil. *PLoS One,* **11,** 8, e0161388 (2016).

Edgerton, M. D. *et al.* Transgenic insect resistance traits increase corn yield and yieldstability. *Nat. Biotechnol.,* **30,** 493–496 (2012).

Fatoretto, J. C., Michel, A. P., Silva Filho, M. C. & Silva, N. Adaptive potential of fall armyworm (Lepidoptera: Noctuidae) limits Bt trait durability in Brazil. *J. Integr. Pest Manag.,* **8,** 17 (2017).

Freeland, J. Molecular ecology. Oxford Wiley-Blackwell Hoboken, NJ, Oxford Hoboken, NJ, 2nd edition. (2011).

Gauffre, B., Mallez, S., Chapuis, M. P., Leblois, R., Litrico, I. et al. Spatial heterogeneity in landscape structure influences dispersal and genetic structure: empirical evidence from a grasshopper in an agricultural landscape. *Mol. Ecolol.,* **24,** 1713-1728 (2015).

Goergen, G., Kumar, P.L., Sankung, S.B., Togola, A., Tamo, M. First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. *PloS One*, **11,** e0165632 (2016).

Gould, F. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. *Annu. Rev. Entomol.,* **43,** 701-726 (1998).

Horikoshi, R. J. *et al.* Large-scale assessment of lepidopteran soybean pests and efficacy of Cry1Ac soybean in Brazil. *Sci. Rep.,* **11,** 15956 (2021).

Hutchison, W.D. *et al.* Areawide suppression of European corn borer with Bt maize reaps savings to non-Bt maize growers. *Science,* **330,** p. 222–225 (2010).

Ingber, D. A., Mason, C. E., & Flexner, L. Cry1 Bt susceptibilities of fall armyworm (Lepidoptera: Noctuidae) host strains. *J. Econ. Entomol.*, **111,** 361-368 (2018).

Jing, D.P. *et al.* Initial detections and spread of invasive *Spodoptera frugiperda* in China and comparisons with other noctuid larvae in cornfield using molecular techniques. *Insect Sci.,* **27,** 780-790, 2019.

Kalleshwaraswamy, C.M. *et al.* First report of the fall armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae), an alien invasive pest on maize in India. *Pest Manage. in Hortic. Ecosyst.,* **24,** 23-29 (2018).

Kergoat, G. J. *et al.* A novel reference dated phylogeny for the genus Spodoptera Guenée (Lepidoptera: Noctuidae: Noctuinae): new insights into the evolution of a pest-rich genus. *Mol. Phylogenet. Evol.,* **161,** 107161 (2021).

Lu, Y. et al. Mirid bug outbreaks in multiple crops correlated with wide-scale adoption of Bt cotton in China. *Science,* **328,** 1151-1154 (2010).

Lu, Y., Wu, K., Jiang, Y., Guo, Y., Desneux, N. Widespread adoption of Bt cotton and insecticide decrease promotes biocontrol services. *Nature,* **487,** 362-365 (2012).

Montezano, D.G., Specht, A., Sosa–Gomez, D.R., Roque–Specht, V.F., Barros, N.M. Immature stages of *Spodoptera eridani*a (Lepidoptera: Noctuidae): developmental parameters and host plants. *J. Insect Sci.,* **14,** 238 (2014).

Nagoshi, R.N., Meagher, R.L. Review of fall armyworm (Lepidoptera: Noctuidae) genetic complexity and migration. *Fla. Entomol.,* **91,** 546-554 (2008).

Panizzi, A. R., Corrêa-Ferreira, B. S. Dynamics in the insect fauna adaptation to soybeans in the tropics. *Trends Entomol.*, **1,** 71–88 (1997).

Pashley, D.P., Martin, J.A. Reproductive incompatibility between host strains of the fall armyworm (Lepidoptera: Noctuidae). *Ann. Entomol. Soc. Am.,* **80,** 731-733 (1987).

Ríos-Díez, J. D., Saldamando-Benjumea, C. I. Susceptibility of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) strains from central Colombia to two insecticides, methomyl and lambda-cyhalothrin: a study of the genetic basis of resistance. *J. Econ. Entomol.*, **104,** 1698-1705 (2011).

Ronce, O. How does it feel to be like a rolling stone? Ten questions about dispersal evolution. *Annu. Rev. Ecol. Evol. Syst.,* **38,** 231-253 (2007).

Silva, C. S. *et al.* Population expansion and genomic adaptation to agricultural environments of the soybean looper, *Chrysodeixis includens*. *Evol. Appl.,* **13,** 2071-2085 (2020).

Silva-Brandao, K. L. *et al.* Loci under selection and markers associated with host plant and host-related strains shape the genetic structure of Brazilian populations of *Spodoptera frugiperda* (Lepidoptera, Noctuidae). *PloS One*, **13,** e0197378 (2018).

Silva-Brandão, K.L. *et al.* Transcript expression plasticity as a response to alternative larval host plants in the speciation process of corn and rice strains of *Spodoptera frugiperda. BMC Genom.*, **18,** 792 (2017).

Soares, P. L., Cordeiro, E. M., Santos, F. N., Omoto, C., Correa, A. S. The reunion of two lineages of the Neotropical brown stink bug on soybean lands in the heart of Brazil. *Sci. Rep.*, **8,** 1-12 (2018).

Sosa-Gómez, D. R. *et al.* *Manual de Identificação de Insetos e Outros Invertebrados da Cultura da Soja.* (Embrapa Soja-Documentos (INFOTECA-E), 2014).

Spark. BIP soja 2021. *Spark Smarter Decisions*, Valinhos, SP, Brazil http://spark-ie.com.br. (2021).

Specht, A., Roque-Specht, V.F. Immature stages of *Spodoptera cosmioides* (Lepidoptera: Noctuidae): developmental parameters and host plants. *Zool.*, **33,** 2016.

Tabashnik, B.E. Communal benefits of transgenic corn. *Science,* **330,** 6001, 189–190 (2010).

Umburanas, R. C. *et al.* Changes in soybean cultivars released over the past 50 years in southern Brazil. *Sci Rep.,* **12,** 1-14 (2022).

USDA - United States Department of Agriculture Oilseeds: World Markets and Trade. (2022). https://usda.library.cornell.edu/concern/publications/tx31qh68h?locale=en. January 2022. Accessed 13 Jan 2022.

Wu, K.M., Lu, Y.H., Feng, H.Q., Jiang, Y.Y., Zhao, J.Z. Suppression of cotton bollworm in multiple crops in China in areas with Bt toxin–containing cotton. *Science,* **321,** 5896, 1676–1678 (2008).

Zhao, J. H., Ho, P.; Azadi, H. Benefits of Bt cotton counterbalanced by secondary pests? Perceptions of ecological change in China. *Environ. Monit. Assess*., **173,** 985-994 (2011).

2. REGIONAL PEST SUPPRESSION ASSOCIATED WITH ADOPTION OF Cry1Ac SOYBEAN BENEFITS PEST MANAGEMENT IN TROPICAL AGRICULTURE

ABSTRACT

Bt crops have been adopted worldwide, providing high-level protection from insect pests. Furthermore, *Bt* crops preserve natural enemies, promote higher yield, and economically benefit farmers. Although regional pest suppression by widespread *Bt* crop adoption has been observed in temperate regions, this possibility remains uncertain in tropical areas due to the high diversity of alternative hosts and mild winters. Here, we present evidence of regional reduction in insecticide use across areas in Brazil where Cry1Ac soybean is grown since 2013, with up to 50% reduction in the number of insecticide sprays for managing lepidopteran pests on non-*Bt* soybean observed at specific locations from 2012 to 2019. We also present pest-monitoring data in four mesoregions across five years of commercial plantings of Cry1Ac soybean from December 2014 to July 2019. Reduced numbers of *Chrysodeixis includens* moths were captured in pheromone traps across years at all locations. The number of *Helicoverpa* spp. moths captured also were reduced at three locations. Thus, we provide evidence for regional suppression of lepidopteran pests and reduced insecticide use with the widespread adoption of Cry1Ac soybean, bringing economic, social and environmental benefits.

Keywords: Intacta soybean, *Chrysodeixis includens*, *Helicoverpa*, IPM

3. LARGE-SCALE ASSESSMENT OF LEPIDOPTERAN SOYBEAN PESTS AND EFFICACY OF Cry1Ac SOYBEAN IN BRAZIL

ABSTRACT

The soybean technology MON 87701 × MON 89788, expressing Cry1Ac and conferring tolerance to glyphosate, has been widely adopted in Brazil since 2013. However, pest shifts, or resistance evolution could reduce the benefits of this technology. To assess Cry1Ac soybean performance and understand the composition of lepidopteran pest species attacking soybeans, we implemented large-scale sampling of larvae on commercial soybean fields during the 2019 and 2020 crop seasons to compare with data collected prior to the introduction of Cry1Ac soybeans. *Chrysodeixis includens* was the main lepidopteran pest in non-*Bt* fields. More than 98% of larvae found in Cry1Ac soybean were *Spodoptera* spp., although the numbers of *Spodoptera* were similar between Cry1Ac soybean and non-*Bt* fields. Cry1Ac soybean provided a high level of protection against *Anticarsia gemmatalis*, *C. includens*, *Chloridea virescens* and *Helicoverpa* spp. Significant reduction in insecticide sprays for lepidopteran control in soybean were observed from 2012 to 2019. Our study showed that *C. includens* and *A. gemmatalis* continue to be primary lepidopteran pests of soybean in Brazil and that Cry1Ac soybean continues to effectively manage the target lepidopteran pests. However, there was an increase in the relative abundance of non-target *Spodoptera* spp. larvae in both non-Bt and Cry1Ac soybeans.

Keywords: MON 87701 × MON 89788, Plusiinae, *Spodoptera*, Integrated Pest Management (IPM), *Bt* plant, pest abundance

4. POPULATION GENETIC STRUCTURE AND DEMOGRAPHIC HISTORY OF EMERGING SOYBEAN PESTS *Spodotera eridania*, *Spodoptera cosmioides* AND *Spodoptera frugiperda*

ABSTRACT

Spodoptera genus is a major problematic and widespread Lepidopteran pest genus worldwide. Understanding the population genetics is an important piece of integrated pest management as lineages or strains might be present in a country of continental proportions. The current study objective was to (i) confirm the larvae identification, using COI marker, and define the genealogic relationship among *Spodoptera* species collected in soybean fields in Brazil; (ii) estimate the genetic diversity and population structure of *Spodoptera eridania*, *Spodoptera cosmioides* and *Spodoptera frugiperda* in different soybean macroregions; and (iii) investigate the population demographic of *S. eridania*, *S. cosmioides* and *S. frugiperda* in different soybean macroregions. A total of 89 *S. eridania*, 32 *S. cosmioides* and 29 *S. frugiperda* were identified and 33, 10 and 14 haplotypes were found for each species, respectively. High genetic diversity was observed for *S. eridania* sampled on soybean in Brazil. The genetic diversity indexes of *S. eridania* was higher than that observed for *S. cosmioides* and *S. frugiperda.* Low genetic structure was observed for the three *Spodoptera* species. The highest Φ_{ST} observed was for *S. cosmioides* (0.058) followed by for *S. eridania* (0.058) and *S. frugiperda* (0.017). Our results evidence that the populations of the three *Spodoptera* species evaluated in this study are in demographic and spatial expansion. The results also suggests that corn strain is the major lineage of *S. frugiperda* occurring in soybean in Brazil. This work increased our understanding of population dynamics of *S. eridania*, *S. cosmioides* and *S. frugiperda*.

Keywords: Phylogeography, *Spodoptera* complex, pest management, ecology

5. FINAL CONSIDERATIONS

The agriculture in Brazil expanded significantly in the last 50 years, bringing new technologies along this time. Insect populations are also adapting to the new agriculture landscapes and this thesis are a step forward to the understanding of population dynamics in Brazil. Here, regional pest suppression of two soybean lepidopteran pests were observed with the increase adoption of *Bt* soybean in Brazil. The lower abundance of insects in the field resulted in reduced insecticide sprays at a farm level. This is a first report of such benefit in a tropical environment and brings a lot of value for farmers to manage pests. The confirmation of pest suppression is an important finding as we can see the how the recently introduced *Bt* crop can bring direct and indirect benefits in the system.

In a large-scale sampling of larvae on commercial soybean fields during the 2019 and 2020 crop seasons, *C. includens* was the main lepidopteran pest in non-*Bt* fields. These results indicated that even with *C. includens* suppression by Bt soybean, this species is still an important pest in soybean fields. Probably, if *Bt* soybean were not present in the system, the abundance of this species could be even higher. Cry1Ac soybean have provided a high level of protection against *A. gemmatalis*, *C. includens*, *C. virescens* and *H. armigera* evidencing that this technology continues to be effective in manage pests in the field. In a near future, if the adoption of Cry1Ac soybean continue high and resistance alleles frequency remained low for these species, the pest suppression will probably continue to be present and will regionally benefit farmers in Brazil.

We also observed that 98% of larvae found in Cry1Ac soybean were *Spodoptera* spp., although the numbers of *Spodoptera* spp. were similar between Cry1Ac soybean and non-*Bt* fields. It is expected to observe larvae of this genera in Cry1Ac soybean as this technology does not confer protection to these species. However, our data showed an increase in the abundance of this species group when compared to the recent past. One hypothesis is that changes in production system might be related to that shift, e.g., winter corn area growth and species adaptation to soybean plants.

These emerging pests of soybean, *S. eridania*, *S. cosmioides* and *S. frugiperda*, have a potential to become a major pest in the soybean, but little information of population genetics is available to these species. We investigated the population genetic diversity, population structure, and demographic pattern, using the mitochondrial COI sequences. A high genetic diversity was observed for *S. eridania* sampled on soybean in Brazil. The genetic diversity index of *S. eridania* was higher than that observed for *S. cosmioides* and *S. frugiperda.* Weak genetic structure was observed for the three *Spodoptera* species. There is evidence that the three *Spodoptera* species evaluated in this study are in demographic and spatial expansion. The results of study also suggest that *S. frugiperda* corn strain is predominant on soybean in Brazil. These are initial steps to future investigations of the *Spodoptera* population genetics on soybean crops, where more abundant markers will be essential to understand this dynamic and guide new management strategies.

Overall, this thesis brought new insights to the population dynamics of the main lepidopteran pests present in soybean crop. After 50 years of agriculture expansion, Brazil became a top producer of soybean. In the current agriculture model, every detail is important to obtain the best result, following best agronomic practices along with sustainability. Further research is still needed to

understand better how the insects will respond to the agriculture landscape changes and the intensification of production systems, but the comprehension of insect dynamics will be a fundamental cornerstone.