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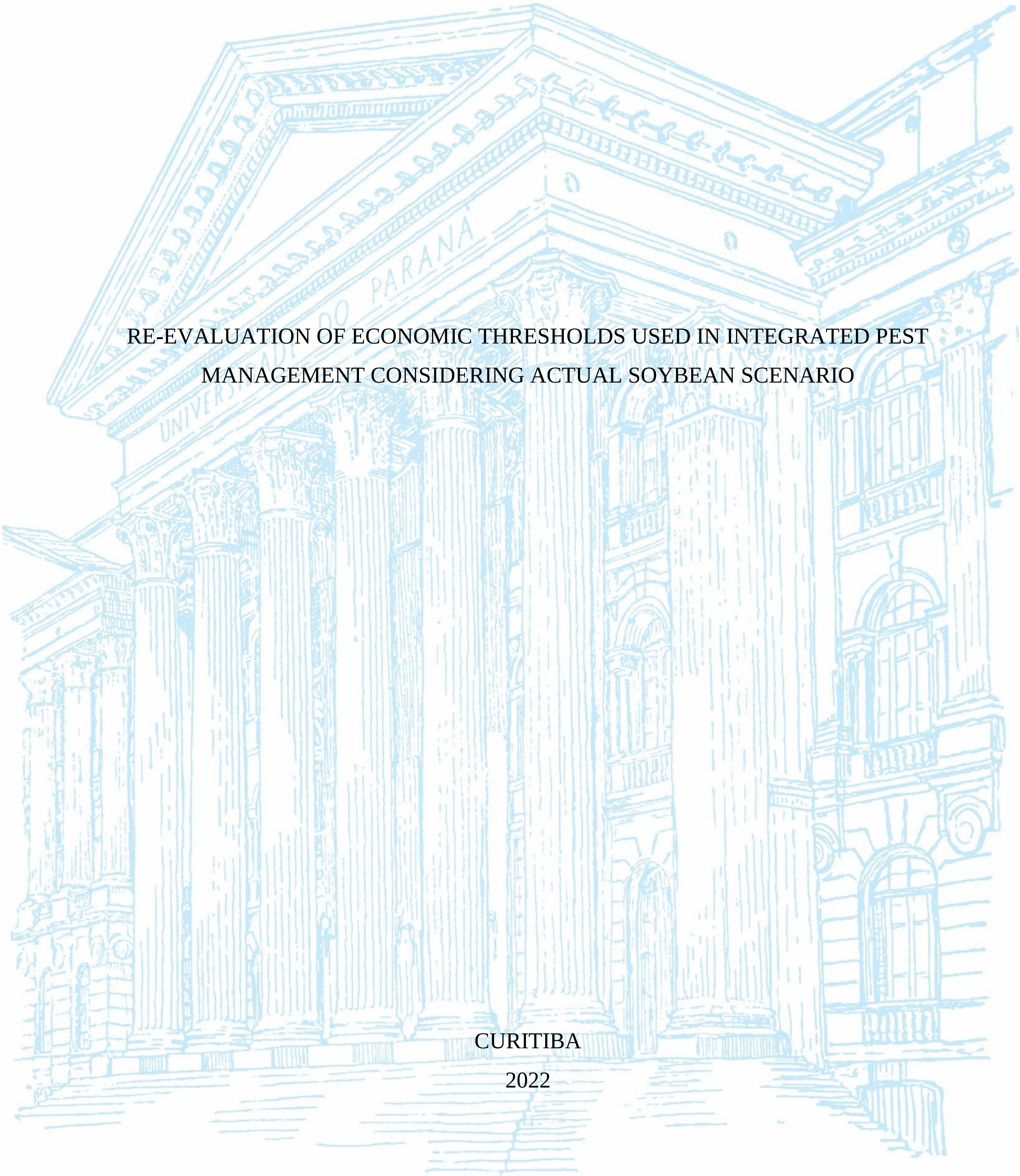

RE-EVALUATION OF ECONOMIC THRESHOLDS USED IN INTEGRATED PEST MANAGEMENT CONSIDERING ACTUAL SOYBEAN SCENARIO

CURITIBA

2022

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RE-EVALUATION OF ECONOMIC THRESHOLDS USED IN INTEGRATED PEST MANAGEMENT CONSIDERING ACTUAL SOYBEAN SCENARIO

Tese apresentada ao curso de Pós-graduação em Entomologia, Setor de Ciências Biológicas, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutor em Ciências Biológicas – Ênfase em Entomologia.

Orientador: Prof. Dr. Adeney de Freitas Bueno

Coorientador: Prof. Dr. William Wyatt Hoback

CURITIBA
2022

DADOS INTERNACIONAIS DE CATALOGAÇÃO NA PUBLICAÇÃO (CIP)
UNIVERSIDADE FEDERAL DO PARANÁ
SISTEMA DE BIBLIOTECAS – BIBLIOTECA DE CIÊNCIAS BIOLÓGICAS

Hayashida, Rafael.
Re-evaluation of economic thresholds used in integrated pest management considering actual soybean scenario. / Rafael Hayashida. – Curitiba, 2022.
1 recurso on-line : PDF.

Orientador: Adeney de Freitas Bueno.
Coorientador: William Wyatt Hoback.

Tese (Doutorado) – Universidade Federal do Paraná, Setor de Ciências Biológicas. Programa de Pós-Graduação em Ciências Biológicas (Entomologia).

1. Desfolhamento. 2. Soja. 3. Percevejo (inseto). 4. Agricultura – Aspectos econômicos. 5. Pentatomidae. I. Título. II. Bueno, Adeney de Freitas. III. Hoback, William Wyatt. IV. Universidade Federal do Paraná. Setor de Ciências Biológicas. Programa de Pós-Graduação em Ciências Biológicas (Entomologia).

Bibliotecária: Rosilei Vilas Boas CRB-9/939

MINISTÉRIO DA EDUCAÇÃO
SETOR DE CIÊNCIAS BIOLÓGICAS
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO CIÊNCIAS BIOLÓGICAS
(ENTOMOLOGIA) - 40001016005P5

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação CIÊNCIAS BIOLÓGICAS (ENTOMOLOGIA) da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **RAFAEL HAYASHIDA** intitulada: **Re-evaluation of economic thresholds used in integrated pest management considering actual soybean scenario**, sob orientação do Prof. Dr. ADENEY DE FREITAS BUENO, que após terem inquirido o aluno e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 28 de Julho de 2022.

Assinatura Eletrônica
01/08/2022 10:08:57.0
ADENEY DE FREITAS BUENO
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Assinatura Eletrônica
01/08/2022 10:00:19.0
YELITZA COROMOTO COLMENAREZ
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Assinatura Eletrônica
02/08/2022 09:36:07.0
PEDRO TAKAO YAMAMOTO
Avaliador Externo (ESCOLA SUPERIOR DE AGRICULTURA LUIZ DE QUEIROZ - UNIVERSIDADE DE SÃO PAULO)

Assinatura Eletrônica
01/08/2022 13:57:24.0
RENATO JUN HORIKOSHI
Avaliador Externo (BAYER S.A.)

I dedicate this work to my grandfather, Mário Hayashida (*In Memoriam*), who always encouraged me to study and keep me curious. Itsumo arigatou gozaimashita!

ACKNOWLEDGMENTS

More than an individual work, this dissertation is the result of the combined efforts of many people that I have had the honour and pleasure of working, discussing, collaborating and sharing experiences and good moments with.

First, I would like to thank my entire family. Especially to my parents, Yoko Nagay and Kiyoshi Hayashida, my sister, Raquel Nagay, my brother-in-law, Adriano dos Santos, my lovely niece, Alice Mayumi. Their faith in me has kept my motivation high during this process. Also, I would also like to thank my dog, Lulu, for all the entertainment and emotional support.

I'm extremely grateful, and here I express my thanks to my advisor, Dr. Adeney de Freitas Bueno, who accepted me as his student, providing me with immeasurable advice, guidance and feedbacks throughout this project. Without his invaluable guidance this project would not have been possible.

Special thanks to my co-advisor, Dr. Wyatt Hoback, for supervising me at Oklahoma State University, during the sandwich programme, and supporting me with extreme care and patience. I would like to extend my sincere thanks to Dr. Astri Wayadande, for teaching me the principles of EPG technique while I was at OSU.

I would like to thank my committee members, Dra. Silvana V. Paula-Moraes, Dra. Yelitza Colmenarez, Dr. Pedro Takao Yamamoto, Dr. Antonio Ricardo Panizzi and Dr. Renato Horikoshi who were more than generous with their expertise and precious time, accepting to participate as examiners of my doctorate defense.

I am grateful to Embrapa soja for providing the facilities for the conduction of the experiments and data analysis. I am also grateful to Embrapa employees, Nivaldo, Elias, Miguel, Sérgio Henrique, Antônio Pavão, Carneiro, Mari Estela, Allan, Rodrigo Leite, Vilma Stroka, Adriana Freitas, Claudinei, Fábio Paro (*in memoriam*) Dr. José Franca Neto, Dr. Irineu and Dr. José Marcos Gontijo Mandarino (*in memoriam*).

I am also grateful to every student I have met in Embrapa soja, Jaciara, Cláudia, Débora, Fernanda, Rodrigo, Paula, Érica, Ana Paula, Marcela, Pâmela, Bruna, Andrea, Jhonathas, Thiago, Larissa, Wellington, Hugo, Alan, Leonardo, Gabriel, Fábio, Vinicius, Everton, Thiago, your friendship and support have added a lot to my life!

I would like to thank the National Council for Scientific and Technological Development CNPq (process No. 142340/2018-9) and CAPES Foundation (No. 88887.374211/2019-00) for the scholarship provided to me.

Finally, I would like to thank my friends, Ricardo Vizo (*in memoriam*), Rebeca Bondiolli, Hugo, Leonardo José, Ana, Gi, Victor, Karen Polanco, Bina , Eloi Teté, Vinicius Bolinho, Marcelo, Giullia, Paulo Cremonez, Guilherme Tanaka, Rodolfo, João Nicoletti, Zé Ricardo, Cindy and Rodrigo Marubayashi for being by my side during this journey!

“Everyone must leave something behind when he dies, my grandfather said.
A child or a book or a painting or a house or a wall built or a pair of shoes made.
Or a garden planted. Something your hand touched some way so your soul has
somewhere to go when you die, and when people look at that tree or that flower you planted,
you're there.

It doesn't matter what you do, he said, so long as you change something from the
way it was before you touched it into something that's like you after you take your hands
away. The difference between the man who just cuts lawns and a real gardener is in the
touching, he said. The lawn-cutter might just as well not have been there at all; the gardener
will be there a lifetime.”
(Ray Bradbury, Fahrenheit 451)

RESUMO

Os níveis de ação (NA) estão bem estabelecidos para as principais pragas de soja e suas injurias; no entanto, eles devem ser frequentemente estudados e atualizados para as condições atuais de cultivo. Com o aumento da adoção de cultivares modernas, com menores índices da área foliar (IAF), torna-se necessário reavaliar os NAs de desfolha. Além disso, há uma necessidade de testar se há interação entre as injúrias por desfolha e por percevejos, e se essa interação também impacta nos NAs previamente estabelecidos para cada grupo individualmente. E, com a crescente ocorrência de *Crocridosema aporema* em campos de soja indeterminada *Bt*, é necessário estudar o impacto desse organismo em cultivares atuais. Portanto neste trabalho, conduziu-se experimentos para avaliadar o impacto da desfolha no IAF e na tolerância de cultivares modernas a essa injúria. Adicionalmente, foram realizados experimentos para testar se há interação entre injúrias causadas por desfolha artificial e percevejos fitófagos. Além disso, a capacidade de dano de *C. aporema* em cultivares indeterminadas de soja *Bt* também foi estudada. Os resultados indicaram haver diferenças de IAF entre cultivares ao longo do desenvolvimento das plantas, além de que os IAF sofreram redução conforme o aumento da intensidade de desfolha; além disso, não houve interação significativa entre as injúrias causadas por desfolha e por percevejo em todos os parâmetros testados; adicionalmente, *C. aporema* apresentou um baixo potencial de dano econômico em soja – especialmente nas novas cultivares indeterminadas *Bt* testadas. Portanto, a desfolha prévia pode afetar a capacidade da planta em tolerar injúrias e isso deve ser levado em consideração para o estabelecimento de NA por desfolha em cultivares moderna; os NA atualmente recomendados por desfolha e percevejos são suficientes e o manejo é recomendado somente quando atingir o NA para cada praga; e, o NA atualmente recomendado de *C. aporema* é muito conservativo, e deve ser aumentado para pelo menos 50% das plantas atacadas, com exceção de quando as plantas estiverem no estágio de florescimento, quando o NA de 30% deve ser adotado. Determinar o nível de tolerância das plantas de soja para os diferentes tipos de injúria é um passo fundamental para o desenvolvimento de NA para as diferentes espécies pragas e seus grupos, além de fundamental para a atualização dos NA para as condições modernas de cultivo.

Palavras-chave: *Glycine max* L. Nível de Dano Econômico. Desfolha artificial. Pentatomidae. *Crocidosema aporema*.

ABSTRACT

Economic thresholds are well-established for the soybean key pests and its injuries; however, it must be frequently studied and updated for current modern farm conditions. Here, experiments were done to study the impact of defoliation on the leaf area index (LAI) and on the tolerance of modern cultivars. The interaction between injuries caused by artificial defoliation and stink bugs on the ET were also tested. In addition to this, the damage potential of *Crocidosema aporema* on *Bt* soybeans were examined. Our results indicated that there were differences among cultivar's LAI during plant development and LAI was also reduced with increasing defoliation intensity; there is no interaction between injuries caused by defoliation and stinkbug for all parameters tested; and, *C. aporema* has low potential to cause economic injure to soybean plants—especially on the evaluated new Bt cultivars. Thus, past defoliation injury can affect the capacity of the plant to cope with injury and must be further evaluated for accurate defoliation ET establishment to modern cultivars; the currently recommended ET for defoliation and stink bugs are sufficient and the management is necessary only when the ET for each pest is reached; and the currently recommended ET of *C. aporema* is too conservative, and should be increased to at least 50% of injured plants, except when plants are in the flowering stage, when the ET of 30% can be adopted. Determining the level of soybean plant tolerance to different types of injuries is a crucial step to develop ETs for different pest species and injury guilds, and fundamental to update the previous stablished ET to the current farm condition.

Keywords: *Glycine max* L. Economic Injury Level. Artificial Defoliation. Pentatomidae. *Crocidosema aporema*.

SUMMARY

1 CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 GENERAL INTRODUCTION

The Economic Injury Level (EIL) and Economic Threshold (ET) are important concepts for the establishment of Integrated Pest Management (IPM) programs. Economic Injury Level is calculated considering the cost-benefit of decision-making regarding pest management, where the costs are related to the pest control (for example, the cost of an insecticide and its application), and the benefits are related to the economic return that this decision would bring (considering the yield market value) (STERN *et al.*, 1959; STONE; PEDIGO, 1972). The ET is, in practical terms, when the control measures should be initiated, in order to prevent an increasing pest population from reaching the EIL (PEDIGO; HUTCHINS; HIGLEY, 1986).

For the soybean key pests, the ET is well-established, and its adoption is proven to bring economic and ecological benefits (BUENO *et al.*, 2021; CONTE *et al.*, 2020). In Brazil, the ET recommended for defoliation is 30% in the vegetative stages and 15% during the reproductive stages (BATISTELA *et al.*, 2012; BUENO *et al.*, 2013). For stink bugs, the recommended ET is 2 insects per meter for grain production and 1 insect per meter for crop seeds (BUENO *et al.*, 2013).

Although the ET for defoliation is established, the development and adoption of modern cultivars with distinct characteristics demands continuous updates to the ET. Newer soybean cultivars with indeterminate growth habits and lower maturity groups have shown small size and lower leaf area index (LAI) (BATISTELA *et al.*, 2012; RICHTER *et al.*, 2014; ZANON *et al.*, 2015) Thus, there is a need for re-evaluation of the previous established ET for new cultivars with a lower leaf area index (HAYASHIDA *et al.*, 2021).

In addition, most previous studies assessed the impact of only one type of injury on soybean plant fitness. Under field conditions, however, plants are usually attacked by lepidopteran defoliators (Lepidoptera: Noctuidae and Erebidae) and stink bugs (Hemiptera: Pentatomidae), and little is known about the impact of both injuries occurring simultaneously on soybean fields on the yield, oil and protein content, and seed quality.

Another change in the soybean fields is the adoption of different *Bt* cultivars, expressing Cry1Ac toxin. With the increase in adoption of this technology without the due refuge area, there is also an increase in the cases of resistance of pests that were initially controlled by this technology (BUENO *et al*., 2021). One of these pests is the soybean bud borer, *Crocidosema aporema* (Walsingham, 1914) (Lepidoptera: Tortricidae). Although the damage potential of this pest was previously studied on soybean (FOERSTER; IEDE; SANTOS, 1983; LOURENÇÃO; MIRANDA, 1983; SIQUEIRA; SIQUEIRA, 2012), there is a lack of studies documenting *C. aporema* on cultivars with indeterminate growth habits containing *Bt* toxins.

Therefore, constant research and revalidation of the ET are crucial for the development of an accurate basis of IPM. The ET must be reliable and updated, considering the modern cultivars' tolerance mechanisms to insect injuries and to the possible interaction of multiple injuries.

1.2 OBJECTIVES

1.2.1 General objective

Assess the interaction between phytophagous insects and soybean plants and then update, if necessary, the established ETs.

1.2.2 Specific objectives

- To investigate the performance of modern soybean cultivars under different defoliation levels.
- To determine whether there is an interaction between defoliation and stink bug injuries, and whether this interaction varies with soybean phenological stage.
- To evaluate the damage potential of *C. aporema* on modern soybean cultivars under field conditions.

1.3 LITERATURE REVIEW

1.3.1 GENERAL SOYBEAN ASPECTS (*Glycine max* [L.])

1.3.1.1 Brazilian productive background

Brazil is currently the world's leading producer of soybeans, which is its major agricultural crop, with the highest gross production value (GAZZONI *et al.*, 2021). In the 2020/2021 crop season, the soybean cultivated area in Brazil was 38.51 million hectares and its national productivity was 3.53 ton/ha (COMPANHIA NACIONAL DE ABASTECIMENTO - CONAB, 2021). Despite climatic problems occurred in different regions of the country, such as over raining during harvest, there was an increase of 8.8% in production of 2020/2021 over the 2019/2020 crop season, reaching a record of 135.86 million tons (COMPANHIA NACIONAL DE ABASTECIMENTO - CONAB, 2021).

To increase soybean production while avoiding crop expansion over new areas, soybean productivity must be increased. There are two different alternatives to achieving such an increase in productivity. First, by genetic improvement (using classical breeding or biotechnological tools), allowing the productive potential to increase. Second, by avoiding or mitigating possible stresses that impact plant fitness, so the crop can reach its full yield potential (GAZZONI *et al.*, 2021).

The Brazilian scenario of soybean production still has some obstacles that do not allow it to reach its full genetic productive potential. Although Brazil has technology to achieve yields even higher than the current ones, this technology has not been fully adopted by most farmers. For most of the fields, there are still lots of possibilities related to what can be done to achieve higher productivity (GAZZONI *et al.*, 2021).

Among the most important obstacles responsible for decreasing productivity, stress caused by biotic agents such as insects, disease and weeds, and by abiotic agents such as drought periods and elevated temperatures must be highlighted (HIRAKURI, 2021). Only during the 2019/2020 crop season, Brazil lost 5.55 million tons of soybeans due to these stresses, amounting to approximately U$1.58 billion (HIRAKURI, 2021). In the state of Paraná, the cost of insect management was estimated at U$84.32 per hectare, or equivalent to 4.9% of its yield (CONTE *et al.*, 2020).

It is estimated that the current adoption of modern technologies for soybean production results in a savings of 71 million hectares. Despite such important results, there are still different challenges to increasing yields in a sustainable soybean production system (GAZZONI *et al.*, 2021) which need to be overcome.

1.3.1.2 Soybean quality, oil and protein content

Among several soybean uses, industrial processing for oil production (degummed, crude, deodorized and refined), meals, flours, and concentrates, in addition to biofuel production and animal feeding, are the most important ones (HIRAKURI *et al.*, 2018). For all these soybean uses to succeed, it is crucial to know the soybean oil and protein contents as well as the qualitative values of the soybean fractions of fatty acids and essential and non-essential amino acids.

The average soybean protein content of the last Brazilian national report was 36.9%, with values ranging from 31.6% to 41.1%, while the average oil content was 22.6%, with variations from 18.4% to 26.1%, depending on the origin of the grains (HENNING *et al.*, 2018). Compared to the USA, the average soybean oil and protein content in Brazil are slightly higher. In the 2020 crop season, the average protein content in the USA was 33.3% ± 1.2% and oil content of 20.5% ± 0.7% (NAEVE; MILLER-GARVIN; NAEVE, 2020). However, the authors considered atypical the last harvest and emphasized that the averages of the last 33 years are 35.0% ± 1.4% for protein and 18.7% ± 0.9% for oil content.

These contents are directly related to the edaphoclimatic conditions, genetics and sowing timing and weakly related to the amount of fertilizer adopted by growers (CARRÃO-PANIZZI *et al.*, 2021; HELMS; ORF, 1998; MOURTZINIS *et al.*, 2017; PIPOLO *et al.*, 2015). Furthermore, there is a positive correlation between yield and grain oil content, with a value of r= 0.40 reported. However, there is an inverse relationship between oil content and protein content, with r= -0.25 being reported (MOURTZINIS *et al.*, 2017).

Classic breeding programs aiming only to increase the protein content may negatively impact the oil content and productivity of the cultivar. It is estimated that, for every 5 to 7 $g.kg^{-}$

^{1} of protein, there is a reduction of 2 to 3 g.kg^{-1} of oil and 70 to 110 kg.ha^{-1}, which can result in a lower gross economic return per hectare (HELMS; ORF, 1998). This inverse relationship between productivity and protein content was also reported by other authors (CARRÃO-PANIZZI *et al.*, 2021; PIPOLO *et al.*, 2015). Breeding programs aiming to increase yields (higher productivity) at the expense of protein content may also be detrimental to the industry that needs high protein content (PIPOLO *et al.*, 2015).

Therefore, a way to optimize soybean production is by foreseeing the future use of the produced grains —for oil or protein— in order to establish the best management recommendation, sowing time, cultivar (and its maturity group), and the crop to be rotated with soybean, since all of them are key components for oil or protein production (CARRÃO-PANIZZI *et al.*, 2021; MOURTZINIS *et al.*, 2017).

With a pioneering work, Hirakuri *et al.* (2018) introduced the discussion about the value paid to protein content and soybean quality in the national market. According to the authors, currently the grain market does not take into consideration soybean protein content, which proportionally implies a lower value paid per unit of protein in soybeans with high protein content. Carrão-Panizzi *et al.* (2021) suggested the possibility of adding value (a premium) to soybeans with higher protein contents, for both the export of grains *in natura* and the crushing industry. Not only is protein content important, but also is oil content, which plays a key role in the national soybean market, responsible for around 82% of vegetable oil consumed in human feeding and also in biofuel, which met about 70% of the sector's national production in 2017 (HIRAKURI *et al.*, 2018).

The soybean grain quality classification in Brazil is currently regulated by the Technical Regulation for Soybean of Normative Instruction No. 11 (IN11) of May 15, 2007, and Normative Instruction No. 37 of July 27, 2011, of the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA) (BRASIL, 2007a, 2007b). These normative instructions define the official standard for classification, labelling, identifying, and defining the intrinsic and extrinsic quality of these grains.

Among the soybean defects described by IN11, there are damaged grains (burnt, moldy, fermented, sprouted, immature, shocked, and damaged by insects and/or disease) and greenish grains. The legal tolerance for each one separately is 8%. Among other defects are crushed, kneaded and broken grains with a tolerance of 30%, and foreign matter and impurities, for which the limit is 1%. This process of soybean classification is carried out by a grain classifier qualified in training approved by MAPA.

Among the defects most commonly found in Brazil, fermented grains, grains damaged by stink bugs, and broken/kneaded grains stand out, with a large variation between producing locations from where the samples were originated (HENNING *et al.*, 2018). It is worth mentioning that from the 2016/2017 crop season, samples with up to 11.69% of grains damaged by stink bugs were found, which reflects the importance of control measures to mitigate the impacts of these insects in the field.

1.3.1.3 Key pests and their economic impacts

Economic losses triggered by insects in Brazil are estimated at 17.7 billion dollars a year, representing 7.7% of the national production (OLIVEIRA *et al.*, 2014). In soybean, there is an estimated annual loss of about 4.31 million tons a year, *i.e.*, 1.51 billion dollars, or 55 dollars per hectare (OLIVEIRA *et al.*, 2014). In addition, discounts for defective grains that exceed the tolerance allowed by IN11 reached US$ 319.96 million in the 2016/2017 crop season, being fermented grains and grains attacked by insects the most important defects (HIRAKURI *et al.*, 2018). Among the soybean key pests that deserve attention in Brazil, two major groups stand out: the phytophagous stink bugs (Hemiptera: Pentatomidae), the caterpillars of the Noctuidae (Lepidoptera: Noctuidae) and Erebidae families (Lepidoptera: Erebidae) (BUENO *et al.*, 2017).

In addition to these key pests, there are some secondary ones that, despite being sporadically present in fields, rarely require control due to low population densities, which in many cases are naturally controlled by biocontrol agents. However, with the reduction of the populations of those beneficial organisms, the population of these pests tends to grow and reach higher densities than previously found, requiring insecticide sprays. Among these species are *Diabrotica speciosa* (Germar, 1824) (Coleoptera: Chrysomelidae) and the whitefly, *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) (HIROSE; MOSCARDI, 2012; POZEBON *et al.*, 2020).

There is also an increasing concern about the potential damage of the soybean bud borer, *Crocidosema aporema* (Walsingham, 1914) (Lepidoptera: Tortricidae), since the recent outbreaks associated with field evolved resistance has been reported (HORIKOSHI *et al.*, 2021). Species such as *Spodoptera litura* (Fabricius, 1775) (Lepidoptera: Noctuidae) and *Aphis* *glycines* Matsumura, 1917 (Hemiptera: Aphididae) are not currently found in Brazil, but they deserve special attention because the ecological and climatic conditions of the country can benefit their biology, and thus if they were accidentally introduced into the country, they could become key pests (POZEBON *et al.*, 2020).

1.3.1.4 Stink bugs (Hemiptera: Pentatomidae)

Among the most abundant phytophagous insects in soybeans are those that belong to the Pentatomidae family. In this complex of stink bugs belonging to the family Pentatomidae, there have been reported at least 54 species from soybean-growing areas (PANIZZI; SLANSKY, 1985). The Neotropical brown stink bug, *Euschistus heros* (Fabricius, 1794) (Hemiptera: Pentatomidae), is the most abundant species in South America, mainly in the central region of Brazil at latitudes between 0° and 23° (PANIZZI; CORREA-FERREIRA, 1997). Not only *E. heros* but also the green-belly stink bug *Diceraeus* spp. (Dallas, 1851) (Hemiptera: Pentatomidae) and the neotropical green stink bug stink bug *Piezodorus guildinii* (Westwood, 1837) (Hemiptera: Pentatomidae) are important stink bugs species found in soybean fields (CONTE *et al.*, 2020; PANIZZI; BUENO; SILVA, 2012). These stink bugs are economic important because they cause quantitative (decreasing yield) and qualitative (reducing seed vigor and grain quality) damage (BUENO *et al.*, 2017; CORRÊA-FERREIRA; KRZYZANOWSKI; MINAMI, 2009; SILVA *et al.*, 2012).

Despite being considered a key pest in Brazil and in the USA in the 1990s, the green stink bug, *Nezara viridula* (Linnaeus, 1758) (Hemiptera: Pentatomidae), is currently of little economic relevance in these countries. Some of the factors that explain the decline of this pest in importance during recent years can be: changes in soybean production management techniques, which reduced the number of host plants and favoured other competing pests, generating interspecific competition; the impact of natural and applied biological control, with parasitoids [especially *Trissolcus basalis* (Wollaston, 1858) (Hymenoptera: Scelionidae)] and predators; and the deleterious effects of global warming on the biology of the green stink bug (PANIZZI; LUCINI, 2016; SMANIOTTO; PANIZZI, 2015).

Less frequently and with little economic relevance to soybean, there are other hemipterans species such as: *Edessa meditabunda* (Fabricius, 1794), *Chinavia* spp., *Thyanta* *perditor* (Fabricius, 1794) (Hemiptera: Pentatomidae) and *Neomegalotomus parvus* (Westwood, 1842) (Hemiptera: Alydidae) (PANIZZI; BUENO; SILVA, 2012). The low economic significance of these species is due to their low frequency of occurrence as well as their low ability to harm soybean (LUCINI; PANIZZI, 2017).

1.3.1.5 *Euschistus heros* (Fabricius, 1798) (Hemiptera: Pentatomidae)

Despite its low abundance in the 1970s, today the Neotropical brown stink bug *E. heros* (FIGURE 1) is the most common species in soybean producing regions in Brazil and has become a serious phytosanitary problem for this and other crops due to the occurrence of high populations associated with the difficulty of their control (CONTE *et al.*, 2020; PANIZZI, 2015). Associated with these factors, the selection of insecticide-resistant populations is noted, possibly in response to indiscriminate insecticide use that directly impacts its ecological balance, causing a rapid pest resurgence (CONTE *et al.*, 2020; CORRÊA-FERREIRA; KRZYZANOWSKI; MINAMI, 2009; CORRÊA-FERREIRA; PANIZZI, 1999).

FIGURE 1 - THE NEOTROPICAL BROWN STINK BUG, *Euschistus heros* (Fabricius, 1794) (Hemiptera: Pentatomidae)

SOURCE: Embrapa Soja (2010).

The beginning of *E. heros* colonization in the field can start during the soybean vegetative stage, coming from other host plants or originating from areas with food shortages, in which the insect is kept in oligopause (MEDEIROS LENICE AMEGIER, 2009). However, its populational density increases after the soybean reproductive stage, from the phenological stage R3 (with the beginning of pod formation), frequently reaching high densities in the seed filling stage R5, coinciding with the most sensitive plant stage to pest injury (PANIZZI; BUENO; SILVA, 2012).

Usually, its population peak is observed at R6 (grain filling); when soybean reaches physiological maturity (R7), there is a decrease in *E. heros* population. At full maturity (R8), stink bugs tend to disperse to other host soybean fields and other host plants or eventually go into oligopause under dry vegetation in the off-season (CORRÊA-FERREIRA; PANIZZI, 1999).

During its lifecycle, the egg-adult stage lasts about 30 to 40 days, and the longevity of adults varies by sex. Females live from 39.3 to 56.7 days, and males live from 58.4 to 92.9 days. Females can oviposit 61 to 312 eggs during their lifetime, depending on the quality and availability of the food they have access to and the climatic conditions in which they are living (HAYASHIDA *et al.*, 2018; MENDOZA; DA ROCHA; PARRA, 2016; PANIZZI; BUENO; SILVA, 2012).

Among the control strategies available for stink bug management, the most widely used is the chemical one. However, biological control by egg parasitoids, such as *T. basalis* and *Telenomus podisi* Ashmead, 1893 (Hymenoptera: Scelionidae) can be a large-scale useful tool, compatible with current planting systems with majority adoption of *Bt* soybean (for lepdopteran pest control) (CORRÊA-FERREIRA, 2003; SILVA *et al.*, 2014). In addition, at field condition, E. heros population is naturally controlled by the parasitoids *Hexacladia smithii* Ashmead, 1891 (Hymenoptera: Encyrtidae), *Hyalomyodes* sp. And *Trichopoda giacomellii* (Blanchard 1966) (Diptera: Tachinidae) (CORRÊA-FERREIRA; NUNES; UGUCCIONI, 1998; PANIZZI; OLIVEIRA, 1998; ZERBINO; PANIZZI, 2019).

1.3.1.5.1 *Diceraeus* spp. (Hemiptera: Pentatomidae)

In the past, the green-belly stink bug, *Diceraeus melacanthus* Dallas, 1851 (FIGURE 2) and *Diceraeus furcatus* (Fabricius, 1775*)* (Hemiptera: Pentatomidae) were initially classified into the genus *Dichelops* Spinola, 1837. However, recently, after a phylogenetic analysis of the group, it was found that the characters previously used to classify the genus are homoplasy, and the genus *Dichelops* should not therefore be considered a natural group, thus proposing the ascension of *Diceraeus* to the genus (BARÃO; FERRARI; GRAZIA, 2020).

FIGURE 2 - THE GREEN-BELLY STINK BUG *Diceraeus melacanthus* (Fabricius, 1775) (Hemiptera: Pentatomidae)

SOURCE: Embrapa Soja (2010).

These two species were considered secondary pests in soybeans and were found infrequently until the 1980s in the Neotropical region. However, a large increase in their abundance and frequency has been observed, possibly due to changes in soybean cultivation techniques, such as succession of host crops, and also with the adoption of the no-till farming system (PANIZZI, 2015; SILVA *et al.*, 2013). In addition, *D. melacanthus* and *D. furcatus* are considered cosmopolitan and polyphagous insects, the former being found on 29 plant species, while *D. furcarthus* is found on 32 plant species (SMANIOTTO; PANIZZI, 2015), and both have corn and wheat as hosts, crops that are commonly used in rotation and succession with soybean by farmers in the second crop season. It seems that *D. furcatus* has a distribution more restricted to the subtropical regions, where temperatures are warmer, while *D. melacanthus* has a wider distribution (SMANIOTTO; PANIZZI, 2015).

1.3.1.6 Key lepidopteran species in soybean

There are several lepidopteran species that cause defoliation in soybeans; among them, the most important are the caterpillars of the Erebidae and Noctuidae families (MOSCARDI *et al.*, 2012). In Brazil, the most common caterpillar species found are the velvetbean caterpillar *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Erebidae) and the soybean looper *Chrysodeixis includens* (Walker, 1858) (Lepidoptera: Noctuidae) (CARVALHO; FERREIRA; BUENO, 2012). There are, however, other caterpillar species that are found sporadically, but that can contribute to soybean injury. They are composed of two groups: *Spodoptera* spp., and *Heliothinae* [*Chloridea virescens* (Fabricius, 1777) (Lepidoptera: Noctuidae), and *Helicoverpa* spp.] groups.

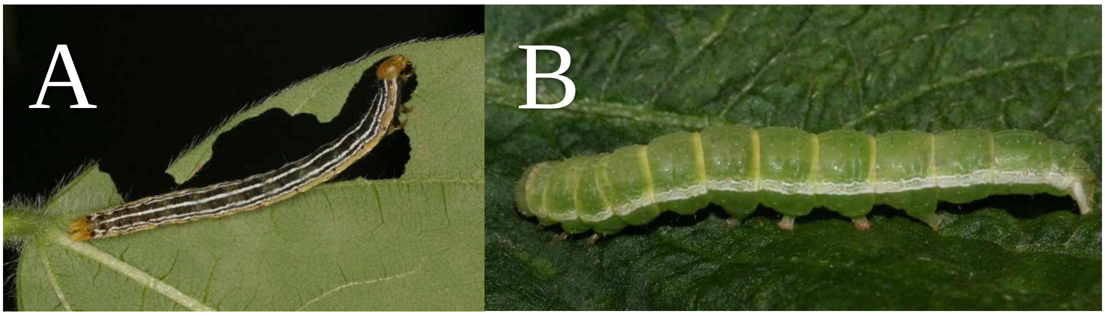
Defoliation injury causes a reduction in the plant's photosynthetically active area, which can compromise soybean productivity. The impact of this injury can vary according to the percentage of defoliation, how long the plant remains under injury, and in which developmental stage the plant is injured (vegetative or reproductive). Therefore, from a practical standpoint, it is possible to adopt an economic threshold (ET) for defoliation, parallel to the ET of each pest, considering that the plant's response to injury by defoliation is the same regardless of the causative agent.

1.3.1.6.1 *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Erebidae)

Among the lepidopteran pests of soybean, the velvetbean caterpillar *A. gemmatalis* is outstanding for its abundance and occurrence in all growing soybean regions in Brazil. Its feeding can trigger different intensities of defoliation, ranging from low defoliation levels to complete plant destruction. This caterpillar prefers new leaves, but it also damages petioles and stems (PRAÇA; NETO; MONNERAT, 2006).

In the field, it is possible to find caterpillars in two colours: black and green, with three longitudinal white lines on the back and four pairs of abdominal prolegs and one anal pair (FIGURE 3A and B). In its development, the velvetbean caterpillar usually passes through 6 instars, then it goes into the pupae stage in the ground, where, after about 11 days, a moth emerges with a variable coloration, from light grey to dark brown (MOSCARDI *et al.*, 2012) .

FIGURE 3 - THE VELVETBEAN CATERPILLAR *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Erebidae) IN BLACK (A) AND GREEN (B) COLOR.



SOURCE: Embrapa Soja (2010).

Besides its significant occurrence in Brazil, the velvetbean caterpillar is also considered the main soybean chewing pest in countries like the USA, Mexico, Colombia, Venezuela and Argentina (HOFFMANN-CAMPO *et al.*, 2000). Besides that, this caterpillar has a great diversity of hosts, among cultivated plants and wild ones, and it is possible to observe a preference for legumes such as soybeans, guandu beans and white lupin, although *A. gemmatalis* can feed on several other species (PANIZZI; OLIVEIRA; SILVA, 2004). In soybean, *A. gemmatalis* consumes about 74 to 95 cm^2 of leaf area during its total life cycle, and the greatest capacity for defoliation can be observed after its fifth instar (BUENO *et al.*, 2011; HUTCHINS; HIGLEY; PEDIGO, 1988).

High rainfall negatively impacts *A. gemmatalis* populations (LUZ *et al.*, 2019), either directly affecting the caterpillar population, preventing individuals from feeding, or supporting some entomopathogen infections and development. Climatic conditions can help the occurrence of epizootics by some fungi, naturally contributing to a large decrease in the number of these insects (SOSA-GÓMEZ, 2017).

For *A. gemmatalis* management, the adoption of IPM is recommended, with the integration of various control strategies rather than only chemical insecticide spraying. Among the strategies, one has a worldwide success: the use of Nuclear Polyhedrosis Virus (AgNPV), Baculovirus anticarsia. A pest control program based on its use was initiated in the 1980s, and the AgNPV reached 2 million hectares of soybean in the 2003/2004 crop season (MOSCARDI *et al.*, 2011; SOSA-GÓMEZ, 2017). However, this tool should be used only when necessary, that is, when it reaches the economic threshold, whose recommendation for this tool is 20 small caterpillars (smaller than 1.5 cm) (MOSCARDI, 1983).

1.3.1.6.2 *Chrysodeixis includens* (Walker, 1858) (Lepidoptera: Noctuidae)

The soybean looper, *C. includens* is found feeding on various agricultural crops, such as soybeans, corn, cotton, sunflower, beans, tomatoes, crucifers, as well as plants of interest in floriculture and non-crop plants (FIGURE 4) (MOSCARDI *et al.*, 2012). Despite this high host range, *C. includens* has a feeding preference for soybean, consuming in average 64 to 93 cm^2 leaf area, with greater consumption starting at the 5th instar (BUENO *et al.*, 2011; HUTCHINS; HIGLEY; PEDIGO, 1988).

FIGURE 4 - THE SOYBEAN LOOPER *Chrysodeixis includens* (WALKER, 1858) (LEPIDOPTERA: NOCTUIDAE)

SOURCE: Embrapa Soja (2010).

In Brazil, the soybean defoliating caterpillars' species has changed in recent years. Previously, *A. gemmatalis* was the most abundant species, however, since the 2002/2003 crop season, *C. includens* has become the predominant one (LUZ *et al.*, 2019; SOSA-GÓMEZ, 2017). The change in the composition of species is due possibly to farmers management changes. For example, with the increasing use of fungicides for Asian Soybean Rust (*Phakopsora pachyrhizi*), there was also a decrease in entomopathogenic fungi that naturally kept *C. includens* populations in balance; as a result, outbreaks of this pest began to be more frequent, thus becoming a key pest (MOSCARDI *et al.*, 2011).

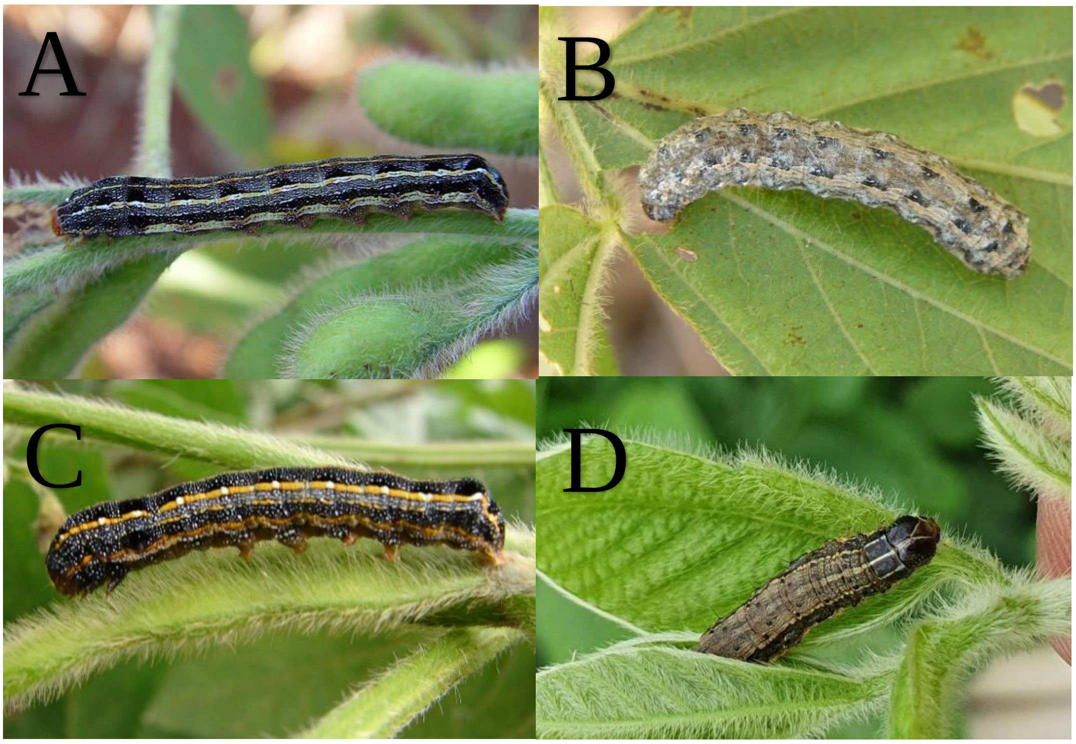
The popular name of *C. includens* is due to the looping movement it makes in its locomotion because it has only two pairs of abdominal prolegs. Egg-adult development is about 43 days, usually going through 6 instars (BARRIONUEVO *et al.*, 2012). The females live for about 15 days and can oviposit about 600 eggs, with a high reproductive capacity (HOFFMANN-CAMPO *et al.*, 2000).

It is primarily controlled through the use of transgenic Bt cultivars and also by chemical insecticide spraying (CARVALHO; FERREIRA; BUENO, 2012; CRIALESI-LEGORI *et al.*, 2014). Besides these methods, biological control by egg parasitoids is also a promising option. The use of micro-hymenoptera such as *Trichogramma pretiosum* (Riley, 1879) (Hymenoptera: Trichogrammatidae) presents several advantages over the chemical insecticides, such as the parasitoid ability to find eggs located in different plant regions, including places where the insecticide spray would hardly reach, ensuring a more efficient control of *C. includens* (BUENO *et al.*, 2008; SIMONATO; GRIGOLLI; OLIVEIRA, 2014)

1.3.1.6.3 *Spodoptera* spp. (Lepidoptera: Noctuidae)

In recent years, there has been a considerable increase in *Spodoptera* spp. frequency in soybean producing areas (BERNARDI *et al.*, 2014; BUENO *et al.*, 2011). This is possibly due to changes in the production system, such as the adoption of *Bt* cultivars, which are designed to control the main soybean caterpillars (*A. gemmatalis* and *C. incluidens*) and consequently decrease the spraying of insecticides for these targets, which indirectly favours the *Spodoptera* spp. outbreaks since this pest is not a target of the Cry1Ac (BERNARDI *et al.*, 2014; CONTE *et al.*, 2020; SORGATTO; BERNARDI; OMOTO, 2015). Among *Spodoptera* spp. found in Brazil there are: *Spodoptera eridania* (Cramer, 1782), *Spodoptera albula* (Walker, 1857), *Spodoptera cosmioides* (Walker, 1858) and *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) (BUENO *et al.*, 2011; LUZ *et al.*, 2019) (FIGURE 5 A-D).

FIGURE 5 – THE *Spodoptera* COMPLEX: THE SOUTHERN ARMYWORM *Spodoptera eridania* (A); *Spodoptera albula* (B); *Spodoptera cosmioides* (C); and *Spodoptera frugiperda* (D) (LEPIDOPTERA: NOCTUIDAE)



SOURCE A-C: Embrapa Soja (2010); D: Revista Cultivar (2021).

Spodoptera spp. eggs are approximately 0.45 mm in diameter and 0.35 mm in height and are laid in masses, with variable coloration depending on the species. Caterpillars will take from 4 to 6 days to emerge, and spend their larval development period in the lower third of the plant, where it lasts from 15 to 23 days, going through 6 or 7 different instars (HARDKE; LORENZ; LEONARD, 2015; MACHADO *et al.*, 2020; PEREIRA-BARROS *et al.*, 2005; SILVA *et al.*, 2017). At the end of the larval period, the caterpillars usually go to the ground to pupate, a period that lasts from 9 to 11 days until adult emergence. Adults will be, in general, nocturnal during the period in which they look for a mate for copulation (PANIZZI; BUENO; SILVA, 2012).

The southern armyworm, *S. eridania*, is the most abundant species found in soybean within the genus in the Brazilian Cerrado region (LUZ *et al.*, 2019). The caterpillars are dark brown, with a longitudinal stripe on the lateral home and interrupted by a spot on the thorax. Adults are grey-brown, measuring about 33-38 cm long, and easily misidentified as adults of *S. albula* (TEIXEIRA *et al.*, 2001).

Spodoptera albula caterpillars in the early instars tend to scrape the leaves, while older ones are able to consume them integrally (TEIXEIRA *et al.*, 2001). This species is considered polyphagous, although it shows a feeding and oviposition preference for soybeans and cotton, which are suitable hosts for the development of this species (SILVA *et al.*, 2017)

Spodopotera cosmioides stands out for its high leaf consumption compared to other *Spodoptera* species, consuming from 175.1 to 185.4 cm² of soybean leaf during its larval cycle (BUENO *et al.,* 2011; SILVA *et al.*, 2017). When its infestation occurs at the reproductive stage, the caterpillars can feed directly on the pods, causing even greater injury (PANIZZI; BUENO; SILVA, 2012). It is possible that, due to its intraspecific similarity and also its sexual dimorphism, this species was often misidentified with *Spodoptera latifascia* Walker, 1856 (Noctuidae, Lepidoptera) in the past (PANIZZI; BUENO; SILVA, 2012)

The fall armyworm, *S. frugiperda,* is the most common species from the genus found in national territory, especially in the central-western region of Brazil, making up about 20% of the caterpillars population (BUENO *et al.*, 2011; STECCA, 2011). Although it is considered a polyphagous species (being found on more than 80 plant species), it shows a greater development and feeding preference for grasses, such as corn, wheat and oats (HARDKE; LORENZ; LEONARD, 2015).

It is worth noting that the *Spodoptera* spp. group does not represent a threat to national soybean production yet (CONTE *et al.*, 2020). However, with the increasing number of *Spodoptera* spp. outbreaks, in addition to the low efficiency of Cry1Ac toxin for the management of these lepidopterans, in addition to the low number of chemicals registered for species such as *S. cosmioides* (Agrofit, 2022), it is important to develop sustainable management strategies for the pest.

Some tools may be useful to *Spodoptera* spp. management: genetic breeding for resistance by antixenosis, attributes sought by physical characteristics such as high density of trichomes, or repellent coloration (QUEIROZ *et al.*, 2020), by antibiosis seeking for a morphological or chemical changes, such as phytoalexins (BOIÇA *et al.*, 2015), in addition to the use of bioinsecticides such as formulations of *Bacillus thuringiensis, Metarhizium rileyi* and viruses of the Nucleopolyhedrovirus (NPV) group (LOUREIRO *et al.*, 2020; SOSA-GÓMEZ, 2017).

1.3.1.6.4 Heliothinae group (Lepidoptera: Noctuidae)

The Heliothinae group (Lepidoptera: Noctuidae: Heliothinae) consists of three species of the subfamily found in soybean: *Helicoverpa armigera* (Hübner, 1808), *Helicoverpa* (=*Heliothis*) zea (Boddie, 1850) and *Cloridea virescens* (Fabricius, 1777) (Lepidoptera: Noctuidae) (FIGURE 6). Because they are phylogenetically related species, interspecific mating is possible, and sometimes fertile offspring (hybrids) are generated (YANG; WANG, 2021).

FIGURE 6. The Heliothinae group (Lepidoptera: Noctuidae: Heliothinae); A: *Helicoverpa armigera* (Hübner, 1808), B: *Helicoverpa* (=*Heliothis*) *zea* (Boddie, 1850) and C: *Heliothis virescens* (Fabricius, 1777)

SOURCE A: Embrapa Soja (2010); B: Clemson University (1999), and C: University of Georgia (2004)

This group has a wide host range and can cause economic damage to crops such as cotton, corn, sunflowers and tomatoes (CUNNINGHAM; ZALUCKI, 2014). The caterpillars can attack soybean from the vegetative stage, feeding on leaves, to the reproductive stage, feeding on flowers and pods (COELHO *et al.*, 2020; PANIZZI; BUENO; SILVA, 2012); as a result, the damage capacity becomes even higher, compared to defoliating species (such as *C. includens* or *A. gemmatalis*).

Within this group, *H. armigera* stands out as the most widely distributed species, totaling 68 host plant families worldwide (CUNNINGHAM; ZALUCKI, 2014). In Brazil, its first occurrence was recorded in 2013 (CZEPAK; ALBERNAZ, 2013), so there has been much concern due to its high capacity for destruction, for its consumption of pods, grains in formation, and the plant's aerial tip, resulting in a loss of soybean tolerance ability and, therefore, having a great capacity to cause economic damage.

The larval development of *H. armigera* lasts about 2 to 3 weeks, passing through 5 to 6 instars. In its last instar, the caterpillar is 30 to 40 cm long. Its body is variable in colour from dark green to light yellow, reddish-brown or black, with lateral lines and brittle along its body (CZEPAK; ALBERNAZ, 2013).

Helicoverpa armigera caterpillars feed mostly on pods in the middle third of soybean plants, and the feeding occurs at full bloom (R2), it can lead to a reduction of 7.72 g of grain per caterpillar, which can be even greater when the attack occurs at the beginning of grain filling (R5.1), with a reduction of 10.61 g per caterpillar (STACKE *et al.*, 2018). Despite the innate plant's tolerance to damage, a small *H. armigera* population density is already impacting the productivity.

The corn earworm, *H. zea*, is considered one of the most important species in the USA because of its damages and its control costs (MUSSER *et al.*, 2020). When the caterpillar feeding occurs on soybean reproductive stage, it can delay the plant maturation due to the injury and/or abscission of reproductive organs, and also can reduce the number of grains per pod; it can damage the grains and, consequently, negatively impacts productivity (SWENSON; PRISCHMANN-VOLDSETH; MUSSER, 2013). However, when the feeding is restricted to the soybean flowers until the R3 stage, it is not possible to observe a reduction in productivity, even under high levels of caterpillar infestation (REISIG *et al.*, 2017).

Helicoverpa zea completes its larval development from 12 to 19 days, passing through 6 instars. Its caterpillars are greenish, reaching 35 mm in length in the last instar. The pupal stage lasts about 16 days, which usually develops below ground in a tunnel up to 10 cm deep. After this period, a moth emerges, varying in colour from light brown to tan brown and measuring 35 to 45 mm in length. These adults have nocturnal habits, when they feed, search for mating, mate and oviposit (SWENSON; PRISCHMANN-VOLDSETH; MUSSER, 2013).

The tobacco budworm, *H. virescens* attacks more than 30 crops of agricultural interest, and, although soybean is not its preferred host, there is a growing concern for soybean growers due to some sporadic cases where the population reaches high levels and it requires control, especially during its vegetative stage (PANIZZI; BUENO; SILVA, 2012), due to the influx of dense populations from other crops (CUNNINGHAM; ZALUCKI, 2014; PANIZZI; BUENO; SILVA, 2012). The caterpillars vary in colour between yellow and yellowish green, with a brown head and body with small brittles, like *H. zea*. Pupae occur on the ground, lasting 11 to 22 days, and a brownish moth emerges, with an average longevity of 15 to 25 days (PANIZZI; BUENO; SILVA, 2012).

Among the management strategies for the *Heliothinae* group is the use of *Bt* plants because these insects are highly susceptible to the Cry1Ac protein (BERNARDI *et al.*, 2014; BORTOLOTTO *et al.*, 2014; DOURADO *et al.*, 2016). These plants express a high concentration of Cry1Ac protein, which contributes to maintaining the technology's longevity, reducing the selection of resistant heterozygotes since high-dose tends to eliminate these organisms as well.

As seen before, these species have a wide host range (they are considered polyphagous species) and their populations tend to grow in one crop and then migrate to another (CUNNINGHAM; ZALUCKI, 2014). As a management strategy, it is possible to choose which crops will be sown after soybean and their sowing date in order to desynchronize with the cycle of these pests.

In addition, there is an important natural suppression by natural enemies in the field. Natural parasitism on *H. armigera* caterpillars is often observed with parasitoids of the Tachinidae family (Diptera: Tachinidae), representing about 75% of the natural enemies found emerging from these caterpillars (WEBER *et al.*, 2021). There are also several predators of *H. zea*, the most commonly found being bed bugs of Nabidae family (Hemiptera: Nabidae), ladybugs (Coleptera: Coccinelidae), and species such as *Geocoris punctipes* (Say, 1832) (Hemiptera: Geocoridae) and *Lygus lineolaris* (Palisot de Beauvois, 1818) (Hemiptera: Miridae) (PFANNENSTIEL; YEARGAN, 2002).

In addition to parasitoids and predators, microorganisms also play a key role in Heliothinae population regulation since caterpillars of these species are susceptible to microbial infections and their populations can potentially be controlled by entomopathogens (SOSA-GÓMEZ, 2017). Besides natural epizootics, there are commercial products such as the baculovirus designed to control *H. zea* (HzSNPV) and the fungus *Metarhizium rileyi* whose effective reduction (up to 77%) of its population in the field (SOSA-GÓMEZ, 2017) can be observed. Another fact worth mentioning is the "cross-infection" effect, when an entomopathogen targeting a specific pest can infect another, as in the case of infection of *H. zea* and *H. virescens* by AgMNPV, often targeting *A. gemmatali*s (SOSA-GÓMEZ, 2017).

1.3.1.7 The Soybean bud borer *Crocidosema aporema* (Walsingham, 1914) (Lepidoptera: Tortricidae)

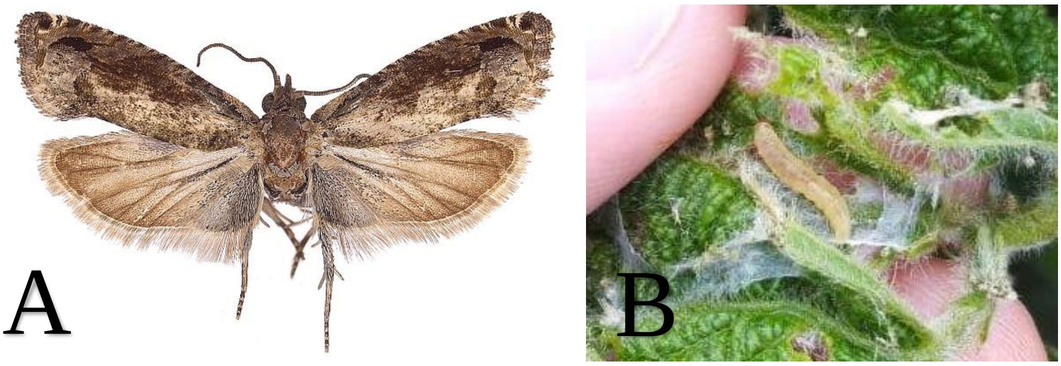
The soybean bud borer *C. aporema* is originated from Costa Rica and is currently widely distributed throughout the Americas, occurring from USA to Argentina (ALTESOR *et al.*, 2010; BIEZANKO, 1961; CORRÊA-FERREIRA, 2012; PEREYRA; SANCHEZ, 1998). In

Brazil, it was first recorded by Biezanko (1961) in the state of Rio Grande do Sul. In addition to the Americas, this species is also reported as a harmful organism in South Korea (PEXD, 2019).

Considered an oligophagous insect, *C. aporema* feeds mainly on leguminous plants, being reported on soybeans (*Glycine max*), pea (*Pisum sativum*), lotononis (*Lotononis bainesii*), clover (*Trifolin polymorphum*), faba bean (*Vicia faba*), peanut (*Arachis hypogaea*), common bean (*Phaseolus vulgaris*) and alfalfa (*Medicago sativa*) (ALZUGARAY, 2003; IEDE; FOERSTER, 1982). Despite soybean is not considered an optimal host, *C. aporema* can pass two generations in each soybean crop cycle (CAPINERA *et al.*, 2008).

The adults of *C. aporema* are small moths, approximately 10-14 mm long (ALZUGARAY, 2003) (FIGURE 7A). The females can oviposit an average of 181 eggs, with an emergence rate of 78.3% (IEDE; FOERSTER, 1982). The eggs are individually laid on both sides of new leaves, and, after egg hatching, the newly hatched larvae feeds on the new buds and new leaflets, forming leaf-rolls around terminal and lateral buds (HOFFMANN-CAMPO *et al.*, 2012; IEDE; FOERSTER, 1982; LILJESTHRÖM; ROJAS; PEREYRA, 2001), which makes the chemical control difficult (IBARRA; ARAYA; ARRETZ V, 1992) (FIGURE 7B).

FIGURE 7 THE SOYBEAN BUD BORER, *Crocridosema* aporema (Walsingham, 1914) (Lepidoptera: Tortricidae). A: ADULT (MALE); B: LARVAE IN SOYBEAN LEAVE.



SOURCE A: Joaquin Baixeras Almela; B: Gustavo Corazza (2021)

The larvae are bright green throughout the first three instars, and their thin cuticle allows to glimpse their midgut (IEDE; FOERSTER, 1982). As the larvae grow, they can also injure the secondary soybean buds by moving to the axils and steaming. Its feeding causes a tunnel that can reach 5 cm in length that obstructs sap movement and compromises the development of the plant (ORTIZ, 1998). Also, the injured plant exhibits stunted growth, with an increase in secondary branches, premature flowering and pod drop (BENTANCOURT; SCATONI, 2006; ORTIZ, 1998).

Depending on temperature and host, the life cycle takes 35-40 days (ALZUGARAY, 2003; HOFFMANN-CAMPO *et al.*, 2012). In its development, the bud borer goes through 5 instars and then pupates in the ground, between 1 and 2 cm deep, close to the base of the plant. The pupae are pinkish-brown in colour, and this stage lasts 8-11 days (IEDE; FOERSTER, 1982).

Its damage to soybeans is usually low. According to Foerster *et al.* (1983), high levels of infestation during vegetative and post-pod-set do not result in yield loss, and even 40-50% infestation during flowering can be tolerated without significant yield loss. The incidence and the injury potential of *C. aporema* in soybean fields is dependent on the genotype and sowing time. Long-cycle genotypes tend to present more injury caused by the soybean bud borer, and this difference is usually observed after soybean V_8 stage (SIQUEIRA; SIQUEIRA, 2012).

Crocidosema aporema is currently considered a secondary pest in soybean in Brazil, with occurrence in southern states with lower temperatures (HOFFMANN-CAMPO *et al.*, 2012). However, there is growing concern about this species after it was discovered in the 2020/2021 crop season that *C. aporema* caused unexpected injuries in MON 87701MON 89788 soybean fields, implying a case of field-evolved resistance to Cry1Ac due to a decrease in pest susceptibility to this protein (HORIKOSHI *et al.*, 2021).

Although C. aporema has a low damage potential, chemical insecticides are typically used to control it early in the growing season, affecting natural enemy populations that would otherwise control it later (ALTESOR *et al.*, 2010). The soybean plant can tolerate and sometimes even recover from the injury caused by *C. aporema* in different plant stages. The correct moment to apply insecticides is indicated by the economic thresholds (ETs). The ET for *C. aporema* in soybean has been studied since the 1980s, and currently it is recommended to start the insecticide spraying when a level of 25 to 30% of attacked plants is reached (HOFFMANN-CAMPO *et al.*, 2012; PANIZZI, 2013).

1.4 SOYBEAN INTEGRATED PEST MANAGEMENT PROGRAM

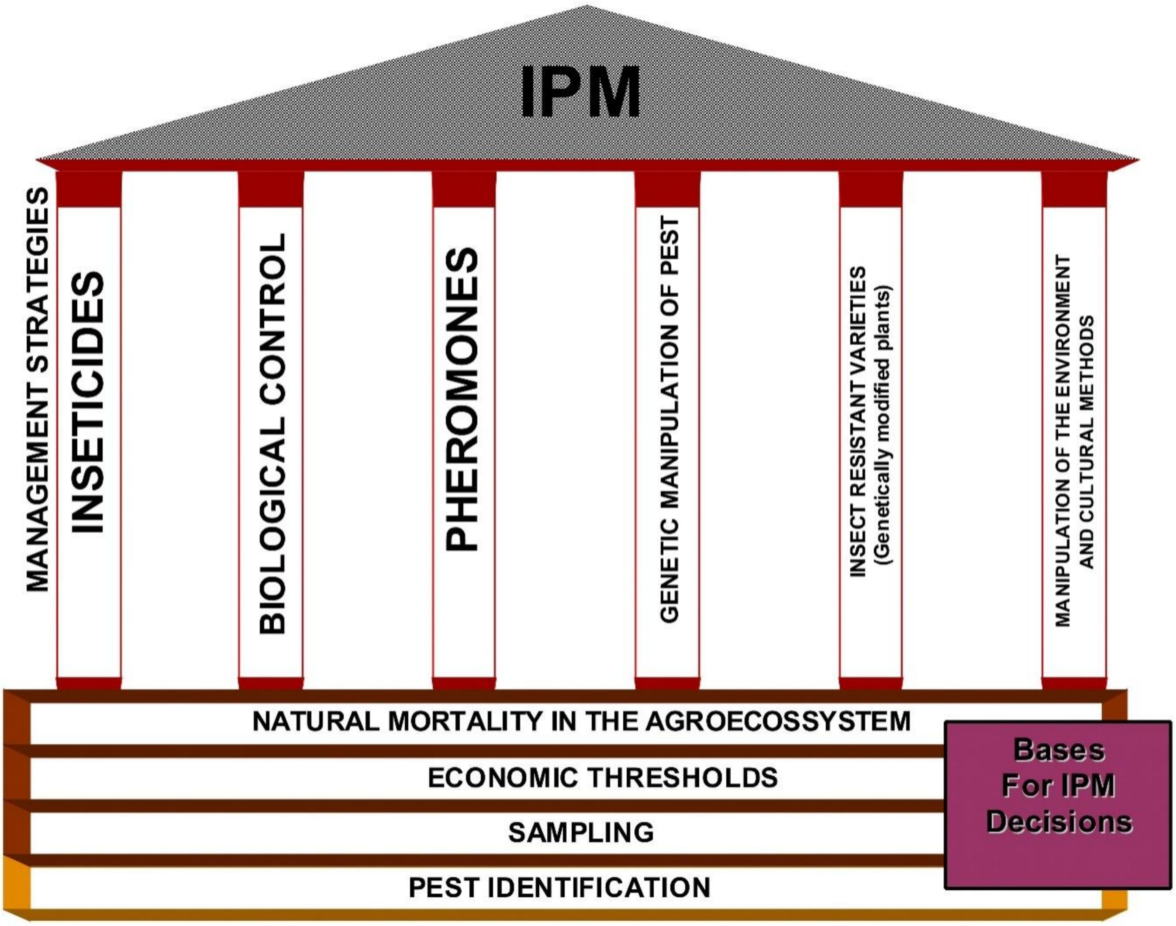
The Integrated Pest Management of soybean (Soybean-IPM) is a grouping of different technologies used on the management of this crop with the goal of preserving the agroecosystem sustainability by keeping it as close to biological equilibrium as possible. This concept was developed at the end of the 1950s and seeks primarily to align the control method with ecological, economic and social principles.

In Brazil, the Soybean-IPM program started in the 1970s and was rapidly disseminated through extension agents and technical publications that allowed its dissemination and adoption by rural growers (BUENO *et al.*, 2021; PANIZZI, 2013; PANIZZI *et al.*, 1977). These publications showed the main soybean pests, with images of each species and its injuries, the natural enemies and entomopathogens present in the fields, and the correct moment to manage these pests (PANIZZI *et al.*, 1977).

However, some people have misunderstood the Soybean-IPM idea throughout time, oversimplifying its complexity. It is sometimes stated that the IPM should only consider using pesticides sparingly or in a sensible way. It is true that implementing the Soybean-IPM results in a more prudent use of pesticides, however this reduction should not be mistaken for the Soybean-IPM itself. This misconception of the IPM intricacy affects a number of other commercially significant crops in addition to the soybean crop.

Due to this oversimplified interpretation of the IPM, various names have also been developed, such as "Ecological Management of Pests", which in essence seeks to reintroduce the underlying complexity of this topic that, regrettably, was lost over time within the IPM concept. It is undoubtedly difficult to sum up the intricacy of the IPM in a concise manner; as a result, its graphical depiction, as illustrated in FIGURE 8, may more clearly demonstrate the complexity that the idea actually possesses.

FIGURE 8 INTEGRATED PEST MANAGEMENT SCHEME.



SOURCE: Adapted from GALLO *et al.*, 2000

The IPM requires a solid foundation to sustain itself, as seen in FIGURE 8, which resembles a "house". Furthermore, to sustain standing with such a degree of complexity, it also needs walls (management strategies). When key structural procedures are skipped or completed insufficiently, it is challenging to succeed with the soybean-IPM. As an illustrative example, the sampling procedures must be carried out meticulously because they will indicate the number of insects present in a specific area of the crop field and, as a result, will provide adequate parameters for the adoption of the economic thresholds (ETs) for the control of the target-pests.

IPM proposes that, prior to any decision, periodic sampling should be carried out in crops, since the correct moment of the management control of any pest is conditioned to its population density or its injury. The recommended method of sampling in soybeans is the shake-cloth (FIGURE 8). This method uses a cloth made of cotton or some synthetic material, with a rigid base 1 meter wide and 1.5 meters long (CORRÊA-FERREIRA, 2012); although its length can be variable, its width must necessarily be 1 meter, because this measurement is associated to the economic threshold (ET) that support decision making.

Currently, it is recommended that the shake-cloth be placed between two rows of soybeans, with the rigid section towards the base of one row and the surface of the cloth covering the other (Figure 9A). Quickly, one row should be inclined and shaken vigorously, resulting in all the insects present on the plants fall directly onto the cloth (Figure 9B) (CORRÊA-FERREIRA, 2012; STÜRMER *et al.*, 2014). After that, the correct identification, counting and recording of the insects found must be done (CORRÊA-FERREIRA, 2012) (Figure 9C).

FIGURE 9. APPROPRIATED SAMPLING PROCEDURE WITH THE AID OF A SAMPLE-CLOTH.

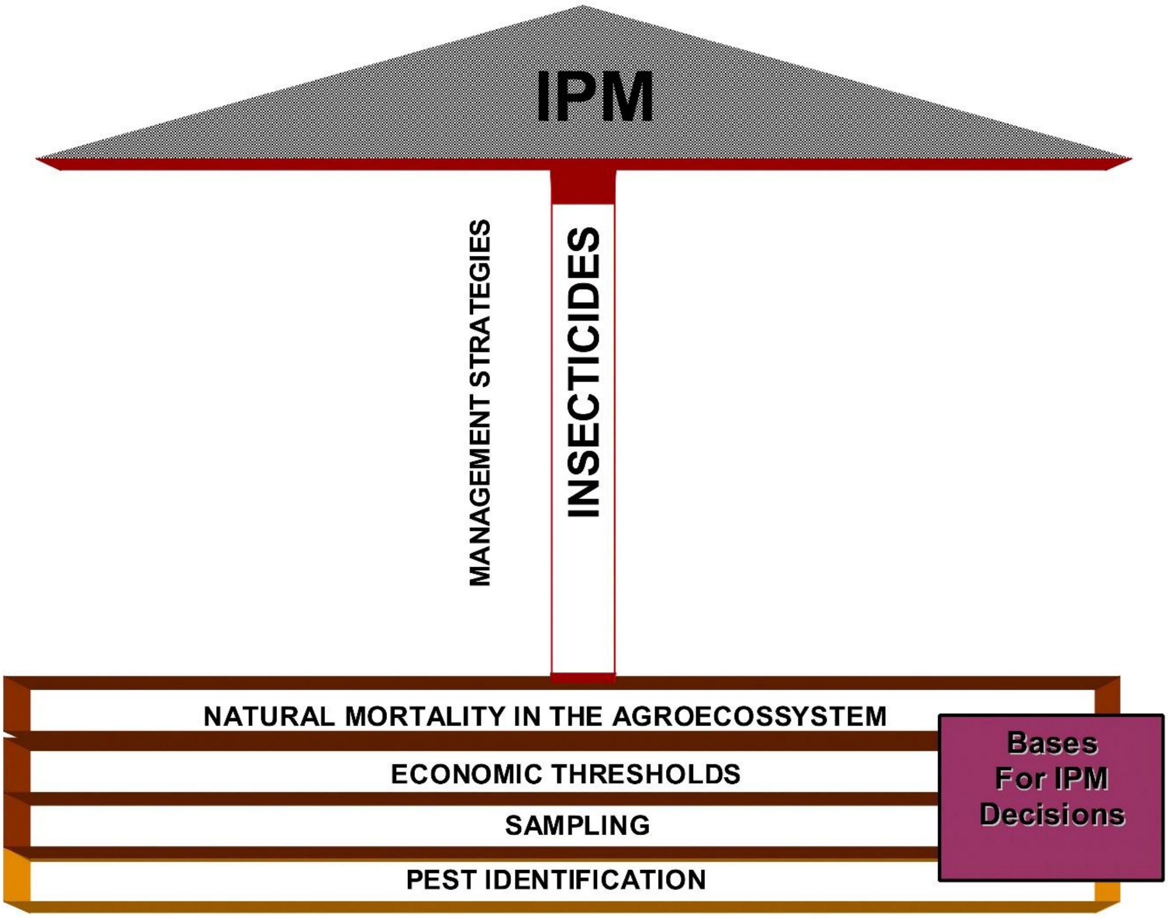
SOURCE: Embrapa (2010)

Sampling should be performed in the milder periods of the day, such as early morning or late afternoon, to avoid excessive movement of insects, especially stink bugs adults, thus preventing their evasion. In addition, it is important to register the date of sampling, also the phenological stage in which the plants are (FEHR *et al.*, 1971), in order to track the development of pest populations in each field.

Unfortunately, recently a number of soybean growers have stopped using sample-cloths. Without a precise sampling method, the grower would undoubtedly use pesticides incorrectly (without a technical support), frequently disregarding the ETs advised by the research. In that situation, poor judgments and superfluous insecticide treatments, which may be utilized as a preventative measure, may be made, increasing the harmful effects of pesticides on the agroecosystem even more.

In this context, it is important to note that chemical spraying is frequently utilized as the single-tool of pest management on soybean crops. The IPM guidelines, which suggest using many management strategies in harmony wherever feasible, do not recommend the adoption of single practice of management. As a result, the Soybean-IPM becomes incredibly fragile without a solid foundation and when it is solely and only attributed to chemical management (FIGURE 10).

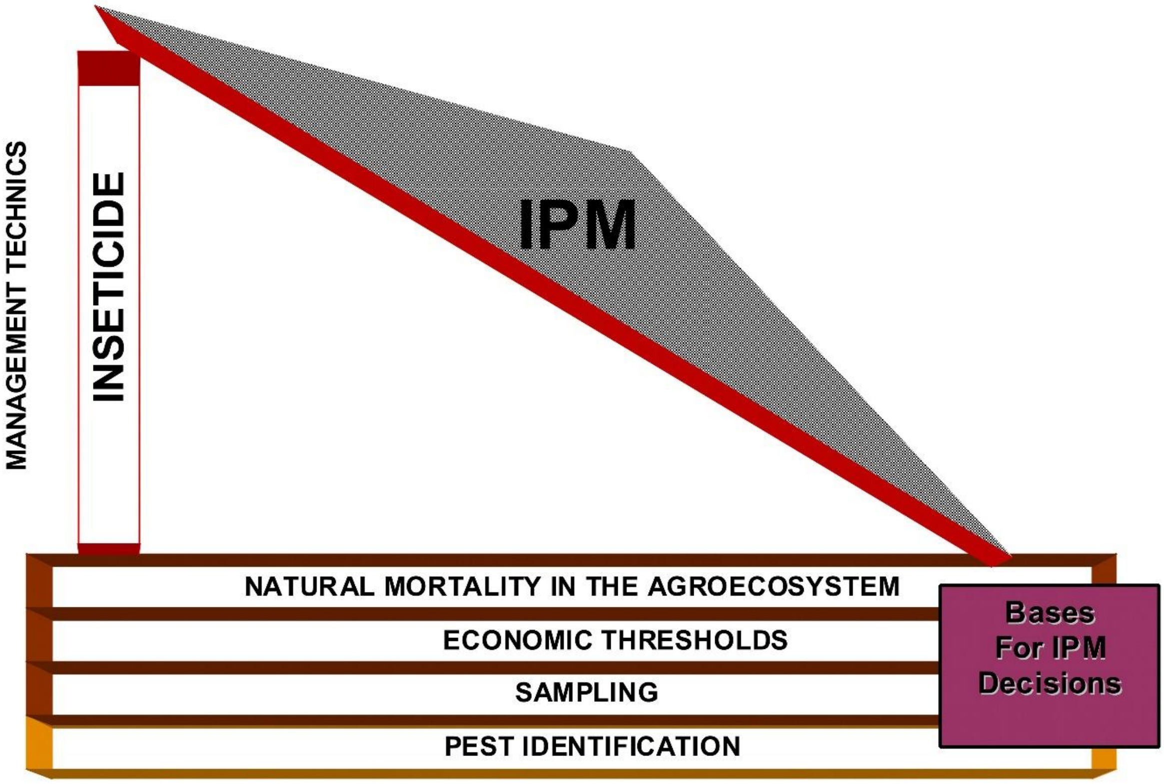
FIGURE 10 ILLUSTRATION OF THE FRAGILITY OF THE INTEGRATED PEST MANAGEMENT WHEN IT IS BASED ON A SINGLE CONTROL METHOD IN ANALOGY TO THE SUSTAINMENT OF A HOUSE ROOF.



SOURCE: Adapted from Gallo *et al.* 2002.

When overuse of chemicals occurs, it could harm all Soybean-IPM technology (FIGURE 11), particularly when non-selective pesticides to beneficial arthropods are sprayed. This happens especially when only pesticides are used to control pest outbreaks, without carrying out the proper sampling and/or not adopting the ETs.

FIGURE 11 ILLUSTRATION OF THE CONSEQUENCES FROM THE WRONG INSECTICIDE USE (NON-SELECTIVE PRODUCT OR THE ABANDON OF ECONOMIC THRESHOLDS = SPRAYING AT THE WRONG TIMING) ENDANGERING SOYBEAN-IPM SUSTAINABILITY.



SOURCE: Adapted from Gallo *et al.* 2002

Therefore, aiming at mitigating the harmful effects that can be caused by pesticides, the adoption of the Soybean-IPM with the use of selective pesticides to beneficial insects (and less harmful to the environment) sprayed only when necessary (at levels equal or above the recommended ET) is of crucial importance, aiming at the economic return with the lowest environmental impact.

It is important that growers replace the concept of pest control with the concept of keeping pest populations below a level that does not cause economic damage (DARA, 2019), because this reflects what is essential in practice: it is often impossible to have absolute control of these organisms, and that the most reasonable option is to try to maintain them at an adequate level, considering all the ecological factors that contribute to this maintenance, including their natural enemies. In addition to predators and parasitoids, epizootics also play a key role in this regulation, since most of these lepidopteran pests are susceptible to microbial infections, and their populations can potentially be controlled by entomopathogens (SOSA-GÓMEZ, 2017).

Despite a large body of scientific work showing the advantages of IPM adoption, its adoption also depends on the instruction of producers and their socioeconomic condition, which reflects environmental concern in addition to economic returns (DARA, 2019). Therefore, the work of scientific research should always be linked to the work of extensionists, so that this knowledge is transferred to the farmers and there is a greater understanding of the importance of adopting ET. The role of chemical insecticides is just one more (and not the only) insect management tool to reduce the negative impacts of this tool on the environment and, at the same time, to ensure a more sustainable and economically viable production.

1.5 ECONOMIC INJURY LEVEL AND ECONOMIC THRESHOLDS

The levels defined for each pest group must be adopted based on the knowledge of the insect population density in each field, then understanding of the concepts of economic injury level (EIL) and economic threshold (ET) is required.

The EIL is defined as the lowest pest population that causes economic damage; to find this level, it is calculated as a trade-off between costs and advantages, i.e., the ratio between costs of the insecticide and its application, per yield market value, multiplied by the damage capacity of each individual pest (or injury unity), multiplied by the control method efficiency (STERN *et al.*, 1959; STONE; PEDIGO, 1972). However, considering that there is a delay between the insecticide spraying and effective insect control, besides the different climatic conditions that can affect its control, the sprays should be made before the EIL is reached, to ensure that this level is not exceeded. Thus, the proposed level for decision making is ET (also known as AT – action threshold), which is defined as the ideal time when the pest population should be controlled so that it does not reach the EIL (PEDIGO; HUTCHINS; HIGLEY, 1986).

In Brazil, the ETs of the main pests of the soybean crop are well established. For lepidopteran caterpillars, the ET recommended for *A. gemmatalis* and *C. includens* is 20 caterpillars.m^{-1}; for the *Spodoptera* group, 10 caterpillars.m^{-1}; and for the *Heliothinae* group, 2 caterpillars.m^{-1}. For decision making based on defoliation, 30% defoliation in the vegetative stage and 15% in the reproductive stage are recommended (MOSCARDI *et al.*, 2012).

The ET for defoliation may slightly vary between nations. For instance, in the US, the defoliation level tolerated, prior to beginning pesticide treatment, is 35% during the crop vegetative stage and 20% during its reproductive stage (ANDREWS *et al.*, 2009). It is important to emphasize that the soybean plant tolerates some defoliation without significant decrease on yield (BATISTELA *et al.*, 2012; HAILE *et al.*, 1998; HAYASHIDA *et al.*, 2021). Earlier results report defoliation levels of until 50% without yield reduction (PICKLE; CAVINESS, 1984).

Many of these studies used to determine the economic threshold currently recommended for controlling the major defoliator pests, however they were carried out in the 1970s or 1980s, although some recently published research papers have shown that these levels are still reliable (BATISTELA *et al.*, 2012; BUENO; BATISTELA; MOSCARDI, 2010; HAYASHIDA *et al.*, 2021; TAGLIAPIETRA *et al.*, 2018). So far, there is no scientific evidence showing that more recent cultivars (early maturity group and indeterminate growth habit and lower leaf area index) are more sensitive to leaf area losses.

It is important to emphasize that the soybean plant has the characteristic of producing a surplus of leaf area. This characteristic, which is shared by other plant species, enables these plants to capture the most solar energy for photosynthesis even after some defoliation (BOARD, 2004; TAGLIAPIETRA *et al.*, 2018). This occurs because a small loss in leaf area can be offset by increased light penetration until the lower leaves, which were previously shaded, increasing the total amount of photosynthetic products produced by the plant and causing it to produce grain yields that are comparable to or even slightly higher than non-defoliated plants (TURNIPSEED, 1972).

It is worth noting that these ETs are indicated for chemical control and may change according to the alternative control method to be used. For example, for control of defoliating caterpillars with commercial AgMNPV-based formulations, the ET of 15 small caterpillars (<1.5 cm) or 5 large caterpillars (≥1.5 cm) is recommended (SOSA-GÓMEZ, 2017), because the time needed for its effective control is longer than chemical control.

In addition to the defoliator insects of the soybean crop, there are the stink bugs, which usually are a complex of distinct species that attack the pods sucking the grain contents. For this complex, ET of 2 stink bugs.m^{-1} is recommended for grain fields and ET of 1 stink bug.m^{-1} for seed production (BUENO *et al.*, 2013). However, there are also concerns about the viability of the ET recommended to start controlling this pest, as well as the interaction between defoliation-induced injury and stink bug feeding.

Similarly to the previously mentioned for defoliators, also for the stink bugs complex the overuse of insecticides brings more harms than benefits, especially given that there is no evidence to suggest that the recommended ET is not safe to ensure the yield related to the sustainability of the crop (BUENO *et al.*, 2015).

Therefore, it is safe to say that controlling preventing stink bug populations is not feasible, primarily because doing so would not substantially increase output quality or yield. It only would increase the number of chemical applications, which would increase production costs and have a negative impact on the environment. On other hand, adopting the ET recommended for stink bugs may reduce environmental risks and lower production cost due to the lower use of chemicals. Therefore, the chemical application at the right moment must always be adopted by growers to reduce the negative impacts caused by agricultural chemicals in the environment since it rationalizes the use of pesticides.

By adopting these ETs, it is possible to spray at the proper time while also preventing additional applications and applying it at the wrong time. An example of the current scenario is illustrated by the work being carried out in Paraná state, which compares areas that adopt IPM (with adoption of weekly monitoring and ET) with areas that do not adopt it. This work is being done for 9 crop seasons, and so far it has been found that it has reduced more than half of insecticide sprayings in assisted areas, in all years (CONTE *et al.*, 2020). This indicates that at least half of the insecticide applications in non-assisted areas is being applied unnecessarily and probably at the incorrect time.

Moreover, this work also evaluated the time between sowing and the first insecticide spraying; authors found that in areas that adopted IPM the first application occurred on average 21 days after the first application of non-assisted areas (CONTE *et al.*, 2020). This gives more time for natural enemies and pollinators to influx and to establish into these areas, since insecticide contamination is one of the major factors responsible for the decline of terrestrial invertebrate species (DOUGLAS; ROHR; TOOKER, 2015; GUNSTONE *et al.*, 2021; REILLY *et al.*, 2020).

Despite several pieces of evidence that prove its effectiveness and economic return, some producers are still reluctant to fully adopt IPM. The review carried out by Bueno *et al.* (2021) indicates two main reasons by growers for the low adoption of IPM: the need for a quick and easy monitoring technique and the fear of productivity loss even after adopting the ET established in research. This concern about adopting ET is because many of them were established in the 1970s from cultivars different from the current ones, which have higher yields. There was also a change of growth habit from determinate to indeterminate, shorter development time, and the modern ones have a smaller size and less leaf area index (ZANON *et al.*, 2015, 2016).

In addition, another concern is the indeterminate growth habit of modern cultivars. The growers assume that flowers, pods and grains would be exposed for a longer time, and the plant would be more susceptible to stink bugs and caterpillar attacks, mainly from the Heliothinae group (REISIG *et al.*, 2017). However, this longer exposure time of reproductive structures is not directly related to the plant susceptibility to pest injuries, and the indeterminate characteristic allows plants to emit new structures to recompose their productive components, which possibly increases the plants tolerance to such injuries (BUENO *et al.*, 2015, 2013; REISIG *et al.*, 2017).

It is of utmost importance that these ETs are continuously updated and refined, since the agricultural scenario is always under improvement. Also, practices and genotypes change, the dynamics of key pests and invasive species change, and the environmental and climatic changes can impact on productivity and crop sensitivity to established ETs (BATISTELA *et al.*, 2012; BUENO *et al.*, 2021; HAYASHIDA *et al.*, 2021; LEATHER; ATANASOVA, 2017; ZANON *et al.*, 2015; ZANON; STRECK; GRASSINI, 2016).

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2 CHAPTER 2. ARE ECONOMIC THRESHOLDS FOR IPM DECISIONS THE SAME FOR LOW LAI SOYBEAN CULTIVARS IN BRAZIL? [1]

Abstract

Economic thresholds (ETs) are well-established for defoliation of soybean, *Glycine max*, and have been updated for many of the newer cultivars; however, there is increasing grower adoption of cultivars with a reduced leaf area index (LAI). It is of theoretical and practical interest to determine low LAI cultivar tolerance to defoliation. We conducted experiments during two consecutive crop seasons (2017/2018 and 2018/2019) using three soybean cultivars (NS 5959 IPRO, NS 5445 IPRO, and DON MARIO 5.8i) and three defoliation levels (0%, 16.7%, and 33.3%) to evaluate the tolerance of reduced LAI soybean cultivars under different defoliation levels. We observed differences among cultivar's LAI during plant development during both years. Soybean LAI was reduced with increasing defoliation intensity. Tested continuous defoliation levels from plant development stages of V2 to R6 reduced the weight of 1000 seeds and yield but did not impact oil or protein content. Despite our findings that current ET for defoliators in soybean (30% defoliation during vegetative stage and 15% defoliation during reproductive stage) are valid, it is important to consider that continuous defoliation injury impacts the capacity of the plant to respond to injury and must be further evaluated for ET refinement in future research.

Key words: action threshold; defoliation; grow tissue removal.

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[1] **Published research article:** HAYASHIDA, R.; GODOY, C. V.; HOBACK, W. W.; FREITAS BUENO, A. Are economic thresholds for IPM decisions the same for low LAI soybean cultivars in Brazil? **Pest Management Science**, v. 77, n. 3, p. 1256–1261, 27 mar. 2021. Disponível em: <https://onlinelibrary.wiley.com/doi/10.1002/ps.6138>.

2.1 INTRODUCTION

Soybean [Glycine max (L.) Merrill] is one of the main oilseed crops produced and processed around the world. In the 2018/2019 crop season, approximately 122.57 million ha were sown, with a production of 358.77 Mt, accounting for a 4.78% increase over the previous worldwide crop season.(MAUPIN; NORTON, 2010). However, this production could be even higher if quality and quantity losses caused by pests mitigated. Pests lower soybean production by an estimated 26–30% annually depending upon region. These losses can be reduced with implementation of Integrated Pest Management (IPM) (OERKE; DEHNE, 2004; OERKE, 2006; CULLINEY, 2014).

Many IPM programs are based on the concepts of economic injury level (EIL), defined as the lowest pest density that cause economic damage, and economic threshold (ET), the timing when the control should begin to prevent pest population density or injury from reaching the EIL (STERN *et al.*, 1959). Preventative applications of insecticides, in the absence of sufficient pest numbers, result in inconsistent economic returns, and can cause pest resistance and environmental damage (HENRY; JOHNSON; WISE, 2011; HIGLEY; PEDIGO, 1996).

The ETs can be established for pest density or degree of injury and are influenced by many factors including pest species, cultivars, climate and other different agroecosystem properties (BUENO *et al.*, 2011a). As a result, the ETs for soybean defoliation can differ by country or even inside the same country. In Brazil, chemical control applications are recommended when defoliation percentage reaches 30% in the vegetative or 15% during the reproductive stages (BATISTELA *et al.*, 2012; BUENO *et al.*, 2013; MOSCARDI *et al.*, 2012a). In contrast, in the United States, ETs for defoliators vary among states. For example, in Georgia, ETs for defoliators are the same as those used in Brazil. Differently, in Ohio, treatment is triggered only when defoliation exceeds 40% prior to bloom, 15% from bloom to pod-fill, or 25% after pod-fill to plant yellowing (ANDREWS *et al.*, 2009). In contrast, chemical control is recommended when 35% defoliation is attained during vegetative and 20% during the reproductive stages in Mississippi (CATCHOT *et al.*, 2016). These differences could result from differences in light interception, photosynthetic efficiency or leaf area index (LAI) among cultivars and therefore, can produce different levels of tolerance to injury (BOARD, 2004; KUMUDINI; HUME; CHU, 2001; RICHTER *et al.*, 2014).

Defoliation ET has been recently re-evaluated for newer cultivars belonging to early maturity groups with indeterminate growth habits (BATISTELA *et al.*, 2012). Development and increasing adoption of cultivars with reduced LAI has increased the need for study of how low LAI cultivars respond to defoliation (ZANON *et al.*, 2015). Therefore, this study aimed to evaluate LAI of new soybean cultivars to determine the impacts of defoliation

2.2 MATERIAL AND METHODS

Experiments were carried out under field conditions during two consecutive soybean seasons (2017/2018 and 2018/2019) at Embrapa, in the municipality of Londrina (S 23° 110 11.700;W51° 100 46.100) in the northern state of Paraná (PR), Brazil. The experiment was carried out in a 3 × 3 factorial randomized block design; three cultivars (NS 5959 IPRO, NS 5445 IPRO, and DON MARIO 5.8i) and three defoliation levels (0%, 16.7%, and 33.3%); with four replicates (six 5-m-long soybean rows). Cultivars' features were indeterminate growth habit with maturity group 5.9 (NS 5959 IPRO), 5.4 (NS 5445 IPRO), and 5.5 (DON MARIO 5.8i).

2.2.1 Experimental Defoliation

Trials were planted on October 17, 2017 (2017/2018) and on October 22, 2018 (2018/2019) with 15 seeds per linear meter and 0.50 m row spacing. Artificial defoliation was carried out twice a week by manually removing the number of leaflets corresponding to each treatment with the aid of scissors, following the method of Gazzoni and Moscardi (GAZZONI; MOSCARDI, 1998). This procedure was performed on all leaves of the plant and on all plants in the plot from phenological stage V2 through R5/R6 (Fehr *et al.*, 1971)To prevent interference from natural defoliators, insecticides and fungicides were applied on a 20-day interval on the plots, using a carbon dioxide (CO2) pressurized backpack sprayer (Herbicat®, Catanduva, São Paulo, Brazil) set for a spray volume of 150 L ha^{-1}. Herbicides were applied during the third and sixth weeks after emergence of soybeans. All pesticides were applied equally over the total area of all treatments, including the control area without defoliation.

2.2.2 Assessments

Throughout soybean development stages, samples were collected from one linear meter and measured for foliar area, using a leaf area meter (Model 3000, LI-COR, Lincoln, NE, USA). The LAI is the ratio between leaf area and the corresponding land area and was calculated from collected material. At the R8 development stage, the two 2-m-long central rows of each plot were separately harvested and threshed for evaluation. The weight and moisture content of each sample was recorded (moisture meter G800, Gehaka Agri, São Paulo, SP, Brazil) and were then corrected to obtain the productivity for 13% seed moisture. In addition to yield, the weight of 1000 seeds was measured, and oil and protein content was quantified using an Antaris II FT-NIR infrared spectroscope (Thermo Fisher Scientific, Dublin, Ireland).

2.2.3 Statistical analysis

Results were submitted to exploratory analysis to verify the assumptions of normality of residuals, homogeneity of treatment variance, and additivity of the model to allow for analysis of variance (ANOVA). When data did not meet ANOVA assumptions, transformations were performed:$\sqrt{x+1}$ (1000 seed mass of 2017/2018 season). When significant differences were detected, they were identified using the Tukey test at 5% probability (SAS Institute, 2001). The cultivar growth equation was made using the polynomial quadratic regression for the LAI development data (R2 more than 89%).

2.3 RESULTS

For all evaluated cultivars, the LAI increased over the season until R5.3 and decreased in the last evaluation (R5.5/R6) (TABLE 1). Defoliation impacted the LAI development for all cultivars during both seasons (2017/2018 and 2018/2019). For both seasons, the quadratic equations closely described LAI with R2 between 0.89 and 0.94 (FIGURE 1A and 1B) for control and defoliated treatments.

LAI differences were observed in both study seasons during crop development among the tested cultivars. In the first season (2017/2018), LAIs were similar among cultivars in the first three evaluations (V4/V5 and, R2/R3) but NS 5959 IPRO showed higher LAI in the last evaluation (R5.5/R6). In contrast, during the second season (2018/2019), NS 5959 IPRO had lower LAI and was similar to DON MARIO 5.8i in the first three evaluations (V2, V4/V5 and, R2/R3) and no differences among cultivars were recorded in the last two evaluations (R5.3 and R5.5/R6). NS 5445 IPRO had the highest LAI in the first three evaluations (V2, V4/V5 and, R2/R3). In the last two evaluations (R5.3 and R5.5/R6), NS 5959 IPRO always had the highest LAI, but only differed significantly for the first season (2017/2018).

Regarding the impact of defoliation over soybean LAI values, plots with 33.33% defoliation had significantly lower LAI than the undefoliated controls from V2 to R5.3 and from V4/V5 to R5.5/R6 in 2017/2018 and 2018/2019 crop seasons, respectively. Plots with 16.7% defoliation had intermediate LAI, being similar to 33.3% defoliation at V2, V4/V5, R5.3, and R5.5/R6 (2017/2018) and V2, V4/V5, R2/R3, and R5.3 (2018/2019) and similar to control (0% defoliation) at V4/V5, R2/R3, R5.3, and R5.5/R6 (2017/2018) and V2, R5.3, and R5.5/R6 (2018/2019) (TABLE 1).

Defoliation significantly reduced yield for both 16.7% and 33.3% defoliation levels during both crop seasons. However, there were no significant differences between treatments. Cultivars also influenced yield. During the first season (2017/2018), NS 5959 IPRO was the most productive (5051.5 ± 100.2 kg ha^{-1}), NS 5445 IPRO was the least productive (4816.8 ± 93.0 kg ha^{-1}) and cultivar DON MARIO 5.8i showed intermediate yield (4841.0 ± 42.2 kg ha^{-1}), but were not significantly different. In 2018/2019 season, the cultivar NS 5959 IPRO showed the lowest yield (3104.4 ± 152.6 kg ha^{-1}) and cultivars DON MARIO 5.8i and NS 5445 IPRO had similar higher yields (3561.9 ± 136.9 kg ha^{-1} and 3740.7 ± 133.5 kg ha^{-1}, respectively) (TABLE 2).

Neither defoliation nor cultivar impacted oil content, which was similar among cultivars and among defoliation levels in both seasons. Protein (%) was also similar among plants with different defoliation levels, but higher for DON MARIO 5.8i than the other studied soybean cultivars. NS 5445 IPRO had intermediate protein (%) but only different from NS 5959 IPRO at the second studied season (2018/2019) (TABLE 2).

In contrast to protein and oil content, the weight of 1000 grains was impacted by both cultivar and defoliation levels. Higher defoliation resulted in the lowest weight of 1000 seeds in the first season and both 33.3% and 16.7% defoliation reduced grain weight during the 2018/2019 trial. For seed weight, the cultivar's response varied. During 2017/2018, DON MARIO 5.8i had the low- est weight of 1000 grains while the lowest wither of 1000 grains was recorded for NS 5959 IPRO in the second season (TABLE 2).

2.4 DISCUSSION

Globally, soybean IPM is based on extensive data that shows soybean plants can tolerate some amount of leaf injury without economically relevant yield reductions (BUENO *et al.*, 2013; HIGLEY; PEDIGO, 1996). Despite this tolerance to defoliation, the response of plants to injury can vary among cultivars, developmental stage of plants, and the timing of exposure to defoliation (BOARD, 2004). Newer soybean cultivars have lower LAI and it tempting to assume that the newer cultivars will be more sensitive to defoliation (BUENO *et al.*, 2013).

The results generated in this study show that despite having shorter maturity periods and lower LAIs than other cultivars cropped in Brazil, the response to defoliation was similar to that of cultivars with higher LAI (BUENO *et al.*, 2013; JIN *et al.*, 2010; TAGLIAPIETRA *et al.*, 2018). Overall, the results for both crop seasons indicate that continuous soybean defoliation of 16.7% and 33.3% significantly reduced yield, as a result of reduced LAI, and that these results are most pronounced during the reproductive developmental stage (HEIFFIG *et al.*, 2006; ZANON *et al.*, 2015).

It is important to note that LAI varies during soybean development and higher or lower LAI soybean cultivars may still behave similarly. For example, a cultivar with higher LAI can be more vulnerable to leaf self-shading, which can trigger earlier leaf senescence relative to lower LAI cultivars. As a consequence, when the plant is at the R5 growth stage, when LAI is most important, newer low LAI cultivars may actually have higher LAI compared to older cultivars because of their ability to retain leaves for longer periods (BUENO *et al.*, 2013; KUMUDINI; HUME; CHU, 2001). Therefore, in addition to LAI, light interception should also be taken into consideration (HAILE *et al.*, 1998).

Soybean sensitivity to defoliation usually peaks at the early R5 growth stage and decreases linearly down to less than 10% of the relative yield loss at the late R6 growth stage(BOARD *et al.*, 2010). Thus, defoliation during the plant reproductive stage has been considered the most critical because photoassimilates produced in this period are intended not only for vegetative growth (in indeterminate cultivars) but also for production and development of reproductive structures, including flowers, pods and seeds. Usually, defoliation during the vegetative and early reproductive stages has less impact on yield, because of leaf regrowth and delayed leaf senescence of remaining tissues that compensate for losses. (HIGLEY, 1992; PETERSON; DANIELSON; HIGLEY, 1992; PETERSON; HIGLEY, 1996; PICKLE; CAVINESS, 1984; WEBER, 1955). Importantly, even during the reproductive period, yield sensitivity to defoliation declines as the seed filling period progresses from stages R5 to R7 (BOARD *et al.*, 2010).

Previous published results indicate an excellent recovery capacity of some soybean cultivars (BEGUM; EDEN, 1965). Soybean has been documented to recover after injury of 50%, 67% and even 75% defoliation with no yield loss, showing that soybean plants are usually tolerant to defoliation (BEGUM; EDEN, 1965; HAILE *et al.*, 1998; PICKLE; CAVINESS, 1984). Previous works evaluated defoliator thresholds for IPM decisions in short-season soybeans using artificial defoliation and concluded that recommended ETs were still valid (ANDREWS *et al.*, 2009; BUENO *et al.*, 2011b; BATISTELA *et al.*, 2012). However, they did not measure LAI or consider the timing and duration of injury exposure which are crucial to determine possible yield loss (BUENO *et al.*, 2011b; BATISTELA *et al.*, 2012; GARCIA; EUBANKS, 2018; PETERSON; HIGLEY, 2001). For plant response to defoliation, when defoliation occurs and how many days the plant has to regenerate leaf area are important considerations for predicting yield (CONLEY *et al.*, 2008; CONLEY; PEDERSEN; CHRISTMAS, 2009; WAGGONER; BERGER, 1987).

In contrast to previous published work, which studied defoliation conducted on a single day or over periods during vegetative or reproductive stages or used cultivars with higher LAI, our study evaluated defoliation levels of 16.7% and 33.3% imposed twice per week from V2 up to R6 (DONATELLI *et al.*, 2017; GAZZONI; MOSCARDI, 1998; PELUZIO *et al.*, 2004). This method imposes standardized defoliation over time (BEGUM; EDEN, 1965; GAZZONI; MOSCARDI, 1998; PELUZIO *et al.*, 2004; PETERSON; VARELLA; HIGLEY, 2017). We found that simulating continuous defoliation from the beginning of crop development (V2) to the end of reproduction (R6), resulted in LAI recovery of defoliated plants, but reduced yield by about 14.01%. Both defoliation treatments studied here (16.7% and 33.3%) were higher than recommend ET of 30% defoliation during soybean vegetative stage and 15% defoliation during soybean reproductive stage (BUENO *et al.*, 2013). Certainly, the capacity to tolerate 15% defoliation during the soybean reproductive stage is impacted by the occurrence of continuous defoliation on those plants over longer periods.

There are two methodological points that deserve highlighting. (i) In some previous studies, plant defoliation was performed on a single date and then plant recovery was observed (BEGUM; EDEN, 1965; GAZZONI; MOSCARDI, 1998; PELUZIO *et al.*, 2004), while our study kept the injuries constant without allowing plants to recover from defoliation (PETERSON; VARELLA; HIGLEY, 2017). (ii) In the present study, defoliation was performed homogeneously over the entire plant, although defoliating insects have different feeding preferences regarding the plant parts (MOSCARDI *et al.*, 2012b).

The difference observed in plant tolerance capacity in our work from the results in the literature may be attributed to the continuous defoliation imposed to the plants during a longer period. Defoliation studied here were imposed twice a week from plant V2 to R6 development stages, which was much more intense (twice a week) and for a longer period than previously reported studies. It is important to mention that this period of defoliation is longer than soybean feeding Lepidoptera would take to complete larval stages (ca 19 days) (ANDRADE *et al.*, 2016). Despite the limitations of this study approach, better understanding of soybean tolerance to longer periods of injury is important not only for areas where continuous Lepidoptera pressure occurs with overlapping generations, but also where multiple defoliating pest species occurs in sequence. Other factors may also contribute to our results including: study location, plant population, climatic differences, different soil and plant fertility, sowing dates and especially, differences in the characteristics of the cultivars studied (HAILE, 2000a, 2000b; TAGLIAPIETRA *et al.*, 2018).

In addition to LAI and overall yield, defoliation also reduced weight of 1000 seeds. It is likely that reduction in seed weight contributed to the observed reduction in yield (DALCHIAVON; PASSOS; CARVALHO, 2012) It is noteworthy that the 1000 seed weight of cultivars differed between the two studied years. In the first year, NS 5959 IPRO had the highest yield, but in the second year its yield was lowest, which may indicate a strong influence of environmental/climatic conditions between years. Both yield quantity and yield quality are essential for soybean production in order to maximize the product delivery value (HIRAKURI *et al.*, 2018). Taking this into consideration, it is important to note that tested defoliation levels did not impact oil or protein content despite records in the literature of both reduction and increase in oil and protein content based on LAI (CONLEY *et al.*, 2008; MCALISTER; KROBER, 1958). Moreover, it is noteworthy that protein content obtained in this experiment was higher than the US national average (34.1% crop 2017) and, with the exception of the cultivar NS 5959 IPRO in 2018/2019 season, it was also higher than Brazilian national average (HIRAKURI *et al.*, 2018; MILLER-GARVIN; NAEVE, 2017).

2.5 CONCLUSION

Our results indicate that continuous defoliation injury for long periods impact soybean yield and must be further considered when developing ETs. Not only is it important for environments where Lepidoptera pressure continuously occurs during the crop season but also for fields where multiple leaf tissue feeding pest species occurs in sequence. However, lowering ETs to account for continuous defoliation injury would certainly increase use of pesticides. Higher use of pesticides result in higher production costs and can be more harmful to humans and to the environment. Furthermore, high pesticide use can lead to pest resurgence, cause secondary pest outbreaks and increase pest resistance to the pesticides (MEISSLE *et al.*, 2010; TANG; TANG; CHEKE, 2010). Although not evaluated in this study, these possible side effects must be taken into consideration for long-term scenario evaluation. Therefore, despite our findings that the current ET for defoliators in soybean (30% defoliation during vegetative stage and 15% defoliation during reproductive stage) are valid, the need for refinement for continuous defoliation injury and low LAI cultivars should be further studied. In addition, it is important to consider that previous work using total dry weight, which has been recorded to be higher in some newer soybean cultivars, can be more closely related to soybean yield than LAI (KUMUDINI; HUME; CHU, 2001). Dry weight was not evaluated in our work and should be further investigated in future research.

Acknowledgments

This study was financed in part by National Council for Scientific and Technological Development (CNPq grant 142340/2018-9) and by CAPES Foundation (88887.374211/2019-00). The authors also thank the department of Entomology and Plant Pathology of Oklahoma State University.

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TABLE 1. Leaf area index (LAI) of three soybean cultivars under different levels of defoliation measured throughout different developmental stages. Londrina, Paraná, Brazil (S 23° 11' 11.7"; WO 51° 10' 46.1"). Crop seasons 2017/2018 and 2018/2019.

Parameter			Soybean developmental stage (Fehr *et al.*, 1971)				
			V2	V4/V5	R2/R3	R5.3	R5.5/R6
2017/2018	Cult[1]	NS 5959 IPRO	0.17 ± 0.01 b	0.47 ± 0.02^{ns}	1.70 ± 0.12^{ns}	4.58 ± 0.22 a	3.70 ± 0.46 a
		NS 5445 IPRO	0.22 ± 0.01 a	0.58 ± 0.04	1.96 ± 0.23	3.82 ± 0.16 b	3.05 ± 0.47 b
		DON MARIO 5.8i	0.21 ± 0.02 ab	0.54 ± 0.06	1.84 ± 0.14	4.00 ± 0.21 ab	2.96 ± 0.53 b
	Def (%)[2]	0	0.23 ± 0.01 a	0.59 ± 0.04 a	2.19 ± 0.19 a	4.51 ± 0.24 a	3.45 ± 0.66^{ns}
		16.66	0.19 ± 0.01 b	0.52 ± 0.04 ab	1.88 ± 0.15 a	4.08 ± 0.15 ab	3.26 ± 0.60
		33.33	0.18 ± 0.01 b	0.47 ± 0.04 b	1.44 ± 0.10 b	3.79 ± 0.22 b	3.00 ± 0.41
	Statistics	$p_{cultivar}$	0.03	0.07	0.32	0.01	<0.01
		$p_{defoliation}$	0.01	0.04	<0.01	0.02	0.05
		$p_{cultivar*defoliation}$	0.25	0.15	0.34	0.66	0.72
		$F_{cultivar}$	4.29	3.01	1.18	5.75	10.95
		$F_{defoliation}$	5.92	3.56	9.87	4.82	3.30
		$F_{cultivar*defoliation}$	1.44	1.88	1.20	0.60	0.52
2018/2019	Cult[1]	NS 5959 IPRO	0.22 ± 0.01 b	0.84 ± 0.07 b	2.31 ± 0.20 b	2.84 ± 0.22^{ns}	2.90 ± 0.23^{ns}
		NS 5445 IPRO	0.30 ± 0.02 a	1.04 ± 0.09 a	2.70 ± 0.19 a	2.64 ± 0.19	2.48 ± 0.13
		DON MARIO 5.8i	0.20 ± 0.01 b	0.77 ± 0.06 b	2.21 ± 0.21 b	2.71 ± 0.22	2.65 ± 0.19
	Def (%)[2]	0	0.26 ± 0.02^{ns}	1.09 ± 0.09 a	2.93 ± 0.21 a	3.17 ± 0.21 a	3.04 ± 0.16 a
		16.66	0.24 ± 0.02	0.84 ± 0.06 b	2.22 ± 0.15 b	2.71 ± 0.20 ab	2.77 ± 0.18 a
		33.33	0.23 ± 0.02	0.72 ± 0.04 b	2.08 ± 0.16 b	2.31 ± 0.13 b	2.22 ± 0.15 b
	Statistics	$p_{cultivar}$	<0.01	<0.01	<0.01	0.72	0.05
		$p_{defoliation}$	0.28	<0.01	<0.01	0.01	<0.01
		$p_{cultivar*defoliation}$	0.81	0.26	0.17	0.43	0.14
		$F_{cultivar}$	14.14	6.71	6.82	0.34	3.39
		$F_{defoliation}$	1.34	12.35	21.49	6.04	13.14
		$F_{cultivar*defoliation}$	0.40	1.42	1.77	0.99	1.93

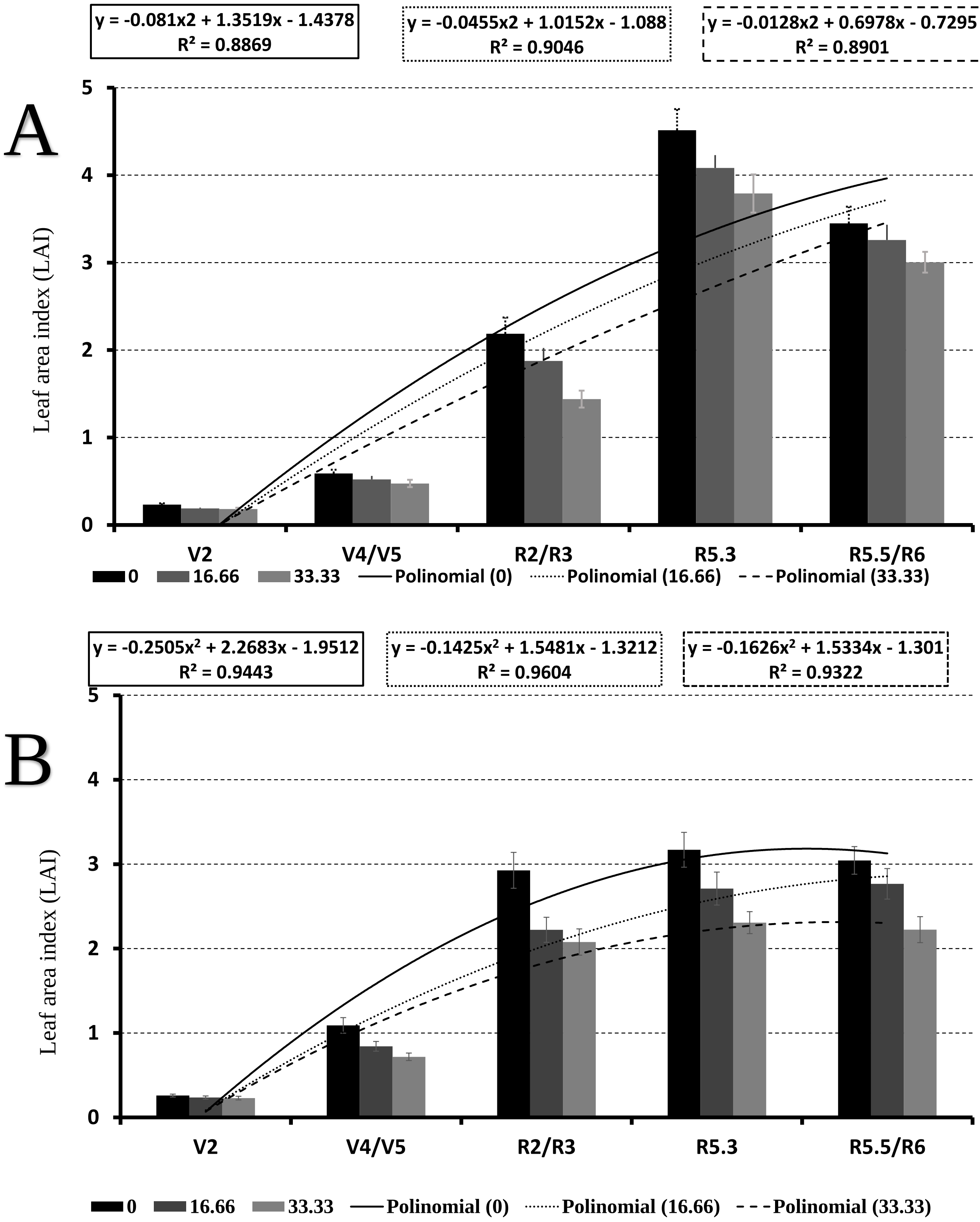
Means (± SE) followed by the same letter in the column for each parameter and crop season do not differ statistically from each other by the Tukey test (p>0.05). [1]Cultivars. [2]Defoliation (%).^{ns}Anova non-significant.

TABLE 2. Yield (kg.ha^{-1}), oil and protein content (%) and weight of 1000 grains of three soybean cultivars under different levels of defoliation. Londrina, Paraná, Brazil (S 23° 11' 11.7"; WO 51° 10' 46.1"). Crop season 2017/2018 and 2018/2019.

Parameter			Yield (kg.ha^{-1})	Oil (%)	Protein (%)	Weight of 1000 grains
2017/2018	Cult^{1}	NS 5959 IPRO	5051.5 ± 100.2 a	22.7 ± 0.2^{ns}	37.0 ± 0.2 b	185.4 ± 2.5 a
		NS 5445 IPRO	4816.8 ± 93.0 b	22.3 ± 0.2	38.3 ± 0.3 b	190.4 ± 2.9 a
		DON MARIO 5.8i	4841.0 ± 42.2 ab	22.4 ± 0.3	39.1 ± 0.3 a	176.1 ± 1.7 b
	Def (%)^{2}	0	5131.3 ± 89.6 a	22.7 ± 0.3^{ns}	38.1 ± 0.4^{ns}	187.1 ± 3.0 a
		16.66	4804.1 ± 66.2 b	22.3 ± 0.3	38.2 ± 0.4	186.3 ± 2.3 a
		33.33	4774.0 ± 63.1 b	22.3 ± 0.2	38.1 ± 0.3	178.5 ± 2.8 b
	Statistics	p_{cultivar}	0.02	0.43	<0.01	<0.01
		$p_{\text{defoliation}}$	<0.01	0.35	0.86	0.02
		$p_{\text{cultivar*defoliation}}$	0.08	0.44	0.27	0.35
		F_{cultivar}	4.34	0.88	18.50	11.37
		$F_{\text{defoliation}}$	10.23	1.09	0.16	4.89
		$F_{\text{cultivar*defoliation}}$	2.36	0.98	1.39	1.18
2018/2019	Cult^{1}	NS 5959 IPRO	3104.4 ± 152.6 b	21.8 ± 0.2^{ns}	36.6 ± 0.2 c	151.4 ± 4.7 b^{3}
		NS 5445 IPRO	3740.7 ± 133.5 a	22.2 ± 0.2	37.9 ± 0.4 b	180.0 ± 2.2 a
		DON MARIO 5.8i	3561.9 ± 136.9 a	21.5 ± 0.3	39.4 ± 0.3 a	163.9 ± 3.3 a
	Def (%)^{2}	0	3929.3 ± 108.3 a	21.9 ± 0.2^{ns}	38.2 ± 0.5^{ns}	174.3 ± 3.6 a
		16.66	3376.7 ± 154.3 b	21.8 ± 0.3	38.0 ± 0.5	160.8 ± 5.7 b
		33.33	3101.0 ± 107.5 b	21.8 ± 0.2	37.6 ± 0.4	160.3 ± 4.4 b
	Statistics	p_{cultivar}	0.00039	0.14	<0.01	<0.01
		$p_{\text{defoliation}}$	<0.01	0.93	0.32	<0.01
		$p_{\text{cultivar*defoliation}}$	0.561	0.55	0.52	0.10
		F_{cultivar}	11.091	2.12	25.83	11.06
		$F_{\text{defoliation}}$	18.325	0.08	1.18	17.66
		$F_{\text{cultivar*defoliation}}$	0.761	0.78	0.83	0.83

Means (± SE) followed by the same letter in the column for each parameter and crop season do not differ statistically from each other by the Tukey test (p>0.05). ^{1}Cultivars. ^{2}Defoliation (%).^{3}Original means followed by statistics performed on the data transformed into $\sqrt{x+1}$. ^{ns}Anova non-significant.

FIGURE 1. Observed leaf area index (LAI), and polynomial regression of three soybean cultivars under different levels of defoliation measured different developmental stages. Londrina, Paraná, Brazil (S 23° 110 11.700;W 51° 100 46.100). (A) Crop season 2017/2018. (B) Crop season 2018/2019



3 CHAPTER 3. A TEST OF ECONOMIC THRESHOLDS FOR SOYBEANS EXPOSED TO STINK BUGS AND DEFOLIATION

Abstract

The economic thresholds (ET) for gross tissue removal and piercing-sucking damage from stink bugs are well-established for soybean (*Glycine max*). However, little is known about the effect of interaction of these injuries on the ET. During the 2017/2018 and 2018/2019 crop seasons, field trials were carried out to assess the defoliation and stinkbug ET interaction and its impact on soybean yield and its components of oil and protein content and quality. During the 2020/2021 crop season, five treatments were selected from previous crop season trials and tested again. No interaction between injuries by defoliation and stinkbug were found, for all parameters tested. When cages were infested with two stink bugs.m^{-1} in vegetative stage, there was a reduction of yield compared to 0 and 1 stink bug.m^{-1}, but it happened only during 2018/2019 crop season. The weight of 1,000 grains was reduced at defoliation ET, but no yield reduction was observed in the same crop season (2017/2018). Although small alterations in tested parameters were observed in some circumstances, overall, the currently recommended ETs for each type of injury are sufficient. Management is necessary only when the ET for each pest is reached.

Keywords: Economic injury level, defoliation, *Euschistus heros*, *Glycine max* L.

3.1 INTRODUCTION

Arthropod injuries on cultivated plants are among the major obstacles of sustainable food chain systems, impacting their production, quality, availability and distribution. The global yield loss of soybean attributed to pests and disease is estimated to be 21.4% (SAVARY *et al.*, 2019). In Brazil, this loss represents over 4.3 million tons annually, and almost 84 million tons of insecticides are used annually for pest management (OLIVEIRA *et al.*, 2014). Integrated pest management (IPM) seeks to maintain yields while conserving natural enemies and only using insecticides when required (BUENO *et al.*, 2021). IPM is based on the premise that plants tolerate certain levels of injuries without reducing yield (HIGLEY; PEDIGO, 1996). Therefore, the use of management tactics, including chemical pesticides, is only appropriate when pest numbers reach Economic Thresholds (ETs) (PETERSON; HIGLEY, 2002). An ET is defined as the most economically appropriate time to start pest management in order to avoid economic yield loss (BUENO *et al.*, 2013, 2021; PEDIGO; HUTCHINS; HIGLEY, 1986). Addopting management as pest numbers lower then ETs can result in inconsistent economic returns (HENRY; JOHNSON; WISE, 2011; HIGLEY; PEDIGO, 1996), while increasing pest resistance (SOSA-GÓMEZ; SILVA, 2010) and disrupting the environment (BUENO *et al.*, 2021).

In Brazil, the most important soybean pests are the defoliating larvae of the lepidopteran families Noctuidae and Erebidae, and the piercing-sucking stink bug complex (Hemiptera: Pentatomidae) (BUENO *et al.*, 2017). Stink bugs include at least 54 different species reported in soybean fields (PANIZZI; SLANSKY, 1985). Among those species, *Euschistus heros* (Fabricius, 1794) (Hemiptera: Pentatomidae) is the most abundant and economically important in South America (SOSA-GÓMEZ *et al.*, 2020).

Soybean ETs slightly vary around the world. While an ET of 30% defoliation (during the soybean vegetative stage) and 15% defoliation (during the soybean reproductive state) is recommended in Brazil (HAYASHIDA *et al.*, 2021), compared with 35% defoliation (during the soybean vegetative stage) and 20% defoliation (during the soybean reproductive state) in the USA (ANDREWS *et al.*, 2009). Similarly, differences in the recommended ET are also observed for stink bugs. The ET for controlling stink bugs in Brazil is two pentatomids per meter for soybean fields cropped for grain production, and only one per meter for soybean fields cropped for seed production (BUENO *et al.*, 2015). In contrast in Mississippi, USA, the stink bug ET in soybeans is 3.3 per meter, regardless of grain or seed production (CATCHOT, 2008).

Despite well-established ETs for both defoliation and stink bug management in soybeans, there is a general lack of information regarding interactions from multiple injury (Hutchins *et al.*, 1988). Under field conditions, soybean plants are frequently attacked by defoliators and stink bugs simultaneously (BUENO *et al.*, 2021), and little is known about the interaction between the two pest groups on soybean yield and thus, on the ET. An ET refinement, or even the development of a multiple-species ET, may be necessary if the interaction between defoliation and stink bug injury on soybean plants causes higher losses in quantity or quality than either pest alone. Therefore, this study evaluated the interaction between different levels of artificial defoliation and stink bugs densities on soybean yield, seed quality, and whether recommended ETs for each pest independently were sufficient to economically reduce losses.

3.2 MATERIAL AND METHODS

Three independent field trials were carried out during the 2017/2018, 2018/2019, and 2020/2021 crop seasons (TABLE 1) to test different scenarios of defoliation and stink bug stress interactions. Trials were located at the Embrapa Soja Experimental Station (Warta District, Londrina County, Paraná, Brazil; 23°11' S, 51° 11' W). During the 2017/2018 and 2018/2019 crop seasons, all three trials were carried out in a factorial randomized block design 3 X 3 (3 defoliation levels X 3 stink bug infestations levels) with 4 replicates. Imposed artificial defoliation levels at the vegetative soybean stage were 0% (control), 16.7% (half current ET) and 33.3% (current ET). At the reproductive soybean stage, studied defoliation levels were 0% (control), 8.3% (half current ET) and 16.7% (current ET). Stink bug infestations were tested at both the vegetative and reproductive soybean stages with 0, 1 and 2 adult *E. heros* per meter (1m-long soybean line with 12 plants). In the 2020/2021 crop season, five treatments were selected from previous crop season trials and carried out in a complete randomized block design with 5 treatments and 4 replicates (TABLE 1).

Each replicate in all trials and seasons was formed by 1m-long soybean line (12 plants) inside a cage. Each cage was 1 m^3 (1m x 1m x 1m) in size and consisted of iron bars covered with nylon screen. The cages included a door fitted with a Velcro strip, allowing for evaluation and maintenance during trials (GOMES; HAYASHIDA; DE FREITAS BUENO, 2020).

The soybean cultivar used was BRS 1010 IPRO; an early-maturing cultivar (maturity group 6.1) with indeterminate growth habit and high yield potential. Each replicate was sowed with 20 seeds per meter and one week after emergence, it was standardized 12 plants per meter.

Trials were evaluated twice a week. The number of stink bugs were counted and the cages were reinfested whenever a lower than previously number (due to insect escape or death) each treatment was noted. Additionally, artificial defoliation was imposed on the newly grown leaves after each evaluation, according to the previously determined treatment. When zero stink bugs were required for the test, we removed any observed insects from cages. Further, to ensure that no other insects were affecting the experiment, thiamethoxam + lambda-cyhalothrin 26.5 + 35.25 g.a.i. ha^{-1} (Engeo Pleno® 250 mL ha^{-1}) was sprayed on a regular basis (each 21 days) using a CO_2 pressurized back sprayer (Herbicat®, Catanduva, SP, Brazil) set to a spray volume of 150 liters ha^{-1}. Additionally, herbicides and fungicides were applied when necessary [two herbicide applications between the third and sixth week after emergence of plants, and three fungicide applications at the reproductive phase, starting between R1 and R2 (FEHR *et al.,* 1971), followed by additional applications at 20 to 30-day intervals]. These applications were performed equally over the total area for all treatments, including the control treatments where plants were not injured.

3.2.1 Defoliation and stink bug injuries

Artificial defoliation was carried out twice per week by manually removing the number of leaflets corresponding to each treatment with scissors, following the method of Gazzoni and Moscardi (1998). This procedure was performed on all leaves of the plant and on all plants inside each cage (replicate), according to the treatment (TABLE 1).

In the trials conducted in 2017/18, adult stink bugs used to infest replicates were from laboratory colonies, reared according to Silva *et al.* (2008). Stink bugs were originally collected from soybean fields in the Embrapa Soybean Experimental Farm, Londrina, State of Paraná, Brazil (23° 11' 11.7" S and 51° 10' 46.1" W). The population has been kept in the laboratory for approximately 7 years; during which new field insects have been introduced each year to maintain colony quality. Insects have been kept in plastic screen containers (20 cm × 20 cm sides × 24 cm tall) (Plasvale Ltda., Gaspar, State of Santa Catarina, Brazil) lined with filter paper and fed *ad libitum* with a mixture of soybeans (*Glycine max* L. Merr.; Fabaceae), peanuts (*Arachis hypogaea* L.; Fabaceae), beans (*Phaseolus vulgaris* L.; Fabaceae), privet fruits (*Ligustrum lucidum* Aiton; Oleaceae), and sunflower seeds (*Helianthus annuus* L.; Asteraceae). A Petri dish (diameter 9 cm) with a cotton wad soaked in distilled water was added to each container. Three days per week, stink bug containers were cleaned, their food was replaced, and their egg masses were collected. 2-day old adults were used to for infestation trials with replacement whenever necessary to keep constant levels of insects based on treatment. During the second and third crop seasons (2018/19 and 2020/21), wild adults of *E. heros* were collected from soybean plants in the surrounding area of the experiment (23° 11' 11.7" S and 51° 10' 46.1" W) and were used to infest trials.

3.2.2 Yield parameters

All plants of each plot were manually harvested and threshed for evaluation. The weight and moisture content of each sample was recorded (moisture meter G800, Gehaka Agri, São Paulo-SP, Brazil) and productivity for 13% seed moisture was calculated. In addition to yield, we recorded the weight of 1,000 grains (g), oil and protein contents (%), number of pods containing 0, 1, 2, 3 and 4 grains, and the total number of pods per replicate.

The protein and oil contents of the soybean samples were determined by Near Infrared Reflectance spectroscopy using the Thermo Scientific™ Antaris™ II FT-NIR analyzer (Thermo Fisher Scientific Waltham, MA, USA), reading three different curves for each sample. The results presented are the mean of the three readings, expressed as percentage on a dry basis (MERTZ-HENNING *et al.*, 2017).

In addition to these parameters, soybean grain quality was also evaluated. For this purpose, 30g of soybean yield were sampled and classified following the national standard quality legislation (BRASIL, 2007). Additionally, tetrazolium test was performed to verify the percentage of dead soybean embryos (6-8 Stink bugs tetrazolium scale), viability, and vigor of grains after different stink bug injury. The tetrazolium test was performed using two subsamples of 50 grains per sample, which were humidified in paper with distilled water for 16 hours at 25ºC. Subsequently, the seeds were submerged in a solution of 0.075% of 2,3,5-triphenyl tetrazolium chloride and were placed in an oven at 40ºC for 2 h and 30 min in the dark. Afterwards, the seeds were washed and individually inspected by cutting longitudinally through the center of the embryonic axis, following the methodology described by França-Neto & Krzyzanowski (2018).

3.2.3 Statistical analysis

Data from all tests were analyzed with R software, using the interface Rstudio and the packages “dplyr” and “ExpDes.pt” (FERREIRA; CAVALCANTI; NOGUEIRA, 2014). Data were subjected to tests for normality (SHAPIRO; WILK, 1965) and homoscedasticity (BURR; FOSTER, 1972), and when necessary, transformed [sin(x) or Box-Cox transformation] prior to the analysis of variance (ANOVA)to examine the main treatment interaction and block effects. Means were compared by a Tukey test when the F statistic showed significant values ($\alpha \leq 0.05$).

3.3 RESULTS

There was no observed interaction among the levels of artificial defoliation and stink bug numbers tested for yield or weight of 1,000 grains for all trials (TABLES 2-5); Thus, the main effects were tested separately for these parameters.

During the 2017/18 crop season, there was no reduction in yield for any of the levels of stink bug and defoliation (%) studied. However, during the 2018/19 crop season, yield reduction was observed when stink bugs were present during the soybean vegetative stage. There was a 15.06 and 13.92% reduction compared to the control, in 2 and 1 stink bug.m^{-1}, respectively (TABLE 2). During the 2020/2021 crop season, when five selected treatments were re-tested, there was yield reduction was not observed for the combination of defoliation and stink bug injuries (TABLE 6).

In both the 2017/18 and 2018/19 crop seasons, defoliation (%) reduced the weight of 1,000 grains compared to the control (0% defoliation) only when imposed during the soybean's reproductive development stage at the ET (16.7% defoliation) (TABLE 2). Despite lower weights of 1,000 grains in the 2020/2021 crop season, significant differences were not observed (TABLE 6).

The number of pods was impacted by both stink bug and defoliation injury but there was no interaction between the two types of injury for this parameter (FIGURE 1). In the 2018/2019 crop season—when 33.3% defoliation ET was imposed at the vegetative stage, there was a significant reduction of about 14% of total pods compared to the control (FIGURE 1D). In general, we observed an increase in the number of pods containing 0, 1, and 2 grains when stink bug density increased during the soybean reproductive stage (FIGURES 1B, 1C, 1E, 1F, 1H); while pods containing 3 and 4 grains (1C, 1D,1F, 1H) decreased in number.

Stink bug and defoliation injuries did not interact to affect protein or oil content (TABLE 3). In the 2018/2019 crop season, there was an increase in protein content when 2 stink bugs.m^{-1} caused injury to the plants during the reproductive stage (Trial 2), compared to the control (0 stink bug) (TABLE 3). Likewise, in the 2020/2021 crop season, the treatment with ½ defoliation ET + 2 stink bugs increased the protein content by 4.12% (TABLE 7). However, the oil content decreased by 2.71% compared to the control, with defoliation and stink bug injuries occurring during the vegetative and reproductive soybean development stages, respectively (trial 2; TABLE 7).

Some parameters of the National Standard Quality Test (BRASIL, 2007) were also impacted by the stink bugs but no interactions with defoliation rates were observed (TABLE 4). In the 2017/2018 crop season, when stink bugs infested soybeans at the plant's reproductive stage (trial 2 and 3), there was an increase in grain mass classified as 'damaged by insects' (Trial 2; Trial 3: TABLE 4). The same year—in trial 3—there was also an increase in fermented grain mass during the treatments with 2 stink bugs.m^{-1}, and a reduction in the mass of immature grains caused by a defoliation level of 16.7% (1 ET) compared to the no injury treatments. In trial 2 (2018/2019), there was an increase of fermented grains in the treatments with a defoliation level of 16.7%, and an increase of immature grains in the treatments with 2 stink bugs compared to the no treatment control.

During the 2020/2021 crop season, in trial 1, there was higher mass of immature grains in treatments with 33.3% defoliation (1ET) + 1 stink bug. In trial 2, the mass of fermented grains was higher in all treatments compared to the check (TABLE 8).

Compared to 0 stink bugs.m^{-1} ,the tetrazolium test revealed a significant increase in the proportion of dead embryos when 2 stink bugs.m^{-1} were present during the reproductive stage (trial 3) for both the 2017/2018 and 2018/2019 crop seasons (TABLE 5). In addition, the vigor was also impacted by 2 stink bugs.m^{-1} in trial 3 (2018/2019)— decreasing by more than 25% when compared to 0 stink bug.m^{-1}. In trial 2 (2020/2021), the treatments of 16.7% defoliation (½ET) + 2 stink bugs.m^{-1}, 33.3% defoliation (1ET) + 1 stink bug.m^{-1} and 33.3% defoliation (1ET) + 2 stink bugs.m^{-1} also lowered the vigor—with a decrease ranging from 32.58% to 39.66% (TABLE 9).

3.4 DISCUSSION

Soybean-IPM has been based on the premise that soybean plants can tolerate a certain level of injury without relevant yield loss (BUENO *et al.*, 2013, 2021; HIGLEY; PEDIGO, 1996). This tolerance of injury was taken into consideration while establishing the ETs for both defoliation (BATISTELA *et al.*, 2012; HAYASHIDA *et al.*, 2021) and stink bug feeding (BUENO *et al.*, 2015). The adoption of such ETs has contributed to the decrease insecticide use (BUENO *et al.*, 2021). However, such ETs were developed for single injury use and growers are concerned when multiple pest guilds are present simultaneously at numbers below individual ETs. Without direct tests, this deeply constrains our understanding and ability to use the traditional ET approach for IPM (Hutchins *et al.*, 1988).

Most tests to establish ET thresholds have focused on species of the same injury guild, producing data on a common injury such as stand reduction, leaf-mass consumption, assimilate removal, water-balance disruption, fruit destruction, or architecture modification (PEDIGO; HUTCHINS; HIGLEY, 1986). As far as we know, this is the first study evaluating a possible interaction between defoliation (gross tissue removal) and piercing-sucking injury (triggered by Hemiptera feeding) in soybean, with plants in the vegetative and reproductive stages of development.

Since there was no interaction between defoliation and stink bug injuries for all trials and evaluated parameters, the results herein reported indicate that defoliation and stink bug ETs can be used independently for soybean IPM decisions. The recommended ETs for lepidopteran larvae (30% defoliation in the vegetative stage or 15% defoliation in the reproductive state) (BATISTELA *et al.*, 2012; HAYASHIDA *et al.*, 2021) should be used independently from the levels of stink bugs in the field. Similarly, the ET of 2 stink bugs.m^{-1} for soybean grain production and 1 stink bugs.m^{-1} for crop seed production (BUENO *et al.*, 2015) should be used independently of the defoliation level of the field.

Trials were carried out during three consecutive crop seasons (2017/2018, 2018/2019, and 2019/2020) with consistent results, although observed small variations deserve more research. Previously, a lack of interaction between defoliation and stink bug feeding in soybean was reported by Simmons & Yeargan (1990), but for different stressors than tested in this study. Simmons & Yeargan (1990) found no significant interaction between artificial defoliation and the green stink bug, *Acrosternum hilare* (Say) feeding on soybean for yield, number of seeds or seed size.

There is considerable evidence in scientific literature that soybean yield and seed quality are more susceptible to injuries imposed during the reproductive stage than in the vegetative stage (BATISTELA *et al.*, 2012; MERTZ-HENNING *et al.*, 2017). Intriguingly, the results for Trial 1 indicate yield loss when two stink bugs.m^{-1} were present during the vegetative stage and it changed the percentage of pods with 2, 3 and 4 grains. However, other important parameters such as the weight of 1,000 grains, total number of pods, oil and protein content, and quality parameters were not impacted by the stink bugs during the vegetative stage. The brown stink bug *E. heros* is able to feed during the soybean's vegetative stage and can trigger the plant's defense response (TIMBÓ *et al.*, 2014) and this possibly could have impacted the pod's composition and yield.

Brazil has adopted the same ET across different species of stink bug pests in soybean. However, the damage potential of other pentatomids species, such as *Piezodorus guildinii,* has been reported to be higher than other stink bug complexes, possibly because of the length of the mouthparts and its unique salivary compounds (DEPIERI; PANIZZI, 2011; SOSA-GÓMEZ *et al.*, 2020). In addition, *P. guildinii* is reported to cause foliar retention (HUSCH; DE OLIVEIRA; SOSA-GÓMEZ, 2014), which can also impact the harvest. Such differences might be considered for future research in order to refine a multiple guild ET. Compared to other stink bug species, and despite being the most common species, *E. heros* is considered less harmful with densities up to 12 adults.m^{-1} for 21 days at the R6 stage not lowering crop yield (SCOPEL *et al.*, 2016).

Our findings indicate that defoliation during the reproductive stage can lower the weight of 1,000 grains. Previous studies have reported that defoliation and the consequent reduction of leaf area index are responsible for decreasing the weight of 1,000 grains (GLIER *et al.*, 2015; HAYASHIDA *et al.*, 2021) and it has direct correlation with yield loss (Dalchiavon *et al.*, 2012). In contrast, the present study found a decrease in weight of 1,000 grains but not an overall yield loss.

Plants adopt different strategies to avoid reduction in fitness. One of these strategies is the reallocation of primary metabolites (ZHOU *et al.*, 2015). It is possible that when our tested plants were experiencing defoliation and stink bug injury, they reallocated photoassimilates from the developing pods and grains to new ones. Our tested cultivar has an indeterminate grown habit and this might explain why the observed decrease in the weight of 1,000 grains had no impact on overall yield.

The soybean grains produced in Brazil contain the highest oil and protein content compared to the other major global exporting countries (THAKUR; HURBURGH, 2007). Thus, beyond soybean yield quantity, soybean quality; particularly the oil and protein content; are important parameters for industry purposes. Soybean oil is the most utilized domestic oil in Brazil, comprising about 90% of total oil and vegetable fat used (HENNING *et al.*, 2018). Further, its protein supply accounts for nearly 60% of the world’s vegetable protein (LIU, 1997).

In the last crop season (2020/2021, trial 2), the protein content was observed to increase with the number of stink bugs. However, the oil content decreased. The inverse correlation between oil and protein content is well-documented in the literature, although its causes are debated (CARRÃO-PANIZZI *et al.*, 2021; MERTZ-HENNING *et al.*, 2017; MOURTZINIS *et al.*, 2017; WIJEWARDANA; REDDY; BELLALOUI, 2019). Despite observed differences in one trial, no changes in oil and protein were observed for the remaining trials. Moreover, the values observed for all trials were very similar to the national average (22.42% oil and 36.69% protein) (HENNING *et al.*, 2018; HIRAKURI *et al.*, 2018).

Despite the differences in soybean quality between treatments for the impact of stink bugs on dead embryos (%), almost all values are within the limit imposed by national legislation (BRASIL, 2007). Dead embryo (%) is a parameter that assesses seed quality. The limit is 6% and the values found in or trials are below the limit. However, in the second and third crop seasons, the values of fermented soybean in all treatments exceed national limits. This increase in fermentation potentially was caused by the cages used in the experiments where cage effects have been previously reported (SIMMONS; YEARGAN, 1990). Further studies are needed to evaluate the impact of cages for early-maturing, indeterminate growth, high yield cultivars, and their interaction with defoliation and stink bug feeding.

As the stink bug density increased, the observed decrease in vigor and viability could be explained by proteases trigged by the insect feeding. This may also contribute to the observed reductions in respiration and seed germination. When interpreting these results, it is necessary to consider that the plants were kept under injury for almost their entire reproductive stage. Infestation time also plays an important role in seed damage and in the intensity of damage (SCOPEL *et al.*, 2016). However, adopting a lower ET does not increase yield and seed quality, nor bring any economic advantages (BUENO *et al.*, 2015).

In conclusion, this study shows that injuries caused by defoliation and stink bug feeding do not have any interaction with the yield and its components, oil and protein content, or seed quality. Although alterations in these parameters were observed by single injury in some circumstances, it was only in a single year and therefore, the currently recommended ETs are still safe and can be used by soybean producers individually for stink bugs and defoliation.

Acknowledgments

The authors thank “Embrapa Soja” (Brazil), Bayer Crop Science Brazil, and the Department of Entomology and Plant Pathology at Oklahoma State University. Thanks are also extended to the sponsor agencies “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” (CAPES-PRINT; grant no. 88887.374211/2019-00), “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq; grants no 142340/2018-9), and with Hatch Project accession no. 1019561 from the USDA National Institute of Food and Agriculture for supporting this research.

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TABLE 1. Studied treatments of three independent field trials evaluating different economic thresholds for IPM decisions in soybeans under the perspective of stress interaction between defoliation and stink bugs (SB). Description of the factorial randomized block design 3 X 3 (3 defoliation levels X 3 stink bug infestations) used during 2017/2018; 2018/2019 crop seasons and selected treatments studied during 2020/2021 crop season.

Trial	2017/2018 and 2018/2019 crop seasons				2020/2021 crop season
	Soybean vegetative stage		Soybean reproductive stage		Selected treatment (soybean development stage)
	Defoliation (%)	Stink bug.m^{-1}	Defoliation (%)	Stink bug.m^{-1}	
1	0; 16.7 and 33.3	0; 1 and 2 adults	0	0	**1)** 0 (control); **2)** 16.7% defoliation (vegetative) + 1 SB (vegetative); **3)** 16.7% defoliation (vegetative) + 2 SB (vegetative); **4)** 33.3% defoliation (vegetative) + 1 SB (vegetative); **5)** 33.3% defoliation (vegetative) + 2 SB (vegetative).
2	0; 16.7 and 33.3	0	0	0; 1 and 2 adults	**1)** 0 (control); **2)** 16.7% defoliation (vegetative) + 1 SB (reproductive); **3)** 16.7% defoliation (vegetative) + 2 SB (reproductive); **4)** 33.3% defoliation (vegetative) + 1 SB (reproductive); **5)** 33.3% defoliation (vegetative) + 2 SB (reproductive).
3	0	0	0; 8.3 and 16.7	0; 1 and 2 adults	**1)** 0 (control); **2)** 8.3% defoliation (reproductive) + 1 SB (reproductive); **3)** 8.3% defoliation (reproductive) + 2 SB (reproductive); **4)** 16.7% defoliation (reproductive) + 1 SB (reproductive); **5)** 16.7% defoliation (reproductive) + 2 SB (reproductive).

TABLE 2. Yield (Kg.ha^{-1}) and weight of 1,000 grains (WTG) at different scenarios of defoliation and stink bug stress interaction evaluated during 2017/2018 and 2018/2019 crop seasons in three independent field trials (Trial 1: 0%; 16.7% (½ET); 33.3% (ET) defoliation and 0; 1; 2 stink bug adults/meter during vegetative soybean development stage; Trial 2: 0%; 16.7% (½ET); 33.3% (ET) defoliation during vegetative stage and 0; 1; 2 stink bug adults/meter during reproductive soybean development stage and Trial 3: 0%; 8.3% (½ET); 16.7% (ET) defoliation and 0; 1; 2 stink bug adults/meter during reproductive soybean development stage).

Injury		**Trial 1**		**Trial 2**		**Trial 3**	
		Yield (Kg.ha^{-1})	WTG (g)	Yield (Kg.ha^{-1})	WTG (g)	Yield (Kg.ha^{-1})	WTG^{1}(g)^{1}
2017/2018 Crop Season							
Stink bug (adult.m^{-1})	0	4240.5 ± 216.2^{ns}	141.8 ± 2.2^{ns}	3679.1 ± 290.^{ns}	131.0 ± 2.5^{ns}	3467.1 ± 206.4^{ns}	130.2 ± 1.9^{ns}
	1	3804.4 ± 275.4	137.6 ± 2.8	3812.5 ± 221.6	131.3 ± 2.1	3855.3 ± 199.1	135.4 ± 2.7
	2	4123.7 ± 199.2	139.7 ± 1.6	3547.0 ± 256.8	135.8 ± 3.9	3859.7 ± 231.7	135.1 ± 2.9
Defoliation (%)	0	4022.8 ± 302.^{ns}	138.4 ± 3.2^{ns}	3664.8 ± 265.2^{ns}	130.4 ± 2.3^{ns}	3892.4 ± 275.2^{ns}	138.8 ± 2.5 A
	½ ET	3918.6 ± 221.9	140.4 ± 1.6	4030.1 ± 189.9	133.5 ± 1.3	3768.0 ± 134.6	133.7 ± 2.2 AB
	ET	4227.2 ± 163.1	140.4 ± 1.8	3343.7 ± 273.0	134.3 ± 4.4	3521.6 ± 211.8	128.3 ± 2.2 B
Statistics	P_{SB}; P_{Defol}; $P_{SBxDefol}$	0.45; 0.68; 0.79	0.50; 0.80; 0.95	0.77; 0.19; 0.87	0.49; 0.66; 0.66	0.36; 0.48; 0.85	0.26; 0.02; 0.68
	$F_{SB;}$ F_{Defol}; $F_{SBxDefol}$	0.82; 0.40; 0.42	0.72; 0.22; 0.18	0.27; 1.80; 0.31	0.73; 0.43; 0.61	1.07; 0.75; 0.33	1.45; 4.73; 0.58
2018/2019 Crop Season							
Stink bug (adult.m^{-1})	0	4415.7 ± 192.4 a	155.0 ± 2.3^{ns}	2958.2 ± 170.2^{ns}	151.6 ± 2.6^{ns}	3246.4 ± 210.1^{ns}	150.4 ± 3.5 a
	1	4358.3 ± 179.1 a	151.6 ± 3.1	3087.2 ± 171.6	151.2 ± 2.6	2827.5 ± 188.0	141.3 ± 2.4 b
	2	3751.8 ± 255.7 b	143.8 ± 5.0	2886.6 ± 163.5	151.8 ± 2.2	3066.6 ± 157.6	147.8 ± 2.5 ab
Defoliation (%)	0	4519.9 ± 213.3^{ns}	150.5 ± 3.2^{ns}	3073.6 ± 139.1^{ns}	155.7 ± 2.2^{ns}	3139.0 ± 198.7^{ns}	149.7 ± 3.2 A
	½ ET	4079.9 ± 253.5	148.5 ± 5.2	3026.2 ± 177.2	149.2 ± 1.9	3037.2 ± 176.7	149.1 ± 2.9 AB
	ET	3926.0 ± 179.0	151.41 ± 2.8	2832.3 ± 182.5	149.7 ± 2.8	2964.3 ± 200.8	140.6 ± 2.2 B
Statistics	P_{SB}; P_{Defol}; $P_{SBxDefol}$	0.04; 0.09; 0.27	0.07; 0.92; 0.50	0.66; 0.52; 0.35	0.98; 0.080.79	0.20; 0.75; 0.05	0.05; 0.03; 0.12
	$F_{SB;}$ F_{Defol}; $F_{SBxDefol}$	3.82; 2.68; 1.38	2.95; 0.08; 0.86	0.42; 0.67; 1.16	0.02; 2.87; 0.43	1.70; 0.30; 2.74	3.53; 4.20; 2.03

Means (± SE) followed by the same letter in the column (low-case letter for stink bugs and upper-case letter for defoliation) do not differ statistically from each other by Tukey test (p>0.05) inside the same crop season. ^{ns}Anova non-significant. ^{1}Original means followed by statistics performed on data transformed by Box-Cox.

TABLE 3. Oil and Protein content (%) (Means ± SE) at different scenarios of defoliation and stink bug stress interaction evaluated during 2017/2018 and 2018/2019 crop seasons in three independent field trials (Trial 1: 0%; 16.7% (½ET); 33.3% (ET) defoliation and 0; 1; 2 stink bug adults/meter during vegetative soybean development stage; Trial 2: 0%; 16.7% (½ET); 33.3% (ET) defoliation during vegetative stage and 0; 1; 2 stink bug adults/meter during reproductive soybean development stage and Trial 3: 0%; 8.3% (½ET); 16.7% (ET) defoliation and 0; 1; 2 stink bug adults/meter during reproductive soybean development stage).

Injury		**Trial 1**		**Trial 2**		**Trial 3**	
		Oil (%)	Protein (%)	Oil (%)	Protein (%)	Oil (%)	Protein (%)
2017/2018 Crop Season							
Stink bug (adult.m^{-1})	0	23.6 ± 0.2^{ns}	33.8 ± 0.3^{ns}	22.7 ± 0.2^{ns}	34.9 ± 0.2^{ns}	23.0 ± 0.2^{ns}	35.0 ± 0.3^{ns}
	1	23.7 ± 0.2	34.2 ± 0.3	22.5 ± 0.3	35.1 ± 0.3	22.8 ± 0.2	35.1 ± 0.3
	2	23.1 ± 0.2	34.6 ± 0.2	23.1 ± 0.2	35.3 ± 0.2	23.1 ± 0.2	34.9 ± 0.2
Defoliation (%)	0	23.4 ± 0.2^{ns}	34.1 ± 0.3^{ns}	22.7 ± 0.2^{ns}	35.3 ± 0.2^{ns}	23.0 ± 0.2^{ns}	35.0 ± 0.3^{ns}
	½ ET	23.4 ± 0.2	34.3 ± 0.3	22.9 ± 0.3	34.9 ± 0.3	22.9 ± 0.2	35.2 ± 0.3
	ET	23.6 ± 0.2	34.1 ± 0.3	22.7 ± 0.2	35.1 ± 0.2	23.0 ± 0.2	34.8 ± 0.2
Statistics	P_{SB}; P_{Defol}; $P_{SBxDefol}$	0.11; 0.60; 0.22	0.21; 0.84; 0.53	0.24; 0.89; 0.59	0.56; 0.42; 0.45	0.32; 0.81; 0.36	0.87; 0.47; 0.36
	$F_{SB;}$ F_{Defol}; $F_{SBxDefol}$	2.44; 0.52; 1.53	1.65; 0.17; 0.81	1.51; 0.12; 0.71	0.60; 0.89; 0.96	1.18; 0.21; 1.15	0.14; 0.77; 1.14
2018/2019 Crop Season							
Stink bug (adult.m^{-1})	0	22.1 ± 0.3^{ns}	35.5 ± 0.4^{ns}	21.2 ± 0.2^{ns}	37.5 ± 0.4 b	21.1 ± 0.4^{ns}	37.1 ± 0.5^{ns}
	1	22.1 ± 0.3	35.8 ± 0.3	20.9 ± 0.4	37.9 ± 0.5 ab	20.8 ± 0.3	37.6 ± 0.4
	2	21.7 ± 0.6	36.3 ± 0.7	20.2 ± 0.3	38.8 ± 0.3 a	20.4 ± 0.3	38.2 ± 0.4
Defoliation (%)	0	22.3 ± 0.4^{ns}	35.2 ± 0.4^{ns}	20.8 ± 0.3^{ns}	38.0 ± 0.4^{ns}	20.8 ± 0.2^{ns}	37.9 ± 0.3^{ns}
	½ ET	21.5 ± 0.5	36.6 ± 0.7	20.7 ± 0.3	38.3 ± 0.4	21.1 ± 0.2	37.4 ± 0.4
	ET	22.0 ± 0.3	35.8 ± 0.3	20.9 ± 0.4	37.9 ± 0.5	20.5 ± 0.4	37.7 ± 0.6
Statistics	P_{SB}; P_{Defol}; $P_{SBxDefol}$	0.44; 0.40; 0.13	0.75; 0.48; 0.11	0.06; 0.84; 3.23	0.04; 0.81; 3.44	0.34; 0.49; 0.79	0.29; 0.79; 1.30
	$F_{SB;}$ F_{Defol}; $F_{SBxDefol}$	0.85; 0.94; 1.97	0.29; 0.76; 2.10	3.23; 0.17; 0.76	3.44; 0.22; 1.74	1.14; 0.73; 0.42	1.30; 0.24; 0.43

^{ns}Anova non-significant.

TABLE 4. Main attributes of National Standard Quality Test (IN11; Brasil, 2007) of 30g samples from different scenarios of defoliation and stink bug stress interaction evaluated during 2017/2018 and 2018/2019 crop seasons in three independent field trials (Trial 1: 0%; 16.7% (½ET); 33.3% (ET) defoliation and 0; 1; 2 stink bug adults/meter during vegetative soybean development stage; Trial 2: 0%; 16.7% (½ET); 33.3% (ET) defoliation during vegetative stage and 0; 1; 2 stink bug adults/meter during reproductive soybean development stage and Trial 3: 0%; 8.3% (½ET); 16.7% (ET) defoliation and 0; 1; 2 stink bug adults/meter during reproductive soybean development stage).

Injury		**Trial 1**			**Trial 2**			**Trial 3**		
		Fermented (g)	Immature (g)^1	DBI (g)^2	Fermented (g)	Immature (g)	DBI (g)^{2,3}	Fermented (g)^1	Immature (g)	DBI (g)^2
2017/2018 Crop Season										
Stink bug (adult.m^{-1})	0	0.0 ± 0.0 ^{ns}	0.1 ± 0.1 ^{ns}	0.6 ± 0.2 ^{ns}	0.1 ± 0.1 ^{ns}	0.1 ± 0.1 ^{ns}	0.9 ± 0.3 b	0.3 ± 0.2 b	0.14 ± 0.06	1.4 ± 0.5b
	1	0.2 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.00 ± 0.00	0.0 ± 0.0	2.5 ± 0.8 ab	0.1 ± 0.1 ab	0.08 ± 0.04	3.0 ± 0.7 ab
	2	0.1 ± 0.1	0.0 ± 0.0	0.6 ± 0.2	0.4 ± 0.3	0.1 ± 0.0	3.0 ± 0.5 a	0.4 ± 0.2 a	0.03 ± 0.01	4.1 ± 0.5 a
Defoliation (%)	0	0.1 ± 0.1 ^{ns}	0.0 ± 0.0 ^{ns}	0.7 ± 0.2 ^{ns}	0.1 ± 0.1 ^{ns}	0.1 ± 0.1 ^{ns}	2.1 ± 0.6^{ns}	0.3 ± 0.2 ^{ns}	0.11 ± 0.04 a	3.0 ± 0.7 ^{ns}
	½ ET	0.2 ± 0.1	0.1 ± 0.1	0.5 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	1.9 ± 0.4	0.1 ± 0.1	0.09 ± 0.05 ab	2.7 ± 0.5
	ET	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.3 ± 0.3	0.0 ± 0.0	2.5 ± 0.7	0.4 ± 0.2	0.05 ± 0.04 b	2.8 ± 0.8
Statistics	P_{SB}; P_{Defol}; $P_{SBxDefol}$	0.37; 0.89; 0.82	0.38; 0.85; 0.45	0.94; 029; 0.14	0.24; 0.68; 0.57	0.57; 0.46; 0.52	0.03; 0.75; 0.95	0.03; 0.18; 0.60	0.57; 0.01; 0.94	0.01; 0.95; 0.19
	F_{SB}; F_{Defol}; $F_{SBxDefol}$	1.04; 0.12; 0.38	1.01; 0.16; 0.96	0.06; 1.29; 1.95	1.53; 0.39; 0.75	0.57; 0.80; 0.82	4.11; 0.30; 0.18	3.96; 1.86; 0.71	0.57; 5.22; 0.20	5.58;0.05; 1.67
2018/2019 Crop Season^1										
Stink bug (adult.m^{-1})	0	16.7 ± 3.8 ^{ns}	0.4 ± 0.2 ^{ns}	3.0 ± 0.6 ^{ns}	23.7 ± 3.8 ^{ns}	3.4 ± 1.0 b	3.1 ± 0.6 ^{ns}	20.2 ± 3.7 ^{ns}	2.06 ± 0.82 ^{ns}	2.8 ± 0.6 ^{ns}
	1	22.1 ± 5.4	0.5 ± 0.2	2.1 ± 0.5	26.7 ± 4.4	2.4 ± 0.6 b	2.7 ± 0.45	25.4 ± 3.8	2.53 ± 0.94	3.9 ± 0.5
	2	23.3 ± 7.8	1.5 ± 1.0	1.5 ± 0.3	31.7 ± 3.4	7.8 ± 1.7 a	3.9 ± 0.5	29.3 ± 3.5	2.29 ± 0.57	4.3 ± 0.5
Defoliation (%)	0	18.0 ± 5.6 ^{ns}	0.4 ± 0.2 ^{ns}	2.7 ± 0.4 ^{ns}	24.2 ± 3.1 B	5.5 ± 1.8 ^{ns}	2.6 ± 0.5 ^{ns}	22.9 ± 3.4 ^{ns}	2.2 ± 0.5 ^{ns}	3.8 ± 0.5 ^{ns}
	½ ET	25.4 ± 7.3	1.5 ± 1.0	2.2 ± 0.6	33.0 ± 3.9 A	3.2 ± 1.0	3.4 ± 0.6	22.5 ± 2.9	1.6 ± 0.5	3.4 ± 0.6
	ET	18.7 ± 4.2	0.5 ± 0.2	1.8 ± 0.5	25.7 ± 4.4 AB	4.9 ± 1.0	3.7 ± 0.6	29.5 ± 4.6	3.2 ± 1.1	3.9 ± 0.5
Statistics	P_{SB}; P_{Defol}; $P_{SBxDefol}$	0.71; 0.63; 0.72	0.20; 0.11; 0.17	0.08; 0.33; 0.20	0.07; 0.04; 0.05	0.01; 0.38; 0.24	0.27; 0.34; 0.71	0.22; 0.33; 0.48	0.93; 0.40; 0.90	0.14; 0.74; 0.95
	F_{SB}; F_{Defol}; $F_{SBxDefol}$	0.34; 0.47; 0.52	1.72; 2.43; 1.75	2.89; 1.15; 1.63	3.04; 3.63; 2.78	5.99; 1.01; 1.49	1.40; 1.13; 0.54	1.60; 1.18; 0.90	0.08; 0.94; 0.27	2.17; 0.30; 0.17

Means (± SE) followed by the same letter in the column (low-case letter for stink bugs and upper-case letter for defoliation) do not differ statistically from each other by Tukey test ($p>0.05$) inside the same crop season. ^{ns}Anova non-significant. ^{1}Data transformed to Box-Cox. ^{2} DBI = Damaged by insects. ^{3}Data transformed to sin(x).

TABLE 5. Results of Tetrazolium test (França-Neto & Krzyzanowski , 2018): Dead embryo (Stink bug scale 6-8) (%), vigor (%) and viability (%) from different scenarios of defoliation and stink bug stress interaction evaluated during 2017/2018 and 2018/2019 crop seasons in three independent field trials (Trial 1: 0%; 16.7% (½ET); 33.3% (ET) defoliation and 0; 1; 2 stink bug adults/meter during vegetative soybean development stage; Trial 2: 0%; 16.7% (½ET); 33.3% (ET) defoliation during vegetative stage and 0; 1; 2 stink bug adults/meter during reproductive soybean development stage and Trial 3: 0%; 8.3% (½ET); 16.7% (ET) defoliation and 0; 1; 2 stink bug adults/meter during reproductive soybean development stage).

Injury		**Trial 1**			**Trial 2**			**Trial 3**		
		Dead embryo (%)	Vigor (%)	Viability (%)	Dead embryo (%)	Vigor (%)	Viability (%)	Dead embryo (%)	Vigor (%)	Viability (%)
2017/2018 Crop Season										
Stink bug (adult.m^{-1})	0	0.5 ± 0.3 ^{ns}	93.2 ± 1. ^{ns}	96.9 ± 0.5 ^{ns}	0.9 ± 0.4 ^{ns}	92.7 ± 1.0 ^{ns}	95.4 ± 0.7 ^{ns}	1.2 ± 0.4 b	90.9 ± 1.6 ^{ns}	95.3 ± 0.5 ^{ns}
	1	0.2 ± 0.1	93.6 ± 0.8	96.5 ± 0.5	2.8 ± 0.9	89.2 ± 1.7	95.3 ± 0.8	2.3 ± 0.5 ab	90.1 ± 1.2	95.1 ± 0.7
	2	0.4 ± 0.2	94.6 ± 1.1	97.2 ± 0.7	2.8 ± 0.5	88.4 ± 1.4	94.4 ± 0.6	3.1 ± 0.4 a	88.6 ± 1.0	95.2 ± 0.5
Defoliation (%)	0	0.7 ± 0.3 ^{ns}	93.3 ± 1.1 ^{ns}	96.8 ± 0.5 ^{ns}	1.9 ± 0.6 ^{ns}	89.3 ± 1.6 ^{ns}	94.8 ± 0.9 ^{ns}	2.7 ± 0.5 ^{ns}	89.5 ± 1.0 ^{ns}	94.3 ± 0.5 ^{ns}
	½ ET	0.3 ± 0.1	92.9 ± 1.1	96.7 ± 0.5	2.3 ± 0.7	90.7 ± 1.5	95.1 ± 0.7	1.7 ± 0.5	90.3 ± 1.3	95.9 ± 0.7
	ET	0.2 ± 0.1	94.8 ± 0.8	97.1 ± 0.7	2.3 ± 0.7	90.3 ± 1.6	95.3 ± 0.7	2.3 ± 0.46	89.8 ± 1.2	95.3 ± 0.2
Statistics	P_{SB}; P_{Defol}; $P_{SBxDefol}$	0.42; 0.14; 0.13	0.75; 0.41; 0.32	0.71; 0.88; 0.81	0.12; 0.93; 0.96	0.12; 0.81; 0.59	0.59; 0.86; 0.94	0.02; 0.28; 0.32	0.36; 0.87; 0.80	0.98; 0.17; 1.00
	$F_{SB;}$ F_{Defol}; $F_{SBxDefol}$	0.91; 2.16; 1.95	0.30; 0.92; 1.24	0.35; 0.13; 0.40	2.29; 0.08; 0.16	2.36; 0.21; 0.71	0.53; 0.15; 0.20	4.90; 1.33; 1.25	1.06; 0.14; 0.41	0.02; 1.89; 0.01
2018/2019 Crop Season										
Stink bug (adult.m^{-1})	0	5.2 ± 1.7^{ns}	74.4 ± 4.4^{ns}	81.3 ± 3.6^{ns}	9.5 ± 2.2^{ns}	49.1 ± 7.6 a	58.9 ± 7.0 a	5.8 ± 1.4 b	53.3 ± 5.9 a	64.0 ± 5.5 a
	1	6.1 ± 2.5	71.8 ± 5.7	80.0 ± 5.1	7.9 ± 1.6	48.6 ± 4.6 a	59.9 ± 4.2 a	10.3 ± 2.2 a	39.2 ± 5.3 ab	49.1 ± 5.5 ab
	2	6.8 ± 3.0	69.8 ± 8.8	77.1 ± 8.4	10.3 ± 1.5	26.9 ± 4.5 b	37.5 ± 4.1 b	10.2 ± 1.2 a	28.2 ± 3.7 b	40.5 ± 3.7 b
Defoliation (%)	0	5.3 ± 2.5 ^{ns}	78.1 ± 6.1 ^{ns}	84.9 ± 5.2 ^{ns}	6.6 ± 0.7 ^{ns}	42.2 ± 6.8 ^{ns}	52.1 ± 6.3 ^{ns}	9.7 ± 2.02^{ns}	37.8 ± 4.2 ^{ns}	48.2 ± 4.4^{ns}
	½ ET	8.2 ± 3.2	63.1 ± 8.0	71.4 ± 7.8	12.1 ± 2.1	37.3 ± 6.5	49.6 ± 5.9	6.8 ± 1.2	47.5 ± 5.3	58.6 ± 5.2
	ET	4.6 ± 1.3	74.9 ± 4.4	82.0 ± 3.6	9.0 ± 1.9	45.2 ± 6.1	54.7 ± 6.1	9.8 ± 1.8	35.3 ± 7.2	46.8 ± 6.7
Statistics	P_{SB}; P_{Defol}; $P_{SBxDefol}$	0.91; 0.59; 0.43	0.87; 0.21; 0.24	0.87; 0.23; 0.16	0.55; 0.58; 0.61	0.01; 0.58; 0.05	0.01; 0.78; 0.08	0.04; 0.38; 080	0.01; 0.25; 0.72	0.01; 0.23; 0.75
	$F_{SB;}$ F_{Defol}; $F_{SBxDefol}$	0.10; 0.53; 1.00	0.14; 1.65; 1.48	0.14; 1.59; 1.79	0.61; 0.56; 0.68	5.63; 0.56; 2.75	6.30; 0.25; 2.42	3.83; 1.01; 0.41	5.65; 1.48; 0.52	5.31; 1.55; 0.48

Means (± SE) followed by the same letter in the column do not differ statistically from each other by Tukey test (p>0.05) inside the same crop season. ^{ns}Anova non-significant.

TABLE 6. Yield (Kg.ha^{-1}) and weight of 1,000 grains (WTG) (Means ± SE) at selected treatments of different scenarios of defoliation and stink bug stress interaction evaluated during 2020/2021 crop season trials (Trials 1 and 2: 16.7% = ½ET and 33.3% = ET for defoliation (%) during vegetative stage; Trial 3: 8.3% = ½ET and 16.7% = ET for defoliation% during reproductive stage).

Treatment		**Trial 1** (defoliation and stink bug injuries during vegetative soybean development stage)		**Trial 2** (defoliation and stink bug injuries during vegetative and reproductive soybean development stages, respectively)		**Trial 3** (defoliation and stink bug injuries during reproductive soybean development stage)	
		Yield (Kg.ha^{-1})	WTG	Yield (Kg.ha^{-1})	WTG	Yield (Kg.ha^{-1})	WTG
Control (0 injury)		4035.4 ± 106.9^{ns}	132.8 ± 1.7^{ns}	3296.8 ± 376.1^{ns}	133.1 ± 4.8^{ns}	2267.9 ± 141.6^{ns}	121.6 ± 3.2^{ns}
½ Defoliation ET + 1 stink bug		3595.4 ± 369.9	137.0 ± 3.0	3120.5 ± 585.5	125.1 ± 3.3	2135.5 ± 64.5	118.3 ± 6.0
½ Defoliation ET + 2 stink bugs		3273.2 ± 195.4	134.8 ± 2.3	2820.3 ± 123.2	125.3 ± 1.6	2512.8 ± 324.1	131.6 ± 0.7
Defoliation ET + 1 stink bug		3500.7 ± 353.8	137.0 ± 2.0	2249.7 ± 262.2	119.4 ± 4.4	2461.9 ± 216.3	126.1 ± 2.6
Defoliation ET + 2 stink bugs		3769.5 ± 197.0	129.0 ± 1.4	2524.9 ± 281.6	124.7 ± 4.8	2261.6 ± 217.3	117.3 ± 7.0
Statistics	$P_{\text{treatment}}$	0.46	0.12	0.07	0.04	0.71	0.27
	P_{block}	0.96	0.54	0.01	0.00	0.44	0.83
	$F_{\text{treatment}}$	0.96	2.29	2.94	3.53	0.54	1.47
	F_{block}	0.09	0.76	6.45	7.62	0.98	0.29
	DF_{residue}	12	12	12	12	12	12

^{ns}Anova non-significant.

TABLE 7. Oil and protein content (%) at selected treatments of different scenarios of defoliation and stink bug stress interaction evaluated during 2020/2021 crop season trials (Trials 1 and 2: 16.7% = ½ET and 33.3% = ET for defoliation (%) during vegetative stage; Trial 3: 8.3% = ½ET and 16.7% = ET for defoliation% during reproductive stage).

Treatment		**Trial 1** (defoliation and stink bug injuries during vegetative soybean development stage)		**Trial 2** (defoliation and stink bug injuries during vegetative and reproductive soybean development stages, respectively)		**Trial 3** (defoliation and stink bug injuries during reproductive soybean development stage)	
		Oil (%)	Protein (%)	Oil (%)	Protein (%)	Oil (%)	Protein (%)
Control (0 injury)		23.0 ± 0.9^{ns}	35.2 ± 1.3^{ns}	23.7 ± 0.3 a	33.8 ± 0.1 b	23.9 ± 0.2^{ns}	32.6 ± 0.5^{ns}
½ Defoliation ET + 1 stink bug		22.5 ± 1.0	35.7 ± 1.2	22.2 ± 0.2 ab	35.00 ± 0.6 b	22.3 ± 0.6	35.5 ± 0.9
½ Defoliation ET + 2 stink bugs		23.1 ± 0.3	35.00 ± 0.2	21.0 ± 0.5 b	38.0 ± 0.3 a	23.7 ± 0.2	34.0 ± 0.3
Defoliation ET + 1 stink bug		23.1 ± 0.5	35.1 ± 1.0	22.8 ± 0.6 a	35.2 ± 0.7 ab	21.9 ± 0.9	36.5 ± 1.6
Defoliation ET + 2 stink bugs		23.8 ± 1.2	34.8 ± 1.0	22.0 ± 1.0 ab	36.5 ± 1.3 ab	22.1 ± 0.4	35.7 ± 0.7
Statistics	$P_{\text{treatment}}$	0.87	0.95	0.01	0.00	0.06	0.07
	P_{block}	0.58	0.06	0.01	0.06	0.43	0.42
	$\text{F}_{\text{treatment}}$	0.30	0.16	6.35	6.64	3.07	2.83
	F_{block}	0.68	3.25	6.74	3.28	0.99	1.01
	$\text{DF}_{\text{residue}}$	12	12	12	12	12	12

Means (± SE) followed by the same letter in the column do not differ statistically from each other by Tukey test (p>0.05). ^{ns}Anova non-significant.

TABLE 8. Main attributes of National Standard quality test (IN11; Brasil, 2007) of 30g samples from scenarios of defoliation and stink bug stress interaction evaluated during 2020/2021 crop season trials (Trials 1 and 2: 16.7% = ½ET and 33.3% = ET for defoliation (%) during vegetative stage; Trial 3: 8.3% = ½ET and 16.7% = ET for defoliation% during reproductive stage).

Treatments		**Trial 1** (defoliation and stink bug injuries during vegetative soybean development stage)			**Trial 2** (defoliation and stink bug injuries during vegetative and reproductive soybean development stages, respectively)			**Trial 3** (defoliation and stink bug injuries during reproductive soybean development stage)		
		Fermented (g)	Immature (g)	DBI (g)^1	Fermented (g)	Immature (g)	DBI (g)^1	Fermented (g)	Immature (g)	DBI (g)^1
Control (0 injury)		9.5 ± 4.3 ^{ns}	0.4 ± 0.2 ab	7.2 ± 2.3 ^{ns}	6.4 ± 0.4 b	1.2 ± 0.4 ^{ns}	6.2 ± 0.8 ^{ns}	4.1 ± 1.1 ^{ns}	0.76 ± 0.50 ^{ns}	5.1 ± 0.6 ^{ns}
½ Defoliation ET + 1 stink bug		12.5 ± 6.7	0.0 ± 0.0 b	8.6 ± 1.7	10.9 ± 2.1 a	1.4 ± 0.5	10.7 ± 1.5	20.8 ± 4.0	0.23 ± 0.06	7.7 ± 0.5
½ Defoliation ET + 2 stink bugs		4.8 ± 2.0	0.1 ± 0.1 ab	6.3 ± 1.5	25.1 ± 2.8 a	2.3 ± 0.2	10.1 ± 1.1	18.2 ± 7.5	0.58 ± 0.32	8.2 ± 2.5
Defoliation ET + 1 stink bug		7.9 ± 2.4	0.7 ± 0.2 a	8.7 ± 2.1	20.2 ± 1.5 a	2.2 ± 0.6	10.1 ± 2.1	19.1 ± 4.9	1.64 ± 0.57	10.6 ± 1.2
Defoliation ET + 2 stink bugs		6.8 ± 2.9	0.2 ± 0.1 ab	7.3 ± 2.9	24.0 ± 3.7 a	3.3 ± 1.9	9.9 ± 0.8	21.4 ± 4.5	3.29 ± 1.63	10.2 ± 2.0
Statistics	$P_{\text{treatment}}$	0.71	0.03	0.94	0.00	0.47	0.22	0.09	0.16	0.21
	P_{block}	0.33	0.23	0.85	0.05	0.23	0.65	0.20	0.70	0.77
	$F_{\text{treatment}}$	0.55	3.81	0.18	17.95	0.96	1.70	2.56	2.02	1.72
	F_{block}	1.26	1.66	0.27	3.50	1.66	0.56	1.79	0.49	0.38
	DF_{residue}	12	12	12	12	12	12	12	12	12

Means (± SE) followed by the same letter in the column do not differ statistically from each other by Tukey test ($p>0.05$). ^{ns}Anova non-significant. ^{1}DBI = Damaged by insects.

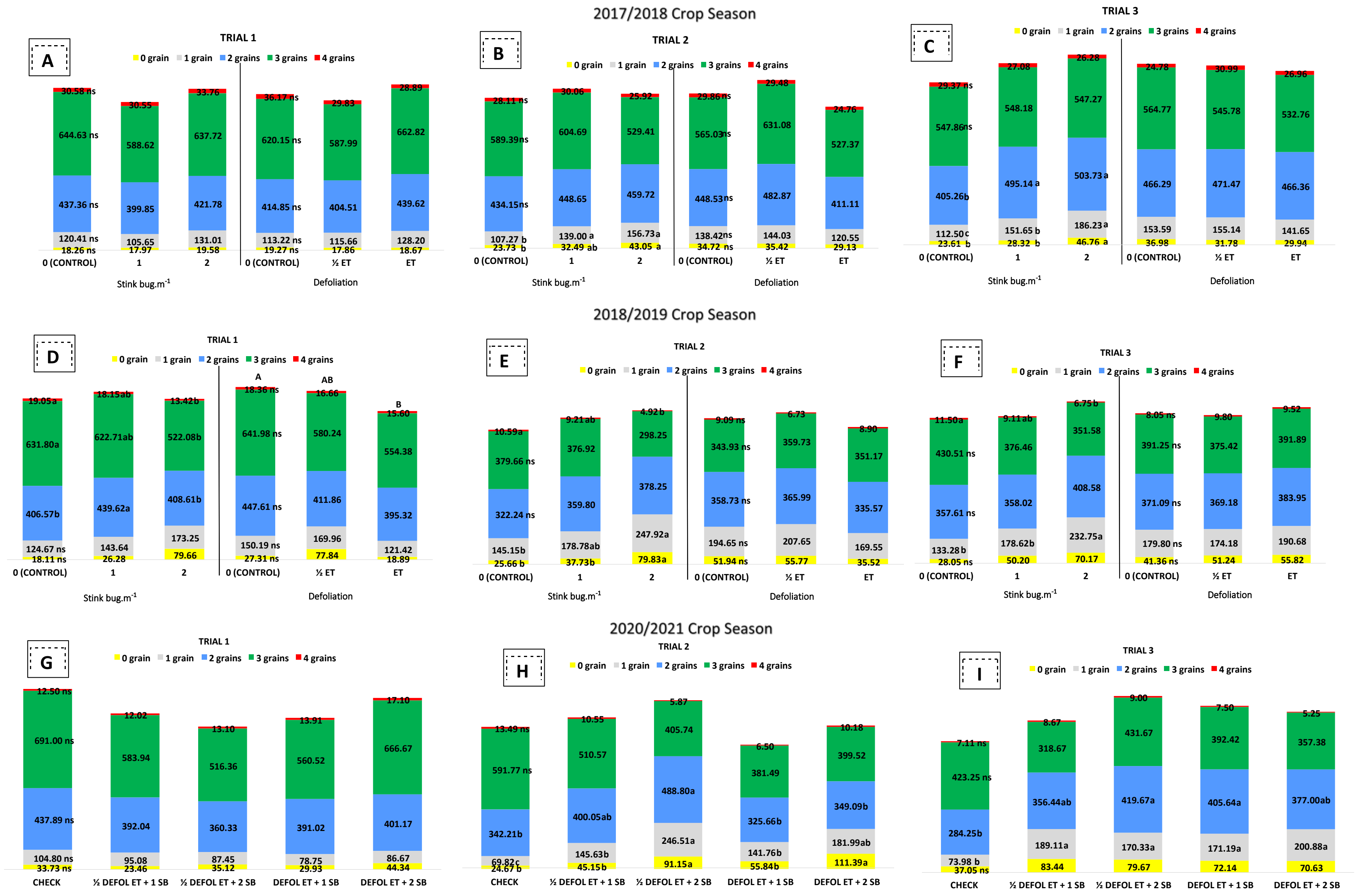
TABLE 9. Results of Tetrazolium test (França-Neto & Krzyzanowski 2018): Dead embryo (Stink bug scale 6-8) (%), vigor (%) and viability (%) from scenarios of defoliation and stink bug stress interaction evaluated during 2020/2021 crop season trials (Trials 1 and 2: 16.7% = ½ET and 33.3% = ET for defoliation (%) during vegetative stage; Trial 3: 8.3% = ½ET and 16.7% = ET for defoliation% during reproductive stage).

Treatments		**Trial 1**			**Trial 2**			**Trial 3**		
		Dead embryo (%)	Vigor (%)	Viability (%)	Dead embryo (%)	Vigor (%)	Viability (%)	Dead embryo (%)	Vigor (%)	Viability (%)
Control (0 injury)		9.5 ± 2.9 ^{ns}	65.5 ± 11.5^{ns}	78.3 ± 7.4^{ns}	9.3 ± 0.9^{ns}	66.3 ± 2.1 a	77.7 ± 2.7 a	4.5 ± 1.9 ^{ns}	74.5 ± 4.0 ^{ns}	83.3 ± 3.7 ^{ns}
½ Defoliation ET + 1 stink bug		11.0 ± 3.8	69.3 ± 8.0	80.8 ± 4.7	16.3 ± 5.7	40.3 ± 6.7 ab	53.8 ± 6.8 ab	18.0 ± 4.5	41.0 ± 5.5	59.3 ± 5.5
½ Defoliation ET + 2 stink bugs		6.3 ± 3.3	75.8 ± 8.2	86.3 ± 5.1	22.0 ± 4.4	26.7 ± 6.3 b	46.0 ± 6.6 b	12.0 ± 4.1	48.5 ± 11.8	62.3 ± 11.3
Defoliation ET + 1 stink bug		11.0 ± 3.7	54.5 ± 12.5	71.5 ± 8.8	19.0 ± 2.4	33.8 ± 7.9 b	49.3 ± 6.2 b	19.0 ± 5.9	43.8 ± 9.6	59.0 ± 9.3
Defoliation ET + 2 stink bugs		10.8 ± 3.8	61.5 ± 12.7	74.0 ± 10.0	20.0 ± 0.4	31.7 ± 8.4 b	52.0 ± 8.5 ab	15.8 ± 4.0	47.0 ± 11.9	61.5 ± 11.7
Statistics	$P_{\text{treatment}}$	0.88	0.76	0.74	0.09	0.01	0.02	0.24	0.16	0.29
	P_{block}	0.78	0.84	0.92	0.13	0.28	0.26	0.89	0.44	0.25
	$F_{\text{treatment}}$	0.29	0.47	0.49	2.60	5.99	4.25	1.61	2.03	1.42
	F_{block}	0.36	0.28	0.16	2.34	1.42	1.54	0.21	0.98	1.56
	DF_{residue}	12	12	12	12	12	12	12	12	12

Means (± SE) followed by the same letter in the column do not differ statistically from each other by Tukey test (p>0.05). ^{ns}Anova non-significant.

Figure captions

FIGURE 1. Number of pods containing 0, 1, 2, 3 and 4 pods at different scenarios of defoliation and stink bug stress interaction evaluated during 2017/2018 and 2018/2019 crop seasons in three independent field (A – F); and during 2020/2021 crop season with selected treatments (G-I). Trials 1 and 2: 16.7% = ½ET and 33.3% = ET for defoliation (%) during vegetative stage; Trial 3: 8.3% = ½ET and 16.7% = ET for defoliation% during reproductive stage. Means followed by the same lowercase letter for stink bug, and uppercase defoliation are not significantly different ($p \geq 0.05$). ns = non-significant.


2017/2018 Crop Season
A
TRIAL 1
0 grain
1 grain
2 grains
3 grains
4 grains
30.58 ns
644.63 ns
437.36 ns
120.41 ns
18.26 ns
30.55
588.62
399.85
105.65
17.97
33.76
637.72
421.78
131.01
19.58
36.17 ns
620.15 ns
414.85 ns
113.22 ns
19.27 ns
29.83
587.99
404.51
115.66
17.86
28.89
662.82
439.62
128.20
18.67
0 (CONTROL)
1
2
0 (CONTROL)
½ ET
ET
Stink bug.m-1
Defoliation
B
TRIAL 2
0 grain
1 grain
2 grains
3 grains
4 grains
28.11 ns
589.39ns
434.15ns
107.27 b
23.73 b
30.06
604.69
448.65
139.00 a
32.49 ab
25.92
529.41
459.72
156.73a
43.05 a
29.86ns
565.03ns
448.53ns
138.42ns
34.72 ns
29.48
631.08
482.87
144.03
35.42
24.76
527.37
411.11
120.55
29.13
0 (CONTROL)
1
2
0 (CONTROL)
½ ET
ET
Stink bug.m-1
Defoliation
C
TRIAL 3
0 grain
1 grain
2 grains
3 grains
4 grains
29.37 ns
547.86ns
405.26b
112.50c
23.61 b
27.08
548.18
495.14 a
151.65 b
28.32 b
26.28
547.27
503.73 a
186.23 a
46.76 a
24.78
564.77
466.29
153.59
36.98
30.99
545.78
471.47
155.14
31.78
26.96
532.76
466.36
141.65
29.94
0 (CONTROL)
1
2
0 (CONTROL)
½ ET
ET
Stink bug.m-1
Defoliation
2018/2019 Crop Season
D
TRIAL 1
0 grain
1 grain
2 grains
3 grains
4 grains
19.05a
631.80a
406.57b
124.67 ns
18.11 ns
18.15ab
622.71ab
439.62a
143.64
26.28
13.42b
522.08b
408.61b
173.25
79.66
A
18.36 ns
641.98 ns
447.61 ns
150.19 ns
27.31 ns
AB
16.66
580.24
411.86
169.96
77.84
B
15.60
554.38
395.32
121.42
18.89
0 (CONTROL)
1
2
0 (CONTROL)
½ ET
ET
Stink bug.m-1
Defoliation
E
TRIAL 2
0 grain
1 grain
2 grains
3 grains
4 grains
10.59a
379.66 ns
322.24 ns
145.15b
25.66 b
9.21ab
376.92
359.80
178.78ab
37.73b
4.92b
298.25
378.25
247.92a
79.83a
9.09 ns
343.93 ns
358.73 ns
194.65 ns
51.94 ns
6.73
359.73
365.99
207.65
55.77
8.90
351.17
335.57
169.55
35.52
0 (CONTROL)
1
2
0 (CONTROL)
½ ET
ET
Stink bug.m-1
Defoliation
F
TRIAL 3
0 grain
1 grain
2 grains
3 grains
4 grains
11.50a
430.51 ns
357.61 ns
133.28b
28.05 ns
9.11ab
376.46
358.02
178.62b
50.20
6.75b
351.58
408.58
232.75a
70.17
8.05 ns
391.25 ns
371.09 ns
179.80 ns
41.36 ns
9.80
375.42
369.18
174.18
51.24
9.52
391.89
383.95
190.68
55.82
0 (CONTROL)
1
2
0 (CONTROL)
½ ET
ET
Stink bug.m-1
Defoliation
2020/2021 Crop Season
G
TRIAL 1
0 grain
1 grain
2 grains
3 grains
4 grains
12.50 ns
691.00 ns
437.89 ns
104.80 ns
33.73 ns
12.02
583.94
392.04
95.08
23.46
13.10
516.36
360.33
87.45
35.12
13.91
560.52
391.02
78.75
29.93
17.10
666.67
401.17
86.67
44.34
CHECK
½ DEFOL ET + 1 SB
½ DEFOL ET + 2 SB
DEFOL ET + 1 SB
DEFOL ET + 2 SB
H
TRIAL 2
0 grain
1 grain
2 grains
3 grains
4 grains
13.49 ns
591.77 ns
342.21b
69.82c
24.67 b
10.55
510.57
400.05ab
145.63b
45.15b
5.87
405.74
488.80a
246.51a
91.15a
6.50
381.49
325.66b
141.76b
55.84b
10.18
399.52
349.09b
181.99ab
111.39a
CHECK
½ DEFOL ET + 1 SB
½ DEFOL ET + 2 SB
DEFOL ET + 1 SB
DEFOL ET + 2 SB
I
TRIAL 3
0 grain
1 grain
2 grains
3 grains
4 grains
7.11 ns
423.25 ns
284.25b
73.98 b
37.05 ns
8.67
318.67
356.44ab
189.11a
83.44
9.00
431.67
419.67a
170.33a
79.67
7.50
392.42
405.64a
171.19a
72.14
5.25
357.38
377.00ab
200.88a
70.63
CHECK
½ DEFOL ET + 1 SB
½ DEFOL ET + 2 SB
DEFOL ET + 1 SB
DEFOL ET + 2 SB

4 CHAPTER 4. RE-EVALUATION OF THE ECONOMIC THRESHOLD FOR *Crocidosema aporema* (WALSINGHAM, 1914) (LEPIDOPTERA: TORTRICIDAE) INJURY TO INDETERMINATE SOYBEANS

Abstract

The recent outbreaks associated with field-evolve resistance of the soybean bud borer, *Crocidosema aporema* (Walsingham, 1914) (Lepidoptera: Tortricidae) to Bt soybeans, resulted in increased concerns of the potential yield impacts of this pest above soybean production in Brazil. Because of larval leaf-rolling and boring, it is difficult to manage with insecticides. Genetically modified *Bt* soybeans has been effective until recently when resistance has emerged and high infestation rates have been observed in Brazilian Bt soybean fields expressing Cry1Ac. The economic threshold (ET) for *C. aporema* was previously studied and established to be 25-30% of plants infested; however, the ET has not been previously tested for Bt soybeans or on indeterminate cultivars. The present work reexamined the potential of C. aporema to injure soybeans. The first experiment compared response of individual, injured plants to uninjured plants in the same field. The two sample T-test showed no effect of injury on the number of pod.plant^{-1} or number of grains.plant^{-1}. In the second experiment, when areas treated with insecticide spray were compared to areas without prayed pesticide, there was a reduction in *C. aporema* in the treated areas but no significative difference in yield was observed. We conclude that *C. aporema* has low potential to cause economic injury to soybean plants. Thus, the ET for *C. aporema* of 25-30% of attacked plants can be increased to 50%, except when plants are in the flowering stage, in which an ET of 30% can still be adopted.

Keywords: *Epinotia aporema*, Economic Threshold, *Glycine max* L., Economic Injury Level, Indeterminate Cultivar.

4.1 INTRODUCTION

The soybean bud borer, *Crocidosema* (=*Epinotia*) *aporema* (Walsingham, 1914) (Lepidoptera: Tortricidae) is an oligophagous pest that occurs from the southeastern United States to Argentina (BIEZANKO, 1961; HOFFMANN-CAMPO *et al.*, 2012; PEREYRA; SANCHEZ, 1998). The species is also listed as a pest in South Korea (PEXD, 2019). Its hosts are apparently restricted to leguminous plants (CORREA-FERREIRA, 1980), including soybean (*Glycine max* L. Merril). Despite not being the pest's optimal host, soybean supports at least two generations of *C. aporema* each crop cycle (CAPINERA *et al.*, 2008). Larval feeding can cause direct damage to the plants, reducing crop yield and seed quality (CORRÊA; SMITH, 1976; FOERSTER; IEDE; SANTOS, 1983).

Not only can *C. aporema* injure soybeans during the plant's vegetative stage, but also during its reproductive stage. Newly hatched larvae feed on the soybean's leaflets and buds, forming leaf-rolls around the apical and lateral buds (IEDE; FOERSTER, 1982; LILJESTHRÖM; ROJAS; PEREYRA, 2001), which also makes chemical control very difficult. As larvae grow, they may also cause secondary injury to the soybean's buds, stems, and axils. The tunnels created by feeding can reach 5 cm (1.97 in) in length within the stem, obstructing sap movement and overall plant development (, Ortiz 1998). During the soybean's vegetative development stage, this type of injury can trigger the number of secondary branches to increase, and reduce overall plant size (FOERSTER; IEDE; SANTOS, 1983). During the soybean's reproductive development stage, larvae can feed on soybean pods, directly damaging the seeds (CORREA-FERREIRA, 1980), and causing flower bud and pod drops (Bentancourt and Scatoni 2006, Ortiz 1998).

Despite being considered key soybean pests in Argentina (BARRIONUEVO *et al.*, 2012; PEREYRA; SANCHEZ, 1998; SANCHEZ; PEREYRA; GENTILE, 1997), *C. aporema* is one of the least studied pests injuring soybean (HOFFMANN-CAMPO *et al.*, 2012; IEDE; FOERSTER, 1985). Cultivation of MON 87701 × MON 89788 soybean technology expressing Cry1Ac *Bt* protein (from *Bacillus thuringiensis*) offers protection against larval injury caused by this pest (MACRAE *et al.*, 2005). In Brazil the MON 87701 × MON 89788 soybean was grown on more than 30 million hectares during the 2020/21 crop season (SPARK, 2021). Surprisingly, during recent monitoring (2020/2021 crop season), unexpected injuries caused by *C. aporema* populations were observed. This is the first evidence of field-evolved resistance to

Cry1Ac and a decrease in pest susceptibility to the insecticide protein (HORIKOSHI *et al.*, 2021). In response, growers overused conventional insecticides over the soybean fields across two crop seasons, negating one of the most important benefits of adopting MON 87701 × MON 89788 soybean technology: chemical insecticide reduction (BUENO; SILVA, 2021).

It is essential to assess the need for additional insecticide treatments in *Bt* soybean production as part of adoption of overall Integrated Pest Management (IPM) strategies. IPM relies on the assumption that cropped plants tolerate certain levels of injury without significant yield losses (HIGLEY; PEDIGO, 1996). Therefore, insecticide sprays are only appropriate in soybean fields when a pest population meets or exceeds the Economic Threshold (ET) (BUENO *et al.*, 2021). The established ET for *C. aporema* ranges from 25 to 30% for attacked plants (Panizzi, 2013). However, this current ET was established in the 1970s and since then it has not been reassessed. Therefore, it is of theoretical and practical interest to evaluate the potential of *C. aporema* injuring current soybeans varieties to determine if conventional treatments are required.

4.2 MATERIAL AND METHODS

To determine *C. aporema's* potential damage to soybeans, two different experiments were carried out repeatedly in commercial fields during the 2020/2021 and 2021/2022 crop seasons in Brazil. The first experiment was repeated 4 and 9 times during the 2020/2021 and 2021/2022 crop seasons, respectively. Trials were conducted in commercial soybean fields that were planted with *Bt* soybean, which expresses the Cry1Ac protein (TABLE 1). In this experiment, injured soybean plants and uninjured soybean plants growing within 1m of each other in the same field, under the same management were compared. The second experiment was carried out in one field during the 2020/2021 crop season and repeated in two different fields in the following crop season (2021/2022; TABLE 1). In these experiments, two areas were identified in each. One of the areas received insecticide to manage *C. aporema*. The other area served as the experimental control, with no insecticide applied for the management of *C. aporema*. The infestation rate of *C. aporema* (%) and yield (kg.ha^{-1}) were compared between the insecticide treated plots and untreated plots.

4.2.1 Experiment 1: Soybean yield components of injured plants versus uninjured plants from the same field

In the first experiment, yield components (number of pods.plant^{-1}, number of grain.plant^{-1} and mass of grains.plant^{-1}) were compared between injured and uninjured plants. During the soybean reproductive stage R_7 (FEHR *et al.*, 1971), three plants with injury and three plants with no injury were manually collected in a random location in the surrounding area (considered a replicate). Each injured plant was sampled at a maximum of a 1-meter radius from the uninjured plant of the same replicate. A total of ten replicates were taken from each commercial field (total of 30 injured and 30 uninjured plants per field) were assessed and a mean for each parameter was calculated for each replicate.

Plants were considered injured by visual diagnosis, when boring injury to the stems or pods was observed. We conducted a careful visual inspection to ensure that no insect injury was present and we only collected uninjured plants that were of a similar size to the injured plant.

4.2.2 Experiment 2: Infestation rate and soybean yield of insecticide treated versus untreated fields

The second experiment was conducted in commercial fields in Tibagi-PR (during the 2020/21 crop season) and repeated twice in Mangueirinha-PR (during the 2021/22 crop season). In each experiment, the area was divided between equal-sized plots of 800 m^2 with one plot receiving insecticide sprays to manage *C. aporema* and the other was not sprayed against the pest. Each area was then divided into 4 pseudo-replicates of 200 m^2, totaling 4 replicates per treatment. At Tibagi-PR, plots were treated three times over the reproductive stage, and at Mangueirinha-PR, plots were treated once at reproductive stage R_5 (TABLE 2). Additionally, to avoid yield losses caused by weeds or disease, herbicides and fungicides were applied when necessary and according to the growers' adopted management [one to two herbicide applications between the third and sixth week after emergence of plants, and two to three fungicide applications at the reproductive phase, starting between R1 and R2 (Fehr *et al.*, 1971), and followed by additional applications at 20 to 30-day intervals].

At the R_8 reproductive stage (Fehr *et al.*, 1971), two 2-m-long rows of each plot were randomly chosen, and the % infestation rate was determined based on the number of injured plants divided by the number of total plants. Afterwards, the rows were separately harvested and threshed for evaluation. The weight and moisture content of each sample was recorded (moisture meter G800, Gehaka Agri, São Paulo, SP, Brazil) and were then used to obtain the yield for 13% seed moisture.

4.2.3 Data analyses

Data were submitted to a Shapiro-Wilk normality test and a Levene's test for homogeneity of variance in order to verify the assumptions prior to analysis by two sample (unpaired) T-test, assuming equal variance. Significance levels were set at 5%, and all tests were performed using RStudio software (version 1.3.1056).

4.3 RESULTS

Yield component results varied among different fields and seasons. Thus, the number of pods.plant^{-1}, the number of grain.plant^{-1}, and the mass of grains.plant^{-1} were compared between injured plants and uninjured plants from the same field (experiment 1). Numbers of pods.plant^{-1} varied from a mean of 37.90 ± 1.02 (Unaí 2; injured plants) to 95.48 ± 8.75 (Itararé; uninjured plants) while the number of grains.plant^{-1} ranged from 95.80 ± 4.83 (Tibagi 2 – Check; uninjured plants) to 225.66 ± 19.64 (Itararé; uninjured plants). Similarly, grain mass.plant^{-1} (g) varied from 11.29 ± 0.56 (Tibagi 2 – Check; uninjured plants) to 31.42 ± 2.58 (Itararé; uninjured plants). Despite this variation among samples, the two sample T-test showed no overall effect of injury on the yield component parameters when the evaluated parameters were compared between injured plants and uninjured plants from the same field. Further, no difference was found in either the numbers of pod.plant^{-1} or number of grains.plant^{-1} in all 13 commercial field (FIGURES 1A and 1B). A significant difference in grain mass.plant^{-1} for uninjured plants compared to injured plants was recorded only in the Cristalina trial (t18 = 2.10; 0.049, FIGURE 1C), and was the only trial which showed an effect among the 13 different tested locations.

In the second experiment, areas with insecticide spraying were compared to areas without spraying. When one insecticide spray was applied in “Mangueirinha 1” and “Mangueirinha 2”, there was no significant difference in yield (kg.ha^{-1}) or infestation rate. There was an observed reduction in infestation rate for the treated field (t10 = 2.65; 0.02) in “Tibagi”, which received three insecticide sprays. However, despite the reduction of *C. aporema* infestation rate from 56.56% in the control to 33.07% in the area sprayed three times, there was no difference in the observed yield (TABLE 3).

4.4 DISCUSSION

Crocidosema aporema is characterized as a major soybean pest in Argentina (IANNONE; PARISSI, 1979; KOCH; WATERHOUSE, 2000) and Brazil (CORRÊA; SMITH, 1976). However, the results of both crop seasons in our study clearly indicate that *C. aporema* has low potential to injure soybean plants despite field infestations exceeding current ET. High soybean plant tolerance to insect injury is well documented in literature (BUENO *et al.*, 2013; PETERSON; VARELLA; HIGLEY, 2017), and newer soybean cultivars, with indeterminate growth habit, have shown the ability to withstand higher levels of injury with no yield loss (BATISTELA *et al.*, 2012; HAYASHIDA *et al.*, 2021). Another change in soybean is the use of varieties containing the Cry1Ac toxin.

The event MON 87701 x MON 89788, expressing Cry1Ac toxin, was first approved in Brazil in 2010, and since it became available in 2013, it has been quickly adopted by growers. Initially, varieties expressing Cry1Ac toxin provided excellent control of primary Lepidopteran soybean pests, including *C. aporema* (BENGYELLA *et al.*, 2018; MACRAE *et al.*, 2005; MURÚA *et al.*, 2018). However, recently, *C. aporema* infestations in *Bt* soybean fields have increased and resistance has been documented (BUENO; SILVA, 2021; HORIKOSHI *et al.*, 2021). However, despite more than 56% of plants being infested by *C. aporema* in our trials, no significant effect on yield was observed, clearly indicating that the excessive use of insecticide applied by soybean growers to control this pest (BUENO; SILVA, 2021) is unnecessary. Similarly, previous studies report that high levels of *C. aporema* incidence during either the vegetative or after the pod-set plant development stages do not reduce soybean yield (FOERSTER; IEDE; SANTOS, 1983). For these plant stages, yield was only reduced at infestation rates of 70% (FOERSTER; IEDE; SANTOS, 1983; IANNONE; PARISI, 1978). The highest level of *C. aporema* infestation recorded in this study was 56.4% with no insecticide use, which may explain the lack of yield reduction in our findings.

Soybean-IPM is based on the assumption that soybean plants can tolerate certain levels of injury without triggering economic yield loss (BUENO *et al.*, 2021; HIGLEY; PEDIGO, 1996). Developing plant tolerance traits as a pest management tool also plays an important role in the management of pest resistance (PETERSON; VARELLA; HIGLEY, 2017). Therefore, determining the level of soybean plant tolerance to different types of injuries is a crucial step to refine ETs for different pest species and injury guilds (JUSTUS *et al.*, 2022). The development of new cultivars that differ in phenology also requires testing to refine ETs, followed by re-testing when pest populations change, as is the case with soybean and *C. aporema*.

Caution should be exercised in interpreting our results because the phenological stage of host plant that is attacked by a pest is highly important to determine the impact of pest injury on final yield (SANCHEZ; PEREYRA; GENTILE, 1997). Despite the shorter period of flowering time compared to both vegetative and pod-set stages in soybeans, yields are more drastically reduced when *C. aporema* injury occurs during flowering, indicating that the flowering stage is most vulnerable. The high tolerance of soybean to *C. aporema* injury observed in our study may be influenced because all *C. aporema* injury in our trials occurred after the pod-set (R3 stage). Nevertheless, it is still important to highlight the use of insecticides should be adopted with caution, mainly when insect outbreak does not occur during flowering as evaluated in our studies.

As far as we know, this is the first study of the impact of *C. aporema* injuries on modern indeterminate soybean *Bt* cultivars. Previous studies documenting *C. aporema* injury potential on soybean plants, investigated the impacts of *C. aporema* on cultivars that have determinate growth and do not contain *Bt* toxins, which are rarely still planted (FOERSTER; IEDE; SANTOS, 1983; LOURENÇÃO; MIRANDA, 1983; SIQUEIRA; SIQUEIRA, 2012). Indeterminate growth habit soybean cultivars are potentially even more tolerant to *C. aporema* injury than older cultivars of determinate growth habit because when injury occurs, plants can more easily develop new buds and branches, replacing injured parts of the plant with new growth. In turn, this helps to reduce the impact of pests on the evaluated parameters. It was previously reported that after a *C. aporema* larval attack, determinate soybean plants grew new secondary branches, compensating injury and avoiding yield loss (FOERSTER; IEDE; SANTOS, 1983).

In summary, this study demonstrated that the currently recommended ET for *C. aporema* of 25 to 30% for attacked plants (Panizzi, 2013) is extremely conservative and could be increased to at least 50%, except when plants are in the flowering stage, in which an ET of 30% (Panizzi, 2013) should still be adopted. Adopting a higher ET also helps to maintain the longevity of conventional insecticides as well as preserve one of the most important benefits of *Bt* soybean adoption which is the reduction of insecticide use. The rapid spread of resistance in populations of *C. aporema* represents a for serious concern to soybean production.

The adoption of structured refuge areas (using varieties that do not express *Bt* toxin) is critical for Insect Resistance Management programs. In Brazilian soybean production it is recommended to plant at least 20% of fields with a non-*Bt* variety, and that the *Bt* plants should not be further than 800 m from the closest non-*Bt* variety (BERNARDI *et al.*, 2016). However, this recommendation is often not followed and the commercial fields used in this study were grown without refuges nearby. Evidence of increasing resistance of lepidopteran pests to *Bt* technology in soybean (BENGYELLA *et al.*, 2018; MURÚA *et al.*, 2018) emphasizes the needs for both IPM and updated ETs.

Acknowledgements

The authors thank “Embrapa Soja” (Brazil), Bayer Crop Science Brazil and Oklahoma State University (USA) for support of this research. Thanks are also extended to the sponsor agencies “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” (CAPES-PRINT; grant no. 88887.374211/2019-00), “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq; grant no 142340/2018-9), and Hatch Project accession no. 1019561 from the USDA National Institute of Food and Agriculture. that supported this research. We also thank Gustavo O. Corazza and Mateus Pretto for field support.

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FIGURE 1. Yield components of plants under *Crocidosema aporema* injury (black) compared to plants with no injury (dotted) of experiment 1 at different fields in crop season 2020/2021 and 2021/2022. A = Number of pods.plant^{-1}; B = Number of grains.plant^{-1}; and C = grain mass.plant $^{-1}$. ^{ns} = deference non-significant. * = treatments differed by two sample (unpaired) T-test (α = 0.05).

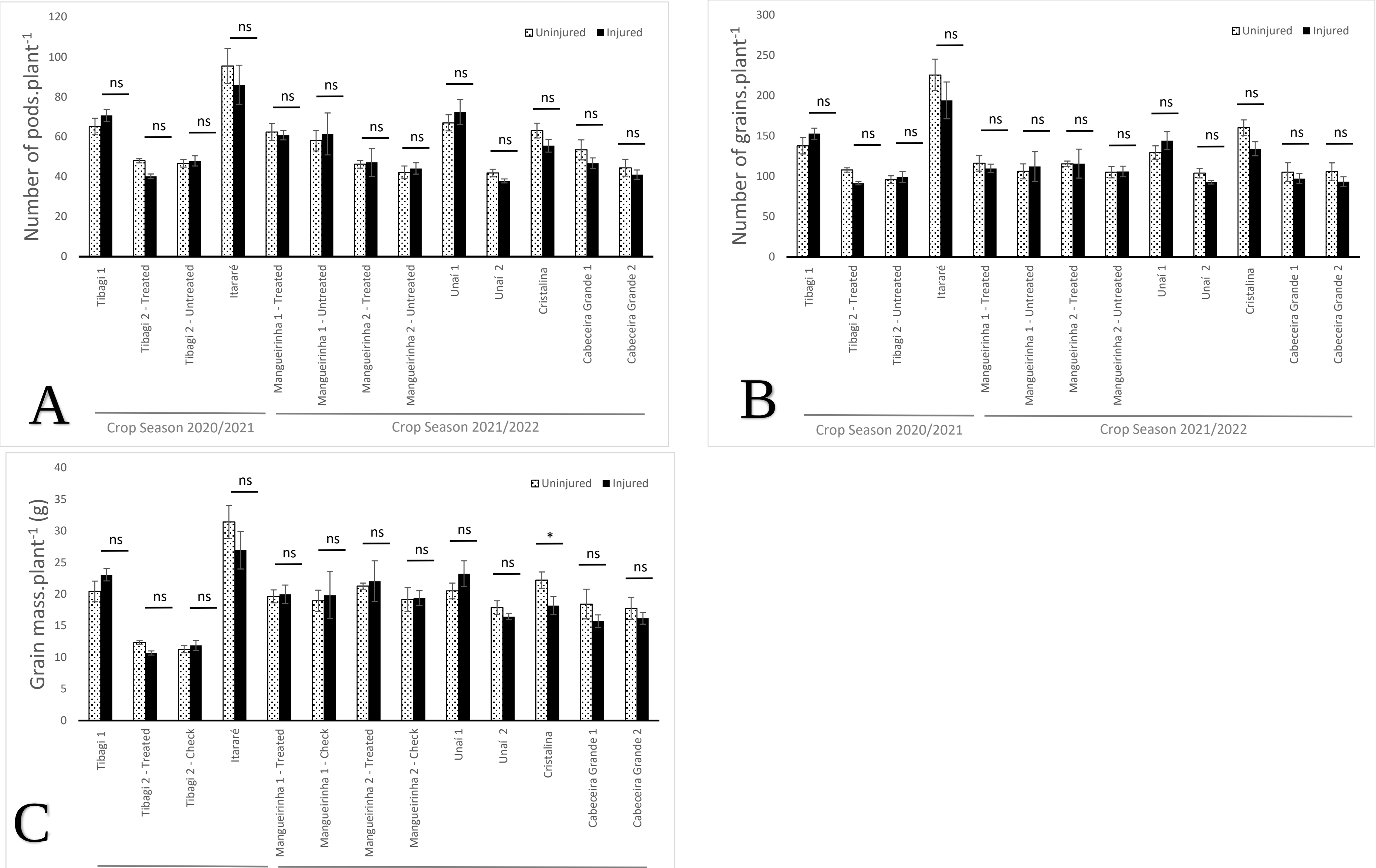

TABLE 1. Field information of experiment 1 trials on 2020/2021 and 2021/2022 crop seasons.

Experiment	Area indentification	County	Cultivar	Geographic coordinates	Crop Season
(1) Soybean yield components of injured plants versus uninjured plants from the same field	Itararé	Itarare, SP	BS 2606 IPRO	24°01'49.6"S 49°21'23.7"W	2020/21
	Tibagi 1	Tibagi, PR	AS 3590 IPRO	24° 40' 38.0"S 50°21'25.0"W	2020/21
	Tibagi 2 – Treated[1]	Tibagi, PR	M6410 IPRO	24° 26' 24.50"S 50°16'9.2"W	2020/21
	Tibagi 2 – Untreated[2]	Tibagi, PR	M6410 IPRO	24° 26' 24.5"S 50°16' 9.2"W	2020/21
	Mangueirinha 1 – Treated[3]	Mangueirinha, PR	DM 66I68 IPRO	26°07'55.4"S 52°07'28.7"W	2021/22
	Mangueirinha 1 – Untreated[2]	Mangueirinha, PR	DM 66I68 IPRO	26°07'55.4"S 52°07'28.7"W	2021/22
	Mangueirinha 2 – Treated[3]	Mangueirinha, PR	BMX Zeus IPRO	26°07'54.4"S 52°07'22.2"W	2021/22
	Mangueirinha 2 – Untreated[2]	Mangueirinha, PR	BMX Zeus IPRO	26°07'54.4"S 52°07'22.2"W	2021/22
	Unaí 1	Unaí, MG	Brasmax Bônus IPRO	16°31'56.4"S 47°18'01.0"W	2021/22
	Unaí 2	Unaí, MG	Brasmax Tormenta SE	16°25'07.2"S 47°21'36.7"W	2021/22
	Cristalina	Cristalina, GO	CZ 37B43 IPRO	16°22'50.5"S 47°24'56.2"W	2021/22
	Cabeceira Grande 1	Cabeceiras, GO	NS 7667 IPRO	15°55'40.9"S 47°05'26.9"W	2021/22
	Cabeceira Grande 2	Cabeceiras, GO	AS3730 IPRO	15°55'46.8"S 47°05'45.7"W	2021/22
(2) Infestation rate and soybean yield of insecticide sprayed fields versus unsprayed fields	Tibagi	Tibagi, PR	M6410 IPRO	24° 26' 24.50"S 50°16'9.2"W	2020/21
	Mangueirinha 1	Mangueirinha, PR	DM 66I68 IPRO	26°07'55.4"S 52°07'28.7"W	2021/22
	Mangueirinha 2	Mangueirinha, PR	BMX Zeus IPRO	26°07'54.4"S 52°07'22.2"W	2021/22

[1] Plots treated with 3 insecticides spraying (commercial name [dose; spray volume]): Voraz® (250 ml.ha-1; 150 L.ha-1), Upmyl®(2 L.ha-1; 150 L.ha-1), and Sperto® (300 ml.ha-1; 150 L.ha-1).

[2] Plots with no insecticide spraying for *Crocidosema aporema* control.

[3] Plots treated with 1 insecticides spraying (commercial name [dose; spray volume]): PREMIO® (50 ml.ha-1; 150 L.ha-1)

TABLE 2. Area name, county, soybean cultivar and geographic coordinates, insecticides (commercial names, dose and spray volume) used in trials of Experiment 2.

Area name	**County**	**Cultivar**	**Geographic coordinates**	**Insecticide (a.i. grams ha^{-1})**
Tibagi	Tibagi, PR	M6410 IPRO	24° 26' 24.480'' S 50° 16' 9.230'' W	Methomyl (110 g.ha^{-1}) + Novaluron (8.75 g.ha^{-1}) + N-Methylpyrrolidone (67.9 g.ha^{-1})+ Dimethyl (72.2 g.ha^{-1}) Methomyl (430 g.ha^{-1}) Acetamiprid (75 g.ha^{-1})+ Biphenthrine (75g.ha^{-1})
Mangueirinha 1	Mangueirinha, PR	DM 66I68 IPRO	26°07'55.4"S 52°07'28.7"W	Chlorantraniliprole (10g. ha^{-1})
Mangueirinha 2	Mangueirinha, PR	BMX Zeus IPRO	26°07'54.4"S 52°07'22.2"W	Chlorantraniliprole (10g. ha^{-1})

TABLE 3. Yield (kg.ha^{-1}) ± SE and infestation rate (%) ± SE of insecticide treated areas and check (non-treated) of experiment 2 at three commercial fields in 2020/2021 and 2021/2022 crop seasons.

Treatments	**Tibagi 2020/21 crop season**			**Mangueirinha 1 2021/22 crop season**			**Mangueirinha 2 2021/22 crop season**		
	Yield (kg.ha^{-1})	Infestation rate (%)	Number of sprays	Yield (kg.ha^{-1})	Infestation rate (%)	Number of sprays	Yield (kg.ha^{-1})	Infestation rate (%)	Number of sprays
Sprayed	3878.8 ± 78.7	33.1 ±7.7	3	1986.5 ± 35.2	14.0 ± 3.1	1	2109.8 ± 43.2	30.8 ± 2.9	1
Non sprayed	3815.8 ± 68.2	56.5 ± 4.6	0	1966.7 ± 62.2	14.4 ± 2.6	0	2119.4 ± 86.3	27.7 ± 4.7	0
DF_{res}	10.00	10.00	-	14.00	14.00	-	14.00	14.00	-
t-statistic	-0.60	2.65	-	0.28	-0.10	-	0.10	-0.56	-
P_{value}	0.56	**0.02**	-	0.79	0.92	-	0.92	0.58	-

5 CHAPTER 5. FINAL CONSIDERATIONS

Soybean-IPM is based on a vast amount of scientific data that shows soybean plants can withstand some considerable amount of injury without any major impact on yield and therefore, with no economic loss. This tolerance can vary among cultivars, developmental stage of plants, timing of exposure to injury, and type of injury. Thus, the ET must be frequently studied and updated for current, modern farm conditions.

Currently, there is an increasing adoption of newer cultivars with a reduced leaf area index and, consequently, a growing concern about their defoliation tolerance. Although our findings suggest that previous defoliation has an effect on the plant's ability to cope with injury, lowering ETs would almost certainly increase pesticide use and may not provide a better control, only increasing management costs. Furthermore, high pesticide use can lead to pest resurgence, cause secondary pest outbreaks, and increase pest resistance to the pesticides.

In addition, most of the tests used to establish ET on soybean have focused on species of the same injury guild or on a single injury. However, in field conditions, soybean plants are usually under attack by multiple pest guilds and their possible interaction must be evaluated. This study shows that there is no interaction between injuries caused by defoliation and stink bug feeding for yield and its components, oil and protein content, or seed quality. Thus, the current recommended ETs for stink bugs and defoliation are still safe and must be independently adopted by soybean producers.

On the other hand, this study points out that *Crocidosema aporema* has low potential damage on soybean and the current recommended ET (PANIZZI, 2013) is extremely rigorous and must be updated. It could be increased to at least 50% of the attacked Bt-plants with no yield loss. The refinement of ET indicates the real need to use insecticides under the current conditions. Increasing ET could help maintain the longevity of Bt technology by delaying the evolution of resistance once insecticide sprayings are less frequent in refuge areas, and it may provide more insects from these areas to mate with individuals from Bt areas, among other benefits.

In conclusion, determining the level of soybean plant tolerance to different types of injuries is a crucial step in developing ETs for different pest species and injury guilds, and updating the previous established ET to the current farm condition. Soybean's high level of tolerance must be considered for effective pest management, which combines a lower environmental impact and potentially lower crop management costs with lower insecticide use.

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