LARAH MARTINS FREITAS

ESTRATÉGIAS DE MANEJO DE *Spodoptera frugiperda* E *Chrysodeixis includens* EM SOJA: RESISTÊNCIA DE CULTIVARES BT E NÃO BT E INDUÇÃO DE RESISTÊNCIA COM JASMONATO DE METILA E EXTRATO DE NIM

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Tese de doutorado apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-graduação em Entomologia, para a obtenção do título de Doutor.

Prof. Dr. Bruno Henrique Sardinha de Souza
Orientador

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MANAGEMENT STRATEGIES FOR *Spodoptera frugiperda* AND *Chrysodeixis includens* IN SOYBEAN: RESISTANCE OF BT AND NON-BT CULTIVARS AND INDUCED RESISTANCE WITH METHYL JASMONATE AND NEEM EXTRACT

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Prof. Dr. Geraldo Andrade de Carvalho	UFLA
Profa. Dr. Maria Fernanda Gomes Villalba Peñaflor	UFLA
Profa. Dr. Joyce Dória Rodrigues	UFLA
Prof. Dr. Eduardo Neves Costa	FCA/UNESP

Prof. Dr. Bruno Henrique Sardinha de Souza
Orientador

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2024

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RESUMO

A soja *Glycine max* (L.) Merrill, é uma cultura de grande importância econômica para o Brasil, que se destaca como um dos principais *players* no mercado internacional. Das espécies de pragas da cultura, merecem destaque as lagartas *Chrysodeixis includens* e *Spodoptera frugiperda* (Lepidoptra: Noctuidae). Há pouco conhecimento quanto aos níveis de resistência em cultivares Bt e não Bt de soja adaptadas a regiões de expansão da cultura, como no estado de Minas Gerais, a *C. includens* e *S. frugiperda*. Além disso, existe a necessidade de encontrar outras estratégias de controle para inserir no manejo integrado dessas pragas em soja, como o uso de elicitores e bioestimulantes. A tese foi dividida em duas partes, sendo a primeira composta por uma introdução geral e referencial teórico, que abrangem as temáticas envolvidas na construção desse trabalho. A segunda parte é composta por dois artigos. No primeiro artigo, foi avaliada a resistência de cultivares Bt e não Bt de soja adaptadas às condições de Minas Gerais no desempenho biológico de *C. includens* e *S. frugiperda*. Os resultados mostraram que a diferença de idade e a espécie de lagarta influenciaram na eficiência de controle das cultivares com proteína Bt e não-Bt, como também observou-se efeitos antinutricionais nos insetos. Além disso, em função do nível de resistência da cultivar Bt NS 6010 IPRO e não Bt UFLA 6301 RR, que se destacaram no trabalho, estas podem ser recomendadas para plantio como culturas principais e áreas de refúgio, respectivamente. Essas informações são importantes para a definição de estratégias regionais de manejo integrado e de manejo de resistência de *C. includens* e *S. frugiperda* em regiões em expansão de cultivo de soja no Brasil. O segundo artigo avaliou os efeitos induzidos de defesa de plantas de soja pela aplicação de formulado à base de nim isolado e associado ao derivado de fitormônio jasmonato de metila (MeJA) a *S. frugiperda*. Como resultados, verificou-se sinergismo entre o extrato de nim e MeJA, demonstrando indução de resistência na soja, de modo que houve um efeito antinutritivo em *S. frugiperda*, que não conseguiu converter o alimento ingerido em ganho de peso nesse tratamento. A herbivoria influenciou nas concentrações de peróxido de hidrogênio, enquanto a atividade enzimática da catalase não teve alteração significativa. Obsevou-se efeitos negativos dos elicitores na redução de parâmetros de crescimento das plantas em parte aérea e raiz. Esses conhecimentos adquiridos quanto ao desempenho das cultivares transgênicas de soja para a região de Minas Gerais no controle de lagartas de *C. includens* e *S. frugiperda* e dos efeitos induzidos de defesa do bioestimulante à base de nim com o elicitor MeJA a *S. frugiperda* são importantes para o manejo integrado de pragas da soja, podendo contribuir para a redução de perdas de produção pelo ataque dessas espécies de lagartas.

Palavras-chave: transgênicos., bioestimulante., resistência de plantas., manejo integrado de pragas.

ABSTRACT

Soybean, Glycine max (L.) Merrill, is a crop of great economic importance to Brazil, which stands out as one of the main players in the international market. The crop pest species, the caterpillars *Chrysodeixis includens* and *Spodoptera frugiperda* (Lepidoptera: Noctuidae) deserve to be highlighted. There is very little knowledge about the levels of resistance in Bt and non-Bt soybean cultivars adapted to regions where this crop is expanding, such as in the state of Minas Gerais, to *C. includens* and *S. frugiperda*. Furthermore, there is a need to find other control strategies to include in the integrated management of these pests in soybeans, such as the use of elicitors and biostimulants. The thesis is divided into two parts, the first of which consists of a general introduction and theoretical reference, which comprehensively covers the themes involved in the construction of this work. The second part consists of two articles. The first of these investigated the efficiency of transgenic Bt and non-Bt soybean cultivars against *C. includens* and *S. frugiperda* adapted to the Minas Gerais region. The results showed that the age difference and the species of caterpillar influenced the control efficiency of cultivars with Bt protein, as well as antinutritional effects on insects in food conversion. Furthermore, the level of resistance and susceptibility of Bt and non-Bt cultivars highlighted in the work can be recommended for planting as main crops and refuge areas, respectively. These findings are important for defining regional strategies for integrated management and resistance to *C. includens* and *S. frugiperda* in regions where soybean cultivation is expanding in Brazil. The second article investigated the effects on soybean plant defenses induced by the application of formulations based on neem alone and in combination with methyl jasmonate (MeJA) to *S. frugiperda*. This study showed synergism between Neem and MeJA, demonstrating an induction of resistance in soybeans, so that there was an antinutritive effect *S. frugiperda* was unable to convert food into weight gain. Herbivory influenced oxidative enzyme concentrations only in hydrogen peroxide, this did not occur in catalase. Negative effects of the elicitors were observed on the reduction of plant growth parameters in shoots and roots. The knowledge gained about the performance of transgenic soybean cultivars for the Minas Gerais region in controlling caterpillars and the discovery of the effects and use of biostimulants associated with elicitors on caterpillars are of paramount importance for integrated pest management and plant resistance and the success of soybean crop production.

Keywords: transgenics., biostimulant., plant resistance., integrated pest management.

INDICADORES DE IMPACTO

O estudo gerou impactos tecnológicos e econômicos significativos para o manejo integrado de pragas na cultura da soja, com foco na resistência de cultivares bt e não bt ao ataque das lagartas *Chrysodeixis includens* e *Spodoptera frugiperda*, em regiões de expansão agrícola de Minas Gerais. A pesquisa revelou o desempenho diferencial das cultivares, destacando a cultivar Bt NS 6010 IPRO e a não Bt UFLA 6301 RR, que podem ser recomendadas como culturas principais e áreas de refúgio, respectivamente. Além disso, foram testadas novas estratégias de controle, como o uso de bioestimulantes à base de nim associado ao fitormônio jasmonato de metila (MeJA), que demonstraram sinergismo no aumento da resistência da planta, reduzindo a capacidade das lagartas de converter alimento em peso. O caráter extensionista da pesquisa se deu pela transferência de conhecimentos práticos sobre o manejo integrado de pragas, impactando diretamente os produtores rurais de Minas Gerais, que enfrentam crescentes desafios no cultivo de soja. Foram beneficiados diretamente grupos de agricultores, bem como pesquisadores e estudantes envolvidos na pesquisa. O trabalho se alinha à temática de "Meio Ambiente" e "Tecnologia e produção" da Política Nacional de Extensão e contribui para os Objetivos de Desenvolvimento Sustentável da ONU, especialmente o ODS 2 (Fome Zero e Agricultura Sustentável) e o ODS 12 (Consumo e Produção Responsáveis), ao propor soluções que equilibram produtividade agrícola e controle sustentável de pragas.

IMPACT INDICATORS

The study generated significant technological and economic impacts for integrated pest management in soybean crops, focusing on the resistance of Bt and non-Bt cultivars to attacks by the larvae *Chrysodeixis includens* and *Spodoptera frugiperda* in expanding agricultural regions of Minas Gerais. The research revealed the differential performance of the cultivars, highlighting the Bt NS 6010 IPRO and non-Bt UFLA 6301 RR cultivars, which can be recommended as main crops and refuge areas, respectively. In addition, new control strategies were tested, such as the use of neem-based biostimulants combined with the phytohormone methyl jasmonate (MeJA), which showed synergism in increasing plant resistance by reducing the larvae's ability to convert food into weight. The extensionist nature of the research was evidenced through the transfer of practical knowledge on integrated pest management, directly impacting rural prFoducers in Minas Gerais, who face growing challenges in soybean cultivation. Directly benefiting were groups of farmers, as well as researchers and students involved in the study. The work aligns with the "Environment" and "Technology and Production" themes of the National Extension Policy and contributes to the UN Sustainable Development Goals, especially SDG 2 (Zero Hunger and Sustainable Agriculture) and SDG 12 (Responsible Consumption and Production), by proposing solutions that balance agricultural productivity with sustainable pest control.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO GERAL

A cultura da soja, *Glycine max* (L.) Merrill, é uma das mais importantes economicamente no mundo, sendo utilizada na produção de óleo, subprodutos como a proteína, ração para alimentação animal e biodiesel (Kidokoro *et al.* 2015). Os Estados Unidos, Brasil e Argentina são os três maiores produtores, representando mais de 82% da produção mundial de soja (Usda/Fas 2020). No Brasil, a estimativa aponta uma produção de 147,68 milhões de toneladas, destancando-se como produção recorde (Conab, 2024). No entanto, o complexo de lagartas consiste em um dos principais fatores na redução da produção, com destaque para *Chrysodeixis includens* (Walker) e *Spodoptera frugiperda* (J.E Smith) (Lepidoptera: Noctuidae).

A lagarta falsa-medideira *C. includens* é uma importante praga, responsável por danos econômicos em culturas agrícolas desde a América do Sul à América do Norte (Alford; Hammond, 1982). No Brasil e em outros países da América do Sul, esta espécie é considerada umas das principais lagartas-praga da soja, algodão e feijão (Bortolotto *et al*., 2015; Panizzi, 2013; Sorgatto *et al*., 2015). Pelo fato de *C. includens* conseguir completar seu desenvolvimento biológico em diferentes plantas cultivadas (Specht *et al*., 2015), possuir alta capacidade de reprodução com diversas gerações ao ano (Moonga; Davis, 2016; Zulin *et al*., 2018) e ter comportamento de dispersão entre várias regiões e culturas (Palma *et al*., 2015), essas características favorecem sua alta ocorrência e infestação nos sistemas agrícolas.

O manejo de *C. includens* na cultura da soja no Brasil tem sido feito principalmente com o uso de plantas transgênicas (Bernardi *et al*., 2012; Marques *et al*., 2016; Muraro *et al*., 2018; Ramos *et al*., 2017; Schilick-Souza *et al*., 2018; Wille *et al*., 2017). Apesar de haver informações a respeito dos efeitos da proteína Cry1Ac e suas concentrações letais para *C. includens*, são escassos os resultados sobre a eficiência de controle da proteína expressa em diferentes cultivares de plantas transgênicas de soja com os eventos MON87701 x MON89788. Sabe-se que a expressão e concentrações das proteínas transgênicas podem ser diferentes em cada cultivar transgênica, influenciando no controle das pragas-alvo.

Além disso, nenhuma informação é disponível sobre os níveis de resistência a lagartas por cultivares Bt e não Bt de soja adaptadas a regiões do Brasil em crescente expansão na produção da oleaginosa, como em Minas Gerais. A área de plantio na safra 2023/2024 neste estado é de 10,6% superior à anterior, atingindo mais de 1,8 milhões de hectares. O uso de cultivares não Bt em áreas de refúgio estruturado com maiores níveis de resistência pode contribuir com a redução populacional de *C. includens* e *S. frugiperda*, redução de perdas e menor gasto com inseticidas nessas áreas. Portanto, essas informações serão de grande importância para os sojicultores de Minas Gerais para a elaboração de estratégias de manejo de resistência e manejo integrado de pragas.

A lagarta-militar ou lagarta-do-cartucho *S. frugiperda* é uma praga-chave conhecida na cultura do milho nas Américas. As espécies de *Spodoptera* são consideradas pragas secundárias da soja nos países da América do Sul (Blanco *et al*., 2016; Conte *et al*., 2019). No entanto, infestações esporádicas dessas espécies podem causar perdas significativas na produtividade da soja devido à sua capacidade de desfolhamento (bueno *et al*., 2011). Devido ao hábito alimentar polífago, no Brasil além da cultura do milho, *S. frugiperda* também pode infestar as culturas de soja, algodão, arroz, trigo e sorgo (Abro *et al*., 2021; Bueno *et al*., 2011; Overton *et al*., 2021; Prassana *et al*., 2021). Os principais métodos de controle de *S. frugiperda* é através de aplicação de inseticidas. Entretanto, essa espécie apresenta alta capacidade adaptativa para a resistência a várias classes de compostos, incluindo às proteínas Cry de *Bacillus thuringiensis* (Bt), expressas por culturas transgênicas Bt (Carvalho *et al*., 2013; Gutiérrez-Moreno *et al*., 2019; Storer *et al*., 2010; Zhu *et al*., 2015). As infestações de *S. frugiperda* têm se tornado cada vez mais frequentes nas plantações de soja e algodão (Overton *et al*., 2021; Prasanna *et al*., 2021; Ramos *et al*., 2022).

Nesses casos, as populações resistentes proliferam-se rapidamente, exigindo medidas emergenciais. Cultivares transências de soja Bt foram lançadas para uso comercial no Brasil em 2010, tornando-se rapidamente uma ferramenta para o manejo de lepidópteros. Cultivares de soja transgênica que expressam a proteína Cry1Ac (evento MON87701 × MON 89788) conferem resistência a certas espécies de lepidópteros (Pomari-Fernandes *et al*., 2015), incluindo *Anticarsia gemmatalis, C. includens* e *Chloridea virescens* (F.) (Lepidoptera: Noctuidae). Uma pesquisa prévia demonstrou que *A. gemmatalis* e *C. includens* ainda são suscetíveis à proteína Cry1Ac expressa na soja (Bernardi, 2012).

Já foi demonstrado que a proteína Cry1Ac expressa em eventos transgênicos de soja possuem baixa especificidade para *S. frugiperda* (Bernardi *et al*., 2014). Além disso, existe resistência cruzada entre proteínas Bt da mesma classe (Bernardi *et al*., 2015; Carrière *et al*., 2015; Santos-Amaya *et al*., 2015; Siqueira *et al*., 2004; Van Den Berg *et al*., 2021b), principalmente entre proteínas Cry1. Assim, a seleção de populações resistentes em culturas de milho e algodão pode contribuir para reduzir a eficácia da soja Bt que expressa Cry1Ac para *S. frugiperda* (Boaventura *et al*., 2020; Machado *et al*., 2020).

Diante desses problemas, deve-se buscar novas estratégias para o manejo de *S. frugiperda* na cultura da soja. Uma alternativa pode ser com a utilização de elicitores de resistência e bioestimulantes. Elicitores de resistência, sejam naturais ou sintéticos, desempenham um papel no aumento dos níveis de defesa das plantas contra estresses, atuando como sinalizadores de condições adversas (Chantini *et al*., 2021; Rani; Murali-Baskaran, 2024). Fitormônios como o ácido jasmônico (JA), ácido salicílico (SA) e etileno estão entre os principais reguladores das respostas das plantas a insetos herbívoros e patógenos (Pieterse *et al*., 2014), como também seu derivado jasmonato de metila (MeJA) (Chen *et al*., 2018; Franceschi *et al*. 2002). Por outro lado, os bioestimulantes, compostos químicos ou microbiológicos, podem promover o crescimento das plantas e aumentar sua resistência a estresses, como evidenciado pelos estudos de Colla & Rouphael (2015) e Du Jardin (2015). Esses produtos, que incluem substâncias húmicas e fúlvicas, bactérias promotoras de crescimento, fungos endofíticos e micorrízicos, aminoácidos, hidrolisados proteicos, vitaminas, extratos de algas e plantas, e micronutrientes, são aplicados para melhorar a adaptação das plantas a condições ambientais adversas (Povero *et al*., 2016).

O nim, Azadiracta indica A. Juss (Meliaceae), é uma planta inseticida amplamente reconhecida, com formulações disponíveis globalmente à base de extratos orgânicos, óleos brutos ou limonoides purificados de suas sementes (Carvalho *et al*., 2015). A azadiractina, principal metabólito secundário, é considerada crucial, encontrando-se em mais de 50 compostos terpenoides presentes nas folhas, sementes e frutos do nim (Forim, 2006; Liu *et al*., 2007; Schneider *et al*., 2017). Esses extratos vegetais não apenas repelem pragas indesejáveis, mas também podem ter efeitos negativos (Carvalho *et al*., 2015). Há evidência de que os óleos essenciais podem desempenhar um papel importante como ativadores de respostas aos estresses bióticos e abióticos das plantas (Bertrand *et al*., 2021), porém, há pouca informação com essa classe de compostos.

Os bioestimulantes causam aumento da tolerância a condições ambientais por melhorar a eficiência nutricional das plantas, dos mecanismos de defesa, das atividades fisiológicas, do uso da água, síntese de metabólitos primários e secundários, e crescimento da parte aérea e sistema radicular, promovendo maior produtividade e produtos agrícolas com maior qualidade (Du Jardin *et al*., 2015; Silva *et al*., 2016). Sendo assim, há possibilidade de uso para respostas induzidas de defesa das plantas proporcionadas pela aplicação de elicitores de resistência e bioestimulantes na alimentação e desempenho de insetos pragas da soja.

Faz-se necessário obter mais informações que auxiliem a recomendação de cultivares de soja com produtividade e resistência para região de Minas Gerais a importantes insetos-praga como *C. includens* e *S. frugiperda*. Também há necessidade de encontrar outras ferramentas de controle que possam ser integradas no manejo do complexo de lagartas da cultura da soja. Para elucidar essas questões, este trabalho de tese de doutorado foi dividido em dois artigos: o primeiro teve o objetivo de obter informações quanto à eficiência de cultivares de soja Bt e não Bt adaptadas às regiões produtoras de Minas Gerais no desempenho das lagartas desfolhadoras *C. includens* e *S. frugiperda*; e o segundo capítulo teve o objetivo de avaliar os efeitos induzidos de defesa e crescimento de plantas de soja pela aplicação de formulações à base de nim isolado ou associado ao fitormônio jasmonato de metila ao ataque de *S. frugiperda*.

2 REFERENCIAL TEÓRICO

2.1 Importância econômica da cultura da soja

A soja, *Glycine max* (L.) Merrill, é uma das oleaginosas de grande importância mundial, com aplicações diretas para alimentação humana, produção de petróleo e ração animal. A produção brasileira na safra agrícola 2023/2024 é estimada em 146,52 milhões de toneladas, representando uma redução de 5,2% em comparação à safra anterior (Conab, 2024). Apesar do aumento na produção, há necessidade de cultivares com maior adaptabilidade às regiões produtoras do Brasil para suprir a alta demanda tanto no mercado interno, quanto internacional (Wang *et al*., 2017).

No Brasil, com a implementação de programas de melhoramento de soja no passado, tornou-se possível o cultivo da cultura em baixas latitudes através do desenvolvimento de cultivares mais adaptadas às novas regiões produtoras e pela incorporação de genes que atrasam o florescimento da cultura, mesmo em condições de fotoperíodo indutivo. Dessa forma, tornou-se possível desenvolver plantas de soja com característica de longo período juvenil, expandindo a cultura em nível significativo nos estados de Santa Catarina, Paraná, São Paulo, Mato Grosso do Sul, Minas Gerais e Goiás (Knorr, 2017).

A área semeada para a soja na safra 2023/2024 teve um ajuste a partir da identificação de novas áreas de cultivo no Maranhão, Goiás, Pará, Mato Grosso, Rio Grande do Sul e Minas Gerais. Com a atualização realizada, área total cultivada soja na safra 2023/24 é de 45,7 milhões de hectares, 3,8% superior ao semeado na safra passada (Conab, 2024).

2.2 Falsa-medideira, *Chrysodeixis includens*

A lagarta falsa-medideira *C. includens* é considerada praga de plantas importantes economicamente como a soja, algodão, tabaco, feijão, girassol e diversas hortaliças (Herzog, 1980). Sabe-se que *C. includens* tem como plantas hospedeiras 174 espécies pertencentes a 39 famílias. No Brasil, há registro de 26 destas plantas hospedeiras que garantem a sobrevivência de *C.*

includens ao longo de todo o ano, mesmo na ausência de extensos cultivos agrícolas (Specht *et al*., 2015)

A partir da safra 2003/2004, a lagarta falsa-medideira deixou de ser considerada praga secundária e atingiu o status de praga primária no Brasil (carvalho, 2015). Essa mudança no status de praga de *C. includens* se deu principalmente a: sua ocorrência em todos os estados produtores do país; alta polifagia, com 175 espécies de plantas hospedeiras; evolução de resistência a inseticidas; e ineficiência do controle químico pelo hábito das lagartas se abrigarem nos terços médio e inferior das plantas, dificultando a penetração dos inseticidas no dossel (Baldin *et al*., 2014; Gómez *et al*., 2014; Hoffmann-Campo *et al*., 2012; Specht *et al*., 2015). Em pouco tempo, a ocorrência de ambas espécies de lagartas desfolhadoras começou a ser relatada nos principais estados produtores de soja, de forma isolada ou associada (Bueno *et al*., 2007) e os ataques relatados mais frequentemente nas regiões centro-oeste, sudeste e sul do Brasil (Botelho *et al*., 2019; Moscardi *et al*., 2012).

Os métodos de manejo de *C. includens* mais utilizados na cultura da soja consistem no cultivo de plantas transgênicas que expressam proteínas Bt (Bernardi *et al*., 2012; Marques *et al*., 2016), aplicação de inseticidas químicos (Perini *et al*., 2019; Ramos *et al*., 2017), e mais recentemente o uso de bioinseticidas à base de baculovírus (Godoy *et al*., 2019; Murano *et al*., 2018). Atualmente, há eventos de soja transgênica liberada comercialmente contendo a tecnologia Intacta RR2 PRO® (MON 87701 (Cry1Ac) x MON 89788 (CP4 EPSPS), proporcionando controle contra as principais lagartas desfolhadoras, *A. gemmatalis, C. includens, Chloridea virescens* Fabricius (Lepidoptera: Noctuidae) e *Crocidosema aporema* Walsingham (Lepidoptera: Tortricidae), além de supressão das lagartas de *Elasmopalpus lignosellus* Zeller (Lepidoptera: Pyralidae), *H. zea e H. armigera* (Bernardi *et al*., 2012). A soja Intacta RR2 PRO® é a segunda geração de soja transgênica, sendo a primeira com característica de resistência a insetos, e foi desenvolvida especialmente para o mercado brasileiro (carvalho, 2015; cib, 2019).

Torna-se necessário avaliar o desempenho biológico de *C. includens* nas cultivares Bt e não Bt adaptadas às regiões produtoras de Minas Gerais, informações ainda muito escassas. Devido à expansão da cultura da soja a diversos estados do país, com destaque para o estado de Minas Gerais, ainda não se têm informações suficientes quanto ao desempenho dessas cultivares ao ataque dos insetos-praga. Assim, faz-se necessário realizar experimentos para recomendações de estratégias de manejo integrado e manejo de resistência das pragas-alvo.

2.3 Lagarta-militar, *Spodoptera frugiperda*

A lagarta-do-cartucho ou lagarta-militar *S. frugiperda* é um inseto-praga economicamente importante para uma diversidade de espécies de plantas cultivadas mundialmente. Este inseto é nativo das Américas, principalmente da América Latina, das ilhas do Caribe e do extremo sul dos EUA, com migrações anuais para o norte dos EUA e para o Canadá (Biondi *et al*., 2018; Kasoma *et al*., 2021). A lagarta-do-cartucho possui várias características que a tornam uma das pragas de maior importância econômica do século XXI, incluindo alta taxa de reprodução, ausência de diapausa e a capacidade de se adaptar rapidamente a novos ambientes, incluindo a evolução de resistência inseticidas (Barros; Bueno, 2010; Nagoshi *et al*. 2015). Há 192 casos relatados de resistência a 43 ingredientes ativos diferentes (Mota-Sanchez *et al*. 2022).

Esse inseto é considerado praga de extrema importância por ser polífago, podendo se alimentar e desenvolver em mais de 353 espécies em 76 famílias de plantas (Abro *et al*. 2021; MonteZano *et al*., 2018). Entre as principais plantas hospedeiras de *S. frugiperda* incluem as culturas da soja, algodão, milho, arroz e sorgo (Montezano *et al*., 2018). As lagartas de *S. frugiperda* são pragas por serem capazes de se alimentar tanto nos estádios vegetativo, quanto reprodutivo das plantas. A espécie possui alta capacidade de adaptação a condições adversas e alto potencial reprodutivo em comparação com outras espécies de lepidópteros. Além disso, os adultos podem migrar mais de mil quilômetros, especialmente em voos noturnos (Kenis *et al*., 2022; Yan *et al*., 2022).

O controle de *S. frugiperda* se baseia principalmente no uso intensivo de inseticidas, o que inevitavelmente resultou na evolução da resistência a vários grupos de inseticidas (Bolzan *et al*., 2019; Carvalho *et al*., 2013; Diez-Rodríguez; Omoto, 2001; Garlet *et al*., 2021; Lira *et al*., 2020; Muraro *et al*., 2021; Nascimento *et al*., 2022; Nascimento *et al*., 2016). A importância do manejo de *S. frugiperda* nas lavouras de soja tem aumentado, pois ela pode ser tão ou mais danosa que *A. gemmatalis*, uma das principais lagartas pragas da soja brasileira (Bueno *et al*. 2011); *S. frugiperda* tem baixa suscetibilidade à proteína Cry1Ac, presente na tecnologia transgênica Bt disponível para o controle de lagartas na soja (Bernardi *et al*. 2014; Santos *et al*. 2009) e tem altas taxas de sobrevivência em culturas hospedeiras alternativas (Santos *et al*. 2009). Além disso, *S. frugiperda* foi indicada como uma das pragas mais difíceis de manejar entre as 31 pragas visadas pelas culturas Bt na América Latina (Blanco *et al*. 2016). Tudo isso somado ao sistema de cultivo duplo (safra de inverno e safra de verão) amplamente adotado em diversas regiões do Brasil causa um aumento no risco de surtos populacionais da praga (Barros *et al*. 2010a, 2012b; Santos *et al*. 2009).

Devido ao intenso uso de inseticidas e plantas transgênicas como principais métodos de controle, e pela rápida evolução de resistência a inseticidas sintéticos e plantas transgênicas que expressam proteínas Bt (Anderson *et al*., 2018; Jones *et al*., 2019; Yang; Li; Wu, 2013), torna-se necessário encontrar outras ferramentas de controle que possam ser integradas no manejo de *S. frugiperda*.

2.4 Soja com resistência a insetos

Aproximadamente 97% da soja cultivada no país é transgênica (Isaaa, 2020). Além disso, do total de 2411 cultivares de soja registradas no Ministério da Agricultura, Pecuária e Abastecimento, 75% são referentes a cultivares geneticamente modificadas (Registro Nacional de Cultivares – RNC, 2019). As cultivares transgênicas de soja mais utilizadas são cultivares que possuem as tecnologias RR® e IPRO®.

As cultivares com a tecnologia IPRO® apresentam os eventos MON87701 x MON89788, e são advindas de cruzamentos por melhoramento clássico de parentais de soja geneticamente modificados para resistência a insetos pela expressão do gene *cry1Ac* da bactéria *B. thuringiensis*. Além disso, apresentam tolerância ao herbicida glifosato através do gene *cp4 epsps* (*Agrobacterium tumefaciens*) (Ferre; Van Rie, 2002; Miklos *et al*., 2007; Rnc, 2020). As cultivares com os eventos MON87701 x MON89788 conferem às plantas a expressão da proteína Cry1Ac, com o intuito de reduzir o uso de inseticidas no controle de lepidópteros pragas em regiões tropicais e subtropicais, sobretudo contra o ataque da lagarta-da-soja *Anticarsia gemmatalis* (Hübner, 1818) (Lepidoptera: Erebidae), e da lagarta-falsa-medideira *Chrysodeixis includens* (Walker, 1858) (Lepidoptera: Noctuidae), como alvos primários; e à broca-das-axilas *Crocidosema aporema*, e lagarta-medideira *Rachiplusia nu*, como alvos secundários (Ferre; Van Rie, 2002; Miklos *et al*., 2007).

Entre os diversos insetos que causam injúrias às plantas de soja, as lagartas desfolhadoras da ordem Lepidoptera constituem um dos complexos de pragas mais importantes da cultura (Ávila; Souza, 2015). Portanto, devido às tecnologias transgênicas serem bem definidas quanto à resistência às pragas-alvo, os níveis de expressão dessas proteínas e suas eficiências podem ser diferentes em diversas localidades, em diferentes genótipos de soja em que o gene *Bt* foi inserido, como também devido às diferenças na suscetibilidade de populações de *C. includens* em função da contínua exposição e pressão de seleção das plantas transgênicas Bt aos insetos. Atualmente, faltam informações sobre a eficiência de controle por plantas transgênicas de soja às principais lagartas pragas em regiões com recente aumento de áreas cultivadas com a cultura, principalmente em relação às cultivares adaptadas a essas regiões, como no estado de Minas

Gerais.

Existem relatos de variedades convencionais de soja, tanto linhagens em fase de melhoramento quanto cultivares comerciais, com níveis moderados de resistência a *S. frugiperda*, e as concentrações de flavonoides e nutrientes podem ter função na expressão da resistência (Bueno *et al*., 2011; Boiça Júnior *et al*., 2015a, 2015b, 2017; Dixon e Steele, 1999; Hoffman-Campo, 1995; Hoffman-Campo *et al*. 2001; Souza *et al*., 2021). Sabendo que cultivares convencionais não Bt são utilizadas principalmente em áreas de refúgio estruturado com soja transgênica Bt (horikoshi *et al*. 2021), torna-se importante conhecer e avaliar os níveis de resistência de cultivares não Bt de soja às lagartas *C. includens* e *S. frugiperda* como objetivo de serem utilizadas com tal propósito. O cultivo dessas cultivares não Bt com maiores níveis de resistência em áreas de refúgio estruturado pode contribuir com a redução populacional de *C. includens* e *S. frugiperda*, redução de perdas e menor gasto com inseticidas nessas áreas. Além disso, esses materiais podem ser inseridos em programas de melhoramento genético visando à resistência a insetos-pragas.

2.5 Elicitores de resistência e bioestimulantes

Os elicitores de resistência são moléculas de origem natural ou sintética que possuem ação de estimular o sistema imune das plantas (Bektas; Eulgem, 2015; Eder; Cosio, 1994; Shinya *et al*., 2016). Alguns exemplos de moléculas elicitores de defesa são a quitina, flagelina e outras moléculas microbianas, compostos da saliva ou fluido de oviposição de insetos. Fitormônios como o ácido salicílico (AS), atua na resistência sistêmica adquirida contra patógenos biotróficos e insetos sugadores de seiva, enquanto o ácido jasmônico (JA) e seu derivado jasmonato de metila (MeJA) medeiam a resistência sistêmica induzida, mais específica contra patógenos necrotróficos e insetos mastigadores (Boutrot; Zipfel, 2017; Gust *et al*., 2017; Stael *et al*., 2015). Portanto, a resistência induzida é regulada por essas moléculas de sinalização como o ácido jasmônico e jasmonato de metila, os quais desempeham um papel crucial na defesa das plantas contra os insetos (Kant *et al*., 2015).

Por outro lado, os bioestimulantes possuem um efeito de promoção do crescimento em situações adversas de estresse, podendo ser ou não acompanhado por um efeito de aumento de resistência (Colla; Rouphael, 2015; Du Jardim, 2015). Os bioestimulantes podem ser considerados uma alternativa ecológica para o aumento do rendimento de diversas culturas na agricultura, principalmente sob cultivo em condições adversas de estresse. Esses produtos podem influenciar no crescimento e desenvolvimento das culturas em diferentes ambientes de cultivo (Ávila *et al*. 2017; Calzada *et al*.; 2016; Hermes *et al*., 2015; Izidório *et al*., 2015; Oliveira *et* *al*., 2016; 2017; Sousa *et al*. 2017; Vendruscolo *et al*., 2016).

O termo “bioestimulante” se refere a compostos químicos ou microbiológicos que quando aplicado nas plantas causam aumentos na eficiência nutricional, tolerância a estresses abióticos e na produtividade e qualidade de produtos agrícolas, independentemente do teor de nutrientes na formulação (Du Jardin, 2015). Os bioestimulantes compõem misturas à base de hormônios, substâncias húmicas e fúlvicas, bactérias promotoras de crescimento de plantas, fungos micorrízicos e endofíticos, aminoácidos e hidrolisados proteicos, vitaminas, extratos de algas marinhas e plantas, e micronutrientes que têm sido usados nas lavouras com intuito de proteger as plantas através da melhoria das respostas adaptativas aos estresses ambientais (Povero *et al*., 2016).

Os bioestimulantes são aplicados em baixas quantidades no solo ou na planta, minimizam os efeitos negativos do estresse, promovendo melhorias no crescimento, desenvolvimento e produtividade das culturas principalmente quando submetidas a algum estresse ambiental (Bulgari *et al*., 2017). Esses produtos promovem aumento da tolerância a condições ambientais, pois melhoram a eficiência nutricional das plantas, aumentam a eficiência dos mecanismos de defesa, das atividades fisiológicas, do uso da água, síntese de metabólitos primários e secundários, e crescimento da parte aérea e sistema radicular, promovendo maior produtividade e produtos agrícolas com maior qualidade (Du Jardin *et al*., 2015; Silva *et al*., 2016).

Os estresses causados por fatores abióticos e bióticos limitam a fixação de CO_2, resultando no excesso de energia nos fotossistemas e podendo causar danos permanentes ao sistema fotossintético, e para isso são necessários mecanismos eficientes que dissipem o excesso de energia. Esse excesso de energia faz com que as cadeias transportadoras de elétrons, tanto da fotossíntese quanto da respiração, gerem o acúmulo de espécies reativas de oxigênio (ERO), modificando a homeostase celular e causando um ambiente oxidativo nas células (Dahal *et al*., 2015; Pyngrope *et al*., 2013; Zhang e al., 2016). O acúmulo das ERO, como o peróxido de hidrogênio (H_2O_2), o radical ânion superóxido ($O2^{\bullet}$), radical hidroxila (HO●) e o oxigênio singleto ($^1O_2$), causa danos oxidativos nas estruturas celulares como proteínas, moléculas de DNA, RNA, lipídeos, membranas, pigmentos fotossintéticos e ativação da morte celular programada (Choudhury *et al*., 2016; Pandy *et al*., 2016).

Como proteção contra os danos oxidativos, as plantas possuem mecanismos de defesa antioxidantes enzimáticos ou não enzimáticos, que podem ou não atuar em conjunto, e que realizam a eliminação das ERO e a manutenção da homeostase celular (choudhury *et al*., 2016; pandey *et al*., 2016). Essa manutenção da capacidade antioxidante para eliminar as ERO tem sido associada ao aumento da tolerância das plantas a uma ampla gama de estresses ambientais

(Caverzan *et al*., 2016; Mishra *et al*., 2012). Assim, a aplicação de bioestimulantes aumenta a capacidade das plantas de eliminar espécies reativas de oxigênio (ERO), sugerindo aumento na tolerância das plantas a fatores de estresse (Shukla *et al*., 2017).

Os bioestimulantes atuam na melhoria do desenvolvimento, crescimento e produtividade de plantas cultivadas em condições adversas de estresse, principalmente abiótico. (Bulgari *et al*., 2017). Por exemplo, na tentativa de mitigar os danos causados pelo estresse hídrico, o uso de bioestimulantes é uma alternativa, pois podem contribuir para a melhoria das propriedades físico-químicas do solo, absorção, translocação e aproveitamento de nutrientes pelas plantas, bem como aumento da resistência ao estresse (calvo; nelson; kloepper, 2014; du jardin, 2015). No entanto, ainda há pouca informação sobre os efeitos dos bioestimulantes de modo geral ao ataque de insetos-praga.

O nim, *Azadiracta indica* A. Juss (Meliaceae), é a planta inseticida mais conhecida e utilizada, e algumas formulações à base de extratos orgânicos (enriquecidos ou não), de óleos brutos ou de limonoides purificados de suas sementes encontram-se disponíveis no mercado de diferentes países (Carvalho *et al*., 2015). *Azadicacta indica* apresenta grande número de metabólitos secundários com atividade biológica, sendo a azadiractina considerada a de maior importância (Forim, 2006). As plantas de nim possuem mais de 50 compostos terpenoides, sendo os principais a azadiractina, salanina, meliantrol e nimbolina, que podem estar presentes nas folhas, sementes e, principalmente frutos (Schneider *et al*., 2017; Liu *et al*., 2007). Características dos extratos vegetais provenientes do nim, é que além de sua capacidade de repelir pragas indesejáveis na cultura, ainda podem apresentar efeitos deletérios.

Pouco se conhece sobre os efeitos fisiológicos, bioquímicos e morfológicos de bioestimulantes originados de extratos formulados à base de plantas de nim em culturas de importância econômica com relação a estresses causados pela herbivoria de insetos. Assim, devido à falta de conhecimentos quanto às respostas induzidas de defesa proporcionadas pela aplicação de formulações à base de nim sobre a alimentação e desempenho de insetos pragas, torna-se importante a condução de estudos que avaliem tais efeitos, bem como dos benefícios no desenvolvimento de plantas de importância agrícola como a soja frente à herbivoria causada por lagartas.

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SEGUNDA PARTE – ARTIGOS

Resistance of Bt and non-Bt soybean cultivars adapted to novel growing regions of Brazil to *Chrysodeixis includens* and *Spodoptera frugiperda*

Larah M. Freitas[1]; Bruno H. S. Souza[1*]; Fernanda S. Ferreira[1]; Ana P.A. Antunes[1]; Adriano T. Bruzi[2]

[1]Department of Entomology, Federal University of Lavras (UFLA), Lavras, MG, Brazil. *brunosouza@ufla.br. [2]Department of Agriculture, UFLA, Lavras, MG, Brazil.

Abstract

Soybean is a highly valuable commodity crop for Brazil's economy. However, it faces significant threats from the attack of a complex of lepidopteran pests, particularly *Chrysodeixis includens* (Walker) and *Spodoptera frugiperda* (J. E. Smith). These pests have been managed primarily using transgenic Bt soybeans, but limited knowledge exists about the resistance levels of Bt and non-Bt cultivars adapted to novel soybean-growing areas in Brazil, such as the Minas Gerais state. This study evaluated the resistance levels of Bt and non-Bt soybean cultivars to *C. includens* and *S. frugiperda*, and whether the Bt cultivars can differentially affect these pests across larval stages. No-choice bioassays were conducted using Bt (NS6010 IPRO and P97R50 IPRO) and non-Bt soybeans (UFLA 6301 RR, P96R90 RR, and ANsc 80111 RR) at V4-stage in the laboratory with neonate (24 h) and third-instar larvae. Larvae were fed leaf discs in Petri dishes, recording the mortality, leaf consumption, and weight gain after 7 days. There was high mortality of *C. includens* neonates on the Bt cultivars, but this trend was not observed for older larvae. For *S. frugiperda* neonates, there was high mortality on the Bt cultivar NS 6010 IPRO and non-Bt cultivar UFLA 6301 RR, but only the former was effective for older larvae. Although the Bt cultivars did not kill the third-instars, antinutritional effects were found, such that leaf tissue consumed was not converted to larval weight gain. These findings are important for defining regional strategies of integrated and resistance management of *C. includens* and *S. frugiperda* in expanding regions of soybean cultivation in Brazil.

Keywords: transgenic, soybean looper, fall armyworm, Cry1Ac, IPM

1. **Introduction**

Soybean, *Glycine max* (L.) Merrill, is one of the most economically important commodity in the world, being used to produce oil, proteins, animal feed, biodiesel, and other by-products (Kidokoro *et al.* 2015; Sun et al. 2018; Marques et al. 2022). A complex of caterpillars of the order Lepidoptera represents a major biotic threat for soybean yield losses, standing out *Chrysodeixis includens* (Walker) and *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) (Bortolotto et al. 2015; Bueno et al. 2021). According to Oliveira et al. (2014), 25 million tons of food, fiber, and biofuels are lost every year in Brazil due to the attack of arthropod pests, including 4.3 million tons of soybean grains that constitute the country's major export commodity.

The soybean looper *C. includens* is an insect pest of many crops of economic importance. *Chrysodeixis includens* can infest diverse host plants from the South to the North America (Blanco et al. 2016). In Brazil and other countries of South America, *C. includens* is considered one of the main insect pests of annual crops like soybeans, cotton, and common beans (Panizzi 2013, Bortolotto et al. 2015; Sorgatto et al. 2015). The fall armyworm *S. frugiperda* is a well-known key pest of maize in the Americas. Because of its polyphagous feeding habit, *S. frugiperda* uses important cultivated plants species as hosts, reaching pest status on several of them. In Brazil, besides maize *S. frugiperda* often infests soybean, cotton, rice, wheat, and sorghum crops (Overton et al. 2021). The fact that *C. includens* and *S. frugiperda* can complete the biological cycle on different plants (Specht et al. 2015), have high reproductive potential (Moonga and Davis 2016; Zulin et al. 2018), dispersion across different regions and crops (Palma et al. 2015), insecticide resistance, and behavior plasticity (Paredes-Sánchez et al. 2021) favor their widespread occurrence.

The management of *C. includens* in soybeans has been carried out mainly with transgenic Bt plants in Brazil (Bernardi et al. 2012; Marques et al. 2016; Ramos et al. 2017; Wille et al. 2017; Muraro et al. 2019; Schlick-Souza et al. 2018). Currently there is no reported field practical resistance in *C. includens* populations to Bt soybeans (Yano et al. 2015; Horikoshi et al. 2021). In the case of *S. frugiperda,* the Cry1Ac protein expressed in the transgenic Bt event MON87701 x MON89788 that is present in most planted soybean crops to date have low specificity to this insect species, not providing efficient control (Bernardi et al. 2014b). Furthermore, because there is cross-resistance between Bt proteins of the same class (Santos-Amaya et al. 2015; Bernardi et al. 2015), especially among the Cry1 proteins, selection of resistant populations in maize and cotton crops may contribute to lowering the efficacy of Bt soybeans expressing Cry1Ac to *S. frugiperda* (Boaventura et al. 2020; Machado et al. 2020). Considering the genetic and bioecological aspects of these pest insects, such as high biotic potential, polyphagy, and continuous generations year round, combined with the low adoption of non-Bt structured refuges, this scenario worsens considerably (Farias et al. 2014; Fatoretto et al. 2017). Therefore, the deployment of insect resistance management strategies is fundamental to extend the durability of Bt transgenic soybean technologies.

It is thought that the expression and concentrations of Bt proteins can vary in different cultivars bearing the same transgenic event because of the potential interaction of the Bt proteins with the genotypic background of each plant genotype (Eghrari et al. 2019). For example, Eghrari et al. (2021) found differences in the concentrations of Vip3Aa20 in homozygous maize hybrids that increased 1.53- to 5.22-fold relative to the hemizygous versions, although substantial increase in *S. frugiperda* control was not so evident due to the great larvae susceptibility to this Bt protein. In another study with maize, Eghrari et al. (2019) found lower injury and survival of *S. frugiperda* on homozygous relative to hemizygous hybrids with Cry1A.105, Cry1F, and Cry2Ab2; in addition, the proteins expression were ~1.5-, 2-, and 2.5-fold higher in the homozygous maize hybrids, respectively. Therefore, there is evidence that the genetic background of plant genotypes can influence the mortality of target pests, but to the best of our knowledge, this was not yet evaluated in Bt soybeans.

Little information is available on the resistance of Bt and non-Bt cultivars adapted to emerging soybean-producing regions in Brazil, such as the Minas Gerais state, to the primary pests *C. includens* and *S. frugiperda*. Despite not being a historical soybean producer, currently Minas Gerais stands out as the sixth national largest producer, contributing nearly 8 million tons of soybean grains in more than 2 million hectares (Conab 2024). In Brazil, soybean production in the 2023/2024 season is estimated at 147.6 million tons, a record production (Conab 2024). Therefore, knowledge on the performance of soybean cultivars against *C. includens* and *S. frugiperda* is important for the adoption of integrated and insect resistance management strategies to these pests at a regional scale.

This study evaluated the levels of resistance of Bt and non-Bt soybean cultivars to *C. includens* and *S. frugiperda*, and whether the Bt cultivars can differentially affect the performance of both insects species across larval stages.

2. Materials and Methods

2.1 Soybean cultivars

Soybean plants used in this study are transgenic cultivars recommended for Minas Gerais state. All the cultivars have RR® technology (event GTS 40-3-2) and two of them have IPRO® technology (event MON87701 x MON89788), which respectively expresses tolerance to the glyphosate herbicide and the Cry1Ac protein of Bt that are toxic to some lepidopteran species. The soybean cultivars used in experiments of this study were as follows: NS6010 IPRO and P97R50 IPRO (Bt); and UFLA 6301 RR, P96R90 RR, and ANsc 80111 RR (non-Bt). All soybean genotypes are commercial cultivars developed by private seed companies (Nidera, Pioneer, and Agro Norte), except UFLA 6301 RR that was the first cultivar developed by the breeding program of the Federal University of Lavras, which was initiated in 2011. Overall, little information is available on the resistance levels of regional soybean germplasm to insect pests.

To obtain leaf material for use in the laboratory experiments, the soybean cultivars were grown in 2.5-L pots containing soil, substrate (Carolina soil®, Sphagnum peat moss, expanded perlite, expanded vermiculite, and roasted rice hulls), and sand (2:1:1). Potted-plants were fertilized with NPK (04-14-08) (Fertipar Sudeste®) following the recommendations of the sixth approximation for soybean cultivation. Each soybean cultivar was tested with 15 replications, which consisted of a single potted plant. The plants were kept on benches in a greenhouse in a complete randomized design until the V4 growth stage, at which the third trifoliate was fully developed. At this point, the leaves were used in the bioassays.

2.2 Insect rearing

The population of *C. includens* used in the bioassays were obtained by purchasing eggs from the national marketplace (Pragas.com, Piracicaba, São Paulo, Brazil). In addition, individuals of *C. includens* were collected from common bean crops in the region of Lavras, Minas Gerais state, to supplement the colony and increase genetic variability. *Spodoptera frugiperda* was obtained from a colony established in the laboratory for five years. Wild egg masses from maize fields in Lavras were annually introduced to the colony. The insect colonies were maintained in a laboratory room under controlled conditions (25 ± 2 °C, 60 ± 10% RH, and 14L:10D h). Larvae of *C. includens* and *S. frugiperda* were reared on artificial diet according to Greene et al. (1976), and the adults were fed a 10% honey solution.

2.3 Larval performance of *C. includens* and *S. frugiperda* on soybean cultivars

To assess the effects of soybean cultivars on the larval performance of *C. includens* and *S. frugiperda*, separate bioassays with each insect species were carried out in Petri dishes (5 cm diameter) lined with moist filter paper. The bioassays were performed using a completely randomized design, for both neonates and third instars of *C. includens* and *S. frugiperda*, for a total of four experiments. All bioassays were conducted in a laboratory climatized room (25 ± 1 °C, 70 ± 10% RH, and 12L:12D h).

For the bioassays, the uppermost and fully expanded trifoliate was collected from V4-stage soybean plants in the greenhouse and brought to the laboratory. Fifteen plants of each cultivar were used as true replicates. Three 0.9-mm leaf discs were cut out from each leaflet of the trifoliate using a metallic puncher, making out three dishes per plant (replicate), totaling 45 plates for each cultivar. Each replicate was composed of three individual Petri dishes containing the leaf discs prepared from the same trifoliate. The dishes containing one leaf disc was infested with one larva of *C. includens* or *S. frugiperda*, closed with lid, and sealed with parafilm to avoid larvae escape.

The effects of soybean cultivars on the larval performance of *C. includens* and *S. frugiperda* at both larval stages were evaluated by recording the mortality, leaf consumption, and larvae weight after 7 days using a methodology adapted from Bernardi et al. (2014b). In the experiments with third-instar larvae, leaf discs were exchanged with fresh leaves from the same cultivar after 4 days because of higher leaf consumption by older larvae. Larval mortality was inspected visually and with a soft touch of larvae by a fine paintbrush. Leaf consumption was recorded on a dry-weight basis; for this, 15 aliquots of leaf discs were prepared with extra plants maintained in the same greenhouse conditions and collected from the same leaf position and stage of soybean. At the end of the experiment, all leaf discs were dried in an oven (EL402, Electrolab, Sao Paulo, Brazil) at 50^{o}C for 72h, and the weight of leaf remnants after larvae feeding was subtracted from the weight of intact leaf discs (aliquots), thus obtaining the leaf dry weight consumed. The weight of larvae was obtained by weighing them individually on an analytical scale (ATY 224R, Shimadzu, Sao Paulo, Brazil).

2.4 Statistical analysis

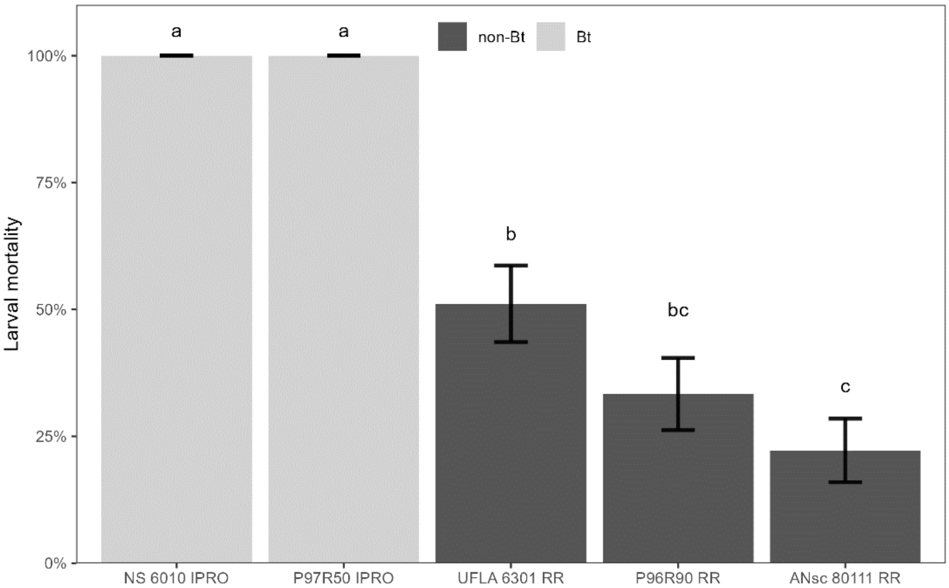
Data were analyzed in R software version 4.2.1 (R Core Team, 2020). For larval mortality, analysis of variance was performed using a binomial distribution and analyzed by GLM. The other biological data were subjected to the Bartlett's test to check the homogeneity of variances, and to the Shapiro-Wilk’s test to check the normality of residuals. Analysis of variance was applied to these data, and the means of treatments were compared by the Tukey’s test ($\alpha=0.05$) using the GLM procedure. Correlation of data of leaf dry weight consumed and fresh weight gain of larvae was performed using Pearson's linear correlation analysis (α=0.05) in Sigmaplot v. 12.5 (Systat Software Inc., San Jose, CA, USA).

3. Results

3.1 Effects of soybean cultivars on *C. includens* and *S. frugiperda* neonate larvae

In the bioassay with *C. includens* neonates, the soybean cultivars significantly differed ($F_{4,\ 220}$ = 46.12; $P<0.001$) for larval mortality. The Bt cultivars NS 6010 IPRO and P97R50 IPRO caused complete mortality of *C. includens* larvae, with greater levels of mortality than those provided by the non-Bt soybeans (Fig. 1). The surviving larvae in the non-Bt cultivars showed differences in the fresh body weight ($F_{2,\ 85}$ = 7.09; $P<0.001$), in that *C. includens* neonates fed on P96R90 RR showed greater weight than those fed ANsc 80111 RR. Greater leaf dry weight was consumed ($F_{2,\ 85}$ = 142.9; $P<0.001$) in P96R90 RR than in the other cultivars (Table 1).

Figure 1 Mortality (% ± SE) of *C. includens* neonate larvae fed on leaf discs of Bt and non-Bt soybean cultivars after seven days



Different letters on bars denote significant differences by Tukey's test ($P<0.05$)

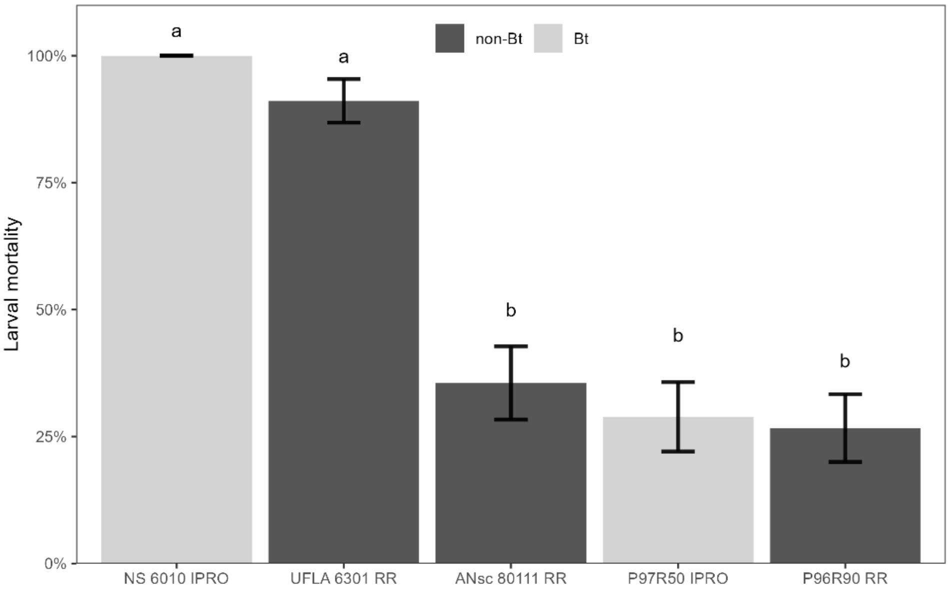
Table 1 Means (mg ± SE) of larvae fresh weight and leaf dry weight consumed of *C. includens* neonate larvae fed on leaf discs of Bt and non-Bt soybean cultivars

Cultivars	Larvae weight	Leaf weight consumed
NS 6010 IPRO	-	-
P97R50 IPRO	-	-
UFLA 6301 RR	4.09 ± 0.57 ab	17.60 ± 0.33 b
P96R90 RR	5.84 ± 0.49 a	24.20 ± 0.29 a
ANsc 80111 RR	3.31 ± 0.46 b	18.40 ± 0.33 b
P-value	0.001*	<0.001*
F	7.091	142.92

Means followed by the same letter in columns do not differ by Tukey test ($P<0.05$)

For the bioassay with *S. frugiperda* neonates, all larvae died when fed on the Bt cultivar NS 6010 IPRO and 91% mortality was observed on the non-Bt cultivar UFLA 6301 RR, which significantly differed ($F_{4,\ 220} = 40.07$; $P<0.001$) from the others (Fig. 2). The surviving larvae showed no differences in the fresh body weight ($F_{2,\ 91} = 0.89$; $P = 0.416$) and leaf dry weight consumed ($F_{2,\ 91} = 1.43$; $P = 0.244$) among cultivars (Table 2).

Figure 2 Mortality (% ± SE) of *S. frugiperda* neonate larvae fed on leaf discs of Bt and non-Bt soybean cultivars after seven days



Different letters on bars denote significant differences by Tukey’s test ($P<0.05$)

Table 2 Means (mg ± SE) of larvae fresh weight and leaf dry weight consumed of *S. frugiperda* neonate larvae fed on leaf discs of Bt and non-Bt soybean cultivars

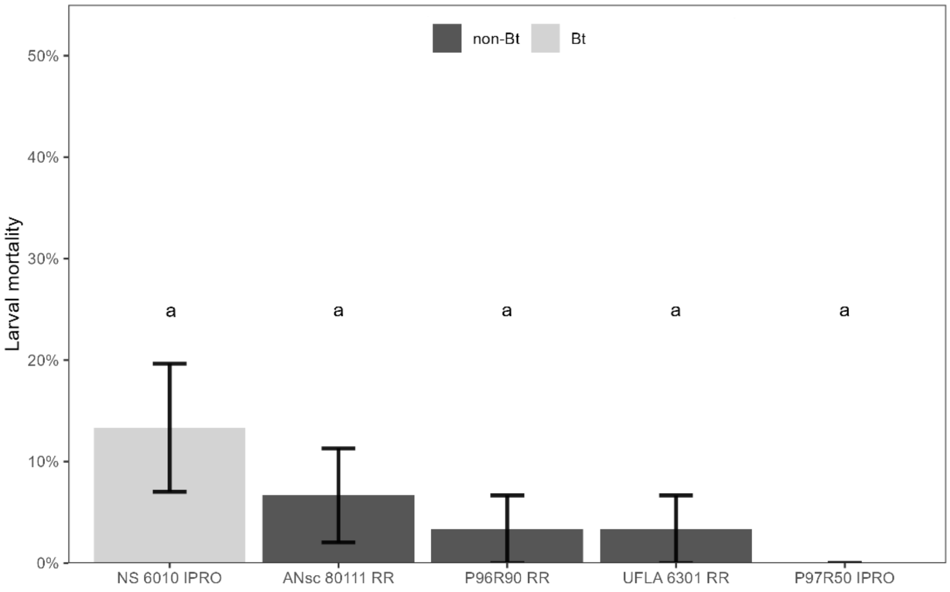
Cultivars	Larvae weight	Leaf weight consumed
NS 6010 IPRO	-	-
P97R50 IPRO	3.33 ± 0.41 a	11.40 ± 1.35 a
UFLA 6301 RR	-	-
P96R90 RR	3.23 ± 0.41 a	9.64 ± 1.33 a
ANsc 80111 RR	3.97 ± 0.43 a	12.91 ± 1.42 a
P-value	0.416	0.244
F	0.885	1.431

Means followed by the same letter in columns do not differ by Tukey test ($P<0.05$)

3.2 Effects of soybean cultivars on *C. includens* and *S. frugiperda* third-instar larvae

In the bioassay with *C. includens* third instars, soybean cultivars did not differ for larval mortality ($F_{4,\,145} = 1.53$; $P = 0.196$) (Fig. 3). Even the Bt cultivars caused low mortality rates of third-instar larvae (~15% mortality at most). For the fresh weight gain of *C. includens*, the non-Bt cultivar ANsc 80111 RR showed higher ($F_{4,\,137} = 80.74$; $P<0.001$) mean weights than NS 6010 IPRO and P97R50 IPRO, followed by UFLA 6301 RR and P96R90 RR; the cultivar P97R50 IPRO showed no difference from NS 6010 IPRO. The cultivar P97R50 IPRO was the least consumed by *C. includens*, followed by ANsc 80111 RR, UFLA 6301 RR, and P96R90 RR, while NS6010 IPRO showed the highest leaf dry weight consumed ($F_{4,\,137} = 1376.4$; $P<0.001$) (Table 3).

Figure 3 Mortality (% ± SE) of *C. includens* third instar larvae fed on leaf discs of Bt and non-Bt soybean cultivars after seven days



Different letters on bars denote significant differences by Tukey's test ($P<0.05$)

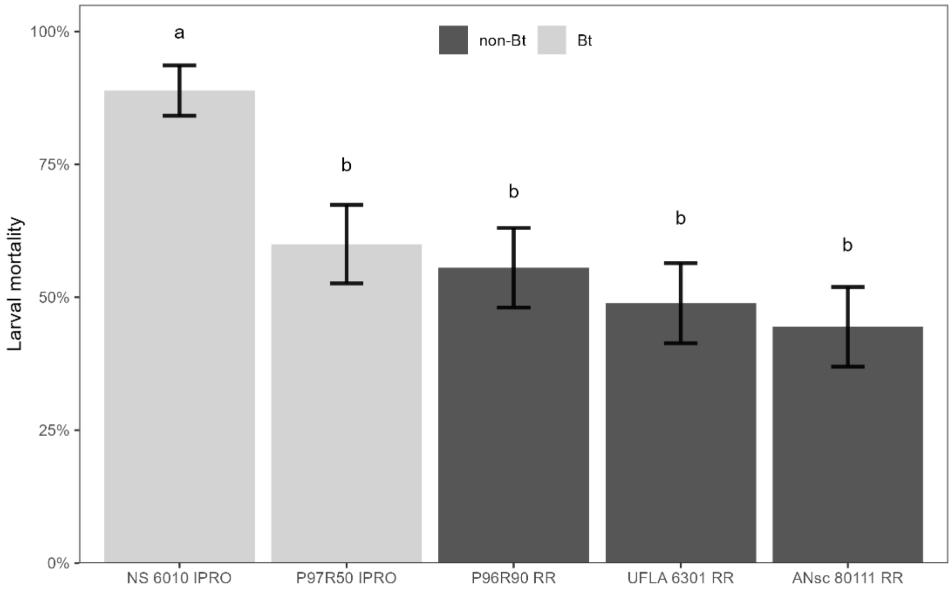
Table 3 Means (mg ± SE) of larvae fresh weight gain and leaf dry weight consumed of *C. includens* third-instar larvae fed on leaf discs of Bt and non-Bt soybean cultivars

Cultivars	Larvae weight gain	Leaf weight consumed
NS 6010 IPRO	-1.60 ± 0.50 d	13.43 ± 0.35 a
P97R50 IPRO	-1.07 ± 0.47 d	10.24 ± 0.32 e
UFLA 6301 RR	3.59 ± 0.48 c	12.19 ± 0.33 c
P96R90 RR	5.81 ± 0.48 b	13.10 ± 0.33 b
ANsc 80111 RR	8.58 ± 0.48 a	11.78 ± 0.33 d
P-value	<0.001*	<0.001*
F	80742	1376.4

Means followed by the same letter in columns do not differ by Tukey test ($P<0.05$)

For *S. frugiperda* third-instar larvae, the Bt cultivar NS 6010 IPRO caused higher larval mortality (90%) than the other cultivars ($F_{4, 220} = 6.19$; $P<0.001$) (Fig. 4). The larvae gained more weight ($F_{4, 177} = 5.07$; $P<0.001$) on the non-Bt cultivars P96R90 RR and UFLA 6301 RR relative to the Bt cultivar P97R50 IPRO, while the cultivars NS 6010 IPRO and ANsc 80111 RR did not differ from the others (Table 4). Leaf dry weight consumed significantly differed among cultivars ($F_{4, 177} = 3826.6$; $P<0.001$), such that *S. frugiperda* third instars showed the greatest leaf consumption on NS 6010 IPRO, followed by UFLA 6301 RR, ANsc 8111 RR, and P96R90 RR, whereas P97R50 RR was the least consumed.

Figure 4 Mortality (% ± SE) of *S. frugiperda* third instar larvae fed on leaf discs of Bt and non-Bt soybean cultivars after seven days



Different letters on bars denote significant differences by Tukey's test (*P*<0.05)

Table 4 Means (mg ± SE) of larvae fresh weight gain and leaf dry weight consumed of *S. frugiperda* third-instar larvae fed on leaf discs of Bt and non-Bt soybean cultivars

Cultivars	Larvae weight gain	Leaf weight consumed
NS 6010 IPRO	14.00 ± 0.73 ab	43.70 ± 0.16 a
P97R50 IPRO	12.50 ± 0.68 b	19.50 ± 0.15 e
UFLA 6301 RR	15.90 ± 0.64 a	28.00 ± 0.14 b
P96R90 RR	15.90 ± 0.64 a	20.60 ± 0.14 d
ANsc 80111 RR	13.70 ± 0.68 ab	23.00 ± 0.14 c
P-value	<0.001*	<0.001*
F	5.073	3826.6

Means followed by the same letter in columns do not differ by Tukey test (*P*<0.05)

Correlation analyses of data of dry leaf consumed and fresh weight gain of third-instar larvae of *C. includens* and *S. frugiperda* were performed individually for each cultivar to gain insight into antinutritional effects of larval feeding on the weight gain (Table 5). For *C. includens*, the correlation results were distinct when the larvae were fed Bt and non-Bt soybean leaf tissue. For the Bt cultivars NS6010 IPRO (r = -0.202; *P* = 0.470) and P97R50 IPRO (r = 0.005; *P* = 0.984), there were no significant correlations, demonstrating that *C. includens* third-instar larvae did not gain weight proportional to the amount of ingested leaf tissue. For the non-Bt cultivars UFLA 6301 RR (r = 0.79; *P*<0.001), P96R90 RR (r = 0.98; *P*<0.001), and ANsc 80111 RR (r = 0.78; *P*<0.001), there were significant and positive correlations, indicating that the amount of leaf tissue consumed by *C. includens* were directly proportional to the larval weight gain.

For *S. frugiperda*, correlation analysis between leaf weight consumed and larvae weight gain indicated that the non-Bt cultivars UFLA 6301 RR (r = -0.204; *P* = 0.214) and P96R90 RR (r = 0.115; *P* = 0.485), and the Bt cultivar NS 6010 IPRO (r = 0.281; *P* = 0.132) did not show significant correlations. On the other hand, for the non-Bt cultivar ANsc 80111 RR (r = 0.497; *P* = 0.001) and the Bt cultivar P97R50 IPRO (r = 0.435; *P* = 0.010), there were significant positive correlations, such that the weight gain of *S. frugiperda* third-instar larvae were proportional to the amount of ingested soybean leaf tissue.

Table 5 Correlation between leaf dry weight consumed and weight gain of *C. inludens* and *S. frugiperda* third-instar larvae in Bt and non-Bt soybean cultivars

C. includens			
Cultivars	Transgenic	r	*P*- value
NS 6010 IPRO	Bt	-0.202	0.470
P97R50 IPRO	Bt	0.005	0.984
UFLA 6301 RR	Non-Bt	0.785	<0.001*
P96R90 RR	Non-Bt	0.984	<0.001*
ANsc 80111 RR	Non-Bt	0.779	<0.001*
S. frugiperda			
Cultivars	Transgenic	r	*P*- value
NS 6010 IPRO	Bt	0.281	0.132
P97R50 IPRO	Bt	0.435	0.010*
UFLA 6301 RR	Non-Bt	-0.204	0.214
P96R90 RR	Non-Bt	0.115	0.485
ANsc 80111 RR	Non-Bt	0.497	0.001*

*Asterisks indicate significant correlation by Pearson´s correlation analysis (*P*<0.05)

4. Discussion

In this study, we evaluated the levels of resistance of Bt and non-Bt soybean cultivars to the soybean looper *C. includens* and fall armyworm *S. frugiperda*. The results indicated that the Bt soybean cultivars differentially affected the larval performance of both insects species across larval stages and that one non-Bt cultivar presented some level of natural resistance to *S. frugiperda*. Studies that evaluate the effects of Bt and non-Bt soybean cultivars adapted to novel growing regions, such as in the Minas Gerais state in Brazil, are of great importance to define and deploy regional strategies of integrated pest management and insect resistance management to the major soybean pests *C. includens* and *S. frugiperda*.

Plants containing Bt technologies are considered highly resistant to targeted lepidopteran larvae species. Some caterpillars are naturally insensitive to Bt-protein binding into insect-midgut receptors, or in some cases, pest populations have evolved resistance to Bt proteins due to the selection pressure caused by extensive transgenics cultivation (Santos-Amaya et al. 2015; Tabashnik and Carrière 2017). The use of Bt cultivars are advantageous for reducing the targeted pest populations, elevating the economic injury levels, benefiting the agroecosystem, and lowering the production costs due to reduced insecticide applications (Brookes and Barfoot 2018).

There was complete larval mortality of *C. includens* neonates on the Bt cultivars NS 6010 IPRO and P97R50 IPRO. This indicates that the Cry1Ac protein expressed in the transgenic event MON87701 x MON89788 has high affinity to *C. includens* and is still considered a high-dose technology for this species. Similar results were found by Bernardi et al. (2012) for *C. includens* neonate larvae from a susceptible lab population, showing high mortality rates on Bt relative to non-Bt leaf discs. A low frequency of resistance alleles in *C. includens* populations has been reported for the Cry1Ac protein expressed in Bt soybeans (event MON87701 x MON89788) in Brazil (Yano et al. 2015; Horikoshi et al. 2021). Although our study was not focused on the evaluation of resistance in the pest populations, it is likely that the status of resistance of *C. includens* has not substantially changed since the previous evaluations by these authors. In addition, in none of these studies were evaluated *C. includens* populations from the soybean-producing region in Brazil where the specimens of this pest and *S. frugiperda* were collected.

The Bt cultivar NS 6010 IPRO (100% mortality) and the non-Bt cultivar UFLA 6301 RR (91% mortality) caused high larval mortality of *S. frugiperda* neonates, being significantly superior than the other cultivars. The other evaluated Bt cultivar, P97R50 IPRO, exhibited low level of *S. frugiperda* neonate mortality, not differing from the other non-Bt cultivars (P96R90 RR and ANsc 80111 RR). The results were only partly expected since *Spodoptera* spp. has higher natural tolerance to Cry1Ac than other Lepidoptera such as *C. includens*, *Chloridea virescens* (F.), and *Helicoverpa zea* (Boddie) (Luttrell et al. 1999a; Santos et al. 2009; Bernardi et al. 2014b). Therefore, the varying effects of Bt cultivars on the *S. frugiperda* mortality found in our study is somewhat different from others in the literature.

Bernardi et al. (2014b) found 41.5, 47.5, and 41.3% mortality of *S. frugiperda* neonate larvae in leaf discs of Bt soybean (MON87701 x MON89788) plants at the V3-V4, V5-V6, and R1-R2 stages, respectively. In the study of Ramalho et al. (2011), *S. frugiperda* larvae fed non-Bt cotton leaves exhibited greater survival (96.7%) than those fed Bt cotton (74.1%), although the mortality on the Bt cultivar was very low. The action of Cry1Ac was not sufficiently lethal for *S. frugiperda* populations in the studies of Luttrell et al. (1999b), Stewart et al. (2001), and Adamczyk and Gore (2004). Plants of Bt soybean effectively control the target pests *A. gemmatalis*, *C. includens*, *H. armigera*, *Crocidosema aporema* (Walsingham), and *C. virescens*, but they show low-to-moderate efficiency against *Spodoptera* spp. (Bernardi et al. 2012, 2014a, 2014b). According to Rahman et al. (2012), the low susceptibility of *Spodoptera* spp. to Cry1Ac may be associated with inactivation of the insecticidal protein by proteases in the larvae's midgut.

Based on the obtained results herein, the high mortality of *S. frugiperda* larvae on the Bt cultivar NS 6010 IPRO was unexpected, and may have synergized with other morphological and chemical soybean plant traits. Host-plant resistance to insects expressed as antixenosis and antibiosis negatively affects insects' feeding and development (Boiça Júnior et al. 2015; Souza et al. 2021). The presence of natural resistance mechanisms may also explain the high *S. frugiperda* mortality on the non-Bt cultivar UFLA 6301 RR, which is the first soybean cultivar developed by the Federal University of Lavras (UFLA). This cultivar also caused moderate mortality in *C. includens* neonates.

Conventional soybean varieties, either breeding lines or commercial cultivars, were previously reported to have moderate resistance levels to *S. frugiperda*, and concentrations of flavonoids and nutrients can underlie the expression of resistance (Hoffman-Campo 1995; Dixon and Steele 1999; Hoffman-Campo et al. 2001; Boiça Júnior et al. 2015, 2017; Souza et al. 2021). Another factor that may explain the antinutrional effects on insects is the activity of protease inhibitors, such as the soybean trypsin inhibitors Kunitz and Bowman-Birk (Gatehouse 2011; Pinheiro et al. 2024). Further research is encouraged to evaluate the potential resistance mechanisms in the cultivar UFLA 6301 RR and in the Bt cultivar NS 6010 IPRO, such as the concentrations of flavonoids, nutrients, and trypsin inhibitors to aid genetic breeding programs to develop resistant and high-yield cultivars adapted to the Minas Gerais state.

In contrast to the high mortality rates of *C. includens* neonates by the Bt cultivars, this was not observed for third-instar larvae. However, sublethal antinutritional effects were found when the older larvae were fed leaf tissue of the Bt soybean cultivars, in that the *C. includens* larvae lost weight after 7 days of feeding. For *S. frugiperda* third-instars, a high mortality rate (90%) was found in NS 6010 IPRO, which agrees with our hypothesis that there may be natural resistance mechanisms in this cultivar to *S. frugiperda*. In addition, the Bt cultivar P97R50 IPRO affected the larval performance of both insects species, showing lower leaf consumption and weight gain.

Correlation analysis was performed between leaf weight consumed and larval weight gain to give insight into antinutritional effects of the Cry1Ac protein on older larvae. For *C. includens*, significant positive correlations were found only for the non-Bt cultivars; these results confirm that, even though Cry1Ac does not kill *C. includens* older larvae, the weight gain is compromised by feeding on the Bt cultivars. For *S. frugiperda*, there was significant positive correlations only for the Bt cultivar P97R50 IPRO and the non-Bt cultivar ANsc 80111 RR; for P97R50 IPRO, the correlation indicated that this cultivar was the least consumed by *S. frugiperda*, and the larvae gained the least weight.

The quantity and quality of food consumed by an insect directly reflects its preference to the host plant, affecting its biological, physiological, and behavioral features (Silva et al. 2017). Ramalho et al. (2011) observed that when *S. frugiperda* was fed on Bt cotton leaves expressing Cry1Ac, lower larval weight was attained due to changes in the nutritional intake, digestion, and food absorption. Other studies reported that although *S. frugiperda* survived on Bt cotton (Cry1Ac), the larvae were smaller and the larval period prolonged (Adamczyk and Sumerford 2001; Stewart et al. 2001). Thus, the sublethal effects of the Bt soybean cultivars on *C. includens* and *S. frugiperda* may result in underdeveloped larvae that may suffer additional environmental effects, be more vulnerable to natural enemies, and have lower reproductive capacity (Bernardi et al. 2014; Barcellos et al. 2022; Machado et al. 2020).

The antinutritional effects of the Bt cultivars found in older larvae may be explained by the fact that Bt endotoxins target cells of the midgut epithelium, which are important for enzyme production and nutrient absorption (Stevens et al., 2013; Howe and Herde et al. 2015). Also, Bt toxins reduce the absorption of amino acids and glucose (Fast and Angus 1965; Gringorten 2001; Jakka et al. 2015). According to Silva et al. (2018), late instars of *H. armigera* larvae more efficiently degrade Cry1Ac proteins due to the higher proteolytic activity of proteases, which may also explain the lower susceptibility of older lepidopteran larvae to Bt proteins.

The contrasting effects of the Bt cultivars on younger and older larvae of *C. includens* and *S. frugiperda* found in our study are important in the context of refuge areas, an insect resistance management strategy essential to keep the resistant alleles in insect populations at low frequencies. The recommended adoption of refuges for transgenic soybeans with the event MON87701 x MON89788 (Cry1Ac) is 20% cultivated area with a non-Bt cultivar (Horikoshi et al. 2021). In the structured refuges with non-Bt cultivars, the lepidopteran larvae can normally feed, grow, and then move to adjacent Bt fields when they are older and more tolerant to the toxic effects of the Bt proteins. Additionally, larvae from the preceding winter and cover crops can survive the off-season and infest recently planted Bt soybeans at advanced larval stages (Nagoshi et al. 2012, 2017). Our results with the non-Bt cultivar UFLA 6301 RR can contribute to the use of cultivars possessing natural insect-resistance levels in structured refuges, in that the soybean plants could be less injured by the attack of lepidopteran larvae and at the same time maintain alive part of the population to allow the reproduction of Bt-resistant with susceptible adult insects in the field.

In Brazil so far only the use of structured-refuge areas (block refuge) is allowed and mandated as insect resistance management, while the use of refuge-in-a-bag (seed-mixture refuge) is permitted in the USA since 2010 (EPA 2011) against *H. zea* (Carrière et al. 2020, 2021) and *Diabrotica virgifera virgifera* (Taylor and Krupke 2018). However, for important caterpillars commonly infesting soybean crops in Brazil, as is the case of *C. includens* and *S. frugiperda*, this strategy is expected not to be effective because of larval movement between plants and because older larvae are more tolerant to the Bt proteins. Although this issue has not yet been evaluated in soybeans, Sorgatto et al. (2015) demonstrated in cotton that both *C. includens* and *S. frugiperda* can survive the toxic effects of Bt events with Cry1Ac+Cry2Ab2 and Cry1Ac+Cry1F when first fed and developed on non-Bt cotton, and that the mortality decreased as the larval age increased. However, using non-Bt cultivars possessing natural insect-resistance levels as seed-mixture refuges could be useful in this scenario, which should be further investigated.

Our study demonstrated that the Bt soybean cultivars expressing the Cry1Ac protein (event MON87701 x MON89788) are efficient to control *C. includens* neonates, but the mortality of *S. frugiperda* neonates is influenced by the genetic background of plant genotype. For third-instar larvae, *C. includens* are more tolerant to the Bt cultivars, but they caused antinutritional effects on the larval performance of both pests. These results are important for expanding soybean-producing regions in Brazil, contributing to deployment of specific strategies of integrated pest management and insect resistance management to *C. includens* and *S. frugiperda*. The highlighted soybean cultivars NS 6010 IPRO and UFLA 6301 RR can be recommended for planting as main crop in the case of the Bt cultivar, and the non-Bt cultivar can be used as structured refuge or in organic production.

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Author contribution

LMF and BHSS designed the study; LMF, FSF, and APAA conducted research; LMF analyzed data; LMF and BHSS wrote the manuscript; ATB bred and provided soybean cultivars; BHSS and ATB revised the manuscript; all authors approved the manuscript for publication.

Declarations

Conflict of interest

The authors declare no competing interest.

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Neem-extract formulation applied singly and mixed with methyl jasmonate on soybean-induced resistance to *Spodoptera frugiperda*

Freitas, Larah M.[1]; Martins, Thayla F.R.[1]; Souza, Bruno H.S.[1]; Keller, E.[2]

[1]Federal University of Lavras, Lavras, MG, Brazil, larah.freitas1@estudante.ufla.br;

[2]Openeem Bioscience, São Paulo, SP, Brazil.

Abstract

Fall armyworm (FAW), *Spodoptera frugiperda*, can defoliate soybeans, reducing the plants yield potential. Biostimulants are chemical or biological products that cause improvements in plants nutritional efficiency, tolerance to stress, and quality of produce. Defense elicitors is another class of compounds that can be used to enhance plant resistance to insect herbivory. This study evaluated the application of neem-extract biostimulant formulation singly and mixed with methyl jasmonate (MeJA) on soybean induced resistance to FAW. Experiments were conducted with foliar application of the following treatments in potted soybean plants: control (water); Neem; MeJA, and Neem + MeJA. In the greenhouse experiment, plants were assigned to the conditions with and without herbivory by FAW after treatments application to soybeans, recording larval survival and weight gain, as well as soybean plant growth; leaf samples were also collected for quantification of hydrogen peroxide concentrations and catalase enzymatic activity. In the laboratory experiment, treatments were applied to soybean plants, and leaflets were subsequently collected to evaluate the effects on FAW larval survival, weight gain, and leaf consumption in Petri dishes. Application of Neem + MeJA in the greenhouse experiment led to reduced root length in the condition without herbivory; in the laboratory Neem + MeJA reduced soybean foliage biomass, while FAW herbivory increased it. Concentrations of hydrogen peroxide were greater in plants subjected to FAW herbivory, indicating stress, but catalase enzymatic activity did not differ among treatments. In the laboratory, FAW consumed more leaf tissue from soybeans treated with Neem + MeJA and MeJA, but this did not result in greater weight gain, suggesting an antinutritive effect caused by elicitors application.

Keywords: Fall armyworm; MeJA; *Azadirachta indica*; Soybean resistance

1. Introduction

Fall armyworm (FAW) *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae) is a highly polyphagous pest, feeding on more than 350 plants species (Kenis et al., 2022; Montezano et al. 2018; Overton et al. 2021; Parra et al., 2022; Prasanna et al. 2021). The main crops where FAW causes economic damage include cereals, forages, and grasses, especially maize, rice, and sorghum, but other annual crops, such as soybean and cotton, have been highly infested by FAW (Barros et al. 2010; Oliveira et al. 2014; Wu et al. 2021a). The success of this pest in causing economic damage to various crops is due to several factors, such as high reproductive potential, adaptability to different host plants, survival on alternative crops and weeds, low susceptibility to Bt proteins, evolution of resistance to insecticides, among others (Barros et al. 2010b; Bernardi et al. 2014; Santos et al. 2009).

There is a need to develop novel and sustainable strategies for managing FAW populations in important cultivated host plants, such as soybeans. One of the options is improving our knowledge on the plants' intrinsic defense mechanisms to support the application of chemical or biological elicitors. Understanding the signaling pathways of plant defense has led to the development of resistance elicitors, either natural or synthetic, that mimic the natural plant defensive responses to herbivory (Cofer et al., 2018; Karban; Kuc, 1999; Mauch-Mani et al., 2017;Rani; Murali-Baskaran, 2024). Induced resistance is regulated by signaling molecules, with jasmonic acid (JA) playing an important role in the plant defense to insects (Kant et al., 2015). Exogenous application of JA, similar to the feeding stress by chewing insects, rapidly increases the endogenous levels of JA, which in turn triggers the expression of defense-related genes (Benevenuto et al., 2019; Pauwels et al., 2009). Exogenous application of methyl jasmonate (MeJA) also elicits JA-related responses after MeJA is demethylated to JA in treated plants (Wu et al., 2008). The use of defense elicitors is considered a promising strategy for suppressing agricultural insect pests (Haq et al., 2021).

Biostimulants comprise another class of compounds that can be used to mitigate plants stresses. Biostimulants are defined as any substances or microorganisms that applied to plants can stimulate natural physiological processes that improve plant growth, nutritional efficiency, tolerance to environmental stresses, and crop quality traits (Calvo et al., 2014; Du Jardim, 2015; Van Oosten et al., 2017; Del Buono, 2021; Monteiro et al., 2022). The positive effects of applying biostimulants to crop plants include increased plant growth and induced biosynthesis of plant secondary compounds (Alvarez et al., 2024), which could be advantageous in integrated pest management.

Plant responses to the various types of stress to which plants are exposed, whether biotic or abiotic, involve the expression of defense mechanisms (Borges et al. 2017; Walters; Heil 2007). Plant defense mechanisms can be classified as constitutive and induced, depending on whether they are constantly expressed or are triggered only after a stress condition (Borges et al., 2017). Tolerance is a resistance category against insect herbivory that is defined as the ability of plants to recover from or compensate for the injury caused by insect herbivory, without their growth and production being substantially affected (Painter 1951, Smith 2005; Stenberg; Muola 2017; Peterson et al. 2017). Tolerance does not affect aspects of insect behavior and development, but rather involves changes in physiological processes of plants species and genotypes that make them better able to mitigate pest injury stress (Chen et al., 2015). Tolerance mechanisms include alterations in photosynthesis and other physiological plant processes such as growth, phenology, reallocation, and use of stored nutrients and photoassimilates (Strauss; Agrawal 1999; Stowe et al. 2000; Tiffin 2000).

Reactive oxygen species (ROS) are common components of the defense responses of plants against pathogens and insect herbivores (He et al., 2011; Sousa et al., 2021). An example of ROS is hydrogen peroxide (H_2O_2), which at low concentrations acts as a signaling molecule in several biological functions, like cell proliferation, differentiation, and defense responses (Zhang et al., 2019). When plants are under stress, physiological processes of plant defenses are triggered, in which antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) maintain the balance of ROS and the homeostasis of plant cells (Sousa et al., 2021). Therefore, plants that express high antioxidant enzyme activities are able to eliminate excess ROS, protecting cells from oxidative damage, allowing these plants to better tolerate stress conditions (Caverzan et al., 2016).

The neem tree *Azaditachta indica* A. Juss. has been recognized as a repository of secondary metabolites for centuries due to their insecticidal and antioxidant properties, particularly azadirachtins (Ezin; Chabi, 2022; George et al., 2007). Azadirachtins showed to be effective against over 540 insects species, including hemipterans, lepidopterans, dipterans, coleopterans, and homopterans (Kilani-Morakchi et al., 2021). Besides azadirachtins, there are various constituents of the neem plants with bioactivity against insects, including salannin, meliantrol, gedunin, nimbidin, nimbin, dacetylsalannin, among others (Depieri; Martínez, 2010). Defense elicitors function as molecules indicative of stress conditions to plants, whose detection in specific receptors at the cell membranes and the consequent signal transduction activates defense-related genes (Chantini et al., 2021). Despite the known direct insecticidal effects of neem extracts and oils, there is little information on the plant induced defense effects of neem formulations to insect herbivory and development.

Because of the multiple types of secondary metabolites produced by neem plants, formulated products based on neem extracts may induce resistance in host plants, meriting further evaluation. This study investigated whether the application of neem-extract biostimulant applied singly and in combination with the phytohormone-derivative methyl jasmonate can induce resistance in soybean plants to *S. frugiperda*.

2. Materials and Methods

2.1 Location of the study

The study was conducted under greenhouse and laboratory conditions in the Laboratory of Plant Resistance and Integrated Pest Management, Department of Entomology, Federal University of Lavras (UFLA), in Lavras, Minas Gerais state, Brazil. In the laboratory the assays were conducted under controlled conditions (25 ± 2°C, 60 ± 10% RH, 14L:10D h) and in the greenhouse under natural environmental conditions and ambient light.

2.2 Insect rearing

FAW larvae used in experiments was obtained from a colony established in the laboratory for five years, with wild egg masses collected from maize fields annually introduced to the colony. FAW colony was maintained in laboratory room under controlled conditions (25 ± 2°C, RH = 60 ± 10%, and 14L:10D h), with the larvae reared on artificial diet (Greene et al., 1976) and the adults fed a 10% honey solution.

2.3 Treatments

This study evaluated the foliar application in soybean plants with the following treatments: Control (water); Neem; Methyl jasmonate (MeJA); and Neem + MeJA. These treatments were also evaluated in both the conditions of herbivory (H+) and without herbivory (H-) by *S. frugiperda* depending on the experiment of this study. The evaluated neem treatment is a formulated product (Openeem Valente®, Openeem Bioscience, São Paulo, Brazil) based on the extraction of the entire neem plant. MeJA evaluated herein was the technical product, with 95% purity (Sigma-Aldrich, St. Louis).

Neem treatment was applied at the proportional dose of 1L ha^{-1}, as recommended by the manufacturer (Openeem Bioscience, SP, Brazil), using 200 L of spray volume. MeJA was applied at 2.0 mM, which was prepared in 0.1 % ethanol, and then dissolved in water to produce the desired concentration. This concentration was chosen based on previous studies that reported induced resistance by MeJA (Cappellari et al., 2019; Senthil-Nathan, 2019).

2.4 Soybean plants cultivation and treatments application

Soybeans were grown in 2.5-L pots containing soil, substrate, and sand (2:1:1). For experiments, we used the non-Bt soybean cultivar Pioneer® P96Y90 (Pioneer Hi-Bred, Johnston, IA) with RR® technology (event GTS 40-3-2), which possesses tolerance to glyphosate herbicide. Potted-plants were fertilized with NPK (04-14-08) following the recommendations of the sixth approximation for soybean cultivation. In total 160 plants were prepared, half of which were used for the greenhouse experiment, while from the other set of plants were obtained leaf material for the laboratory experiment. Treatments were foliar-applied at the V2 growth stage (Fehr et al., 1971) with a hand-held sprayer until runoff, amounting ~50 ml per plant. The plants were kept on benches in a covered greenhouse until the V3-V4 growth stage, when they were used in the experiments.

2.5 Greenhouse experiment

The experiment was installed in a complete randomized design in a 4 x 2 factorial scheme, consisting of the applied treatments (Control, Neem, MeJA, and Neem + MeJA) in the conditions with and without FAW herbivory (H+ and H-), in a total of eight treatments. Ten plants were prepared for each treatment, five of which were infested with FAW larvae and five plants were uninfested, and were used as replicates. Seven days after the treatments were applied to the plants, the oldest trifoliate from the lower part of V3-V4 plants was infested with three third-instar larvae, being confined in a voile-fabric cage closed with twist ties. Uninfested plants were also covered with voile fabric in the same leaf position.

The larvae were allowed to feed on the trifoliates for seven days, after which larval survival were recorded. The surviving larvae were taken to the laboratory to assess the fresh weight using an analytical scale (ATY 224R, Shimadzu, Sao Paulo, Brazil). The fresh weight gain of larvae was obtained by subtracting the initial weight from the final weight.

Additional five plants of each treatment were used as replicates for assessment of soybean plant growth parameters in function of treatments application in both herbivory conditions. After the larvae were removed from the plants at the V3-V4 growth stage, the plants were allowed to develop until the V4-V5 stage, which took an average of 4 to 7 days when the experiment was ended. Plant height was measured with the aid of a ruler, from the soil surface in pots to the insertion of the uppermost trifoliate. The relative chlorophyll index was recorded using a SPAD-502 portable chlorophyll meter (Konica Minota Sensing, TECNAL, Piracicaba, Brazil). Readings were taken on the youngest trifoliate, recording the mean value out of three readings in each leaflet of the trifoliate. Next, the soil of potted plants was watered, and the plants carefully removed from the pots. The roots were carefully washed in running water, after which their length was measured with a ruler. The root nodules of the soybean plants were visually counted. The plant and root materials that had been washed were stored in paper bags and taken to a forced circulation oven (EL402, Electrolab, Sao Paulo, Brazil) at a temperature of 50^{o}C for 72 hours, in order to obtain their dry mass. Dry foliage and root samples were weighed on an analytical scale (ATY 224R, Shimadzu, Sao Paulo, Brazil).

2.6 Hydrogen peroxide concentration and antioxidant enzyme activity

In the end of the greenhouse experiment, two trifoliates of the upper part of the plants were collected from the eight treatments, stored in aluminum envelopes, and immediately placed in liquid nitrogen to conserve the physiological processes. The samples were stored in ultra-freezer (-80 ^{o}C) until chemical analysis. Five plants of each treatment were used as replicates.

Leaf samples were used for extraction of hydrogen peroxide (H_2O_2) and catalase (CAT) following the protocol of Buege and Aust (1978). Frozen leaves (0.2 g) were ground in liquid nitrogen with 50% polyvinylpolypyrrolidone (PVPP). The samples were homogenized in 1500 μM of 0.1% trichloroacetic acid (TCA) and centrifuged at 12,000 *g* for 15 minutes at 4°C. Subsequently, the supernatant was collected and submitted to analysis. All analyses were performed in duplicates for each plant replicate.

The quantification of H_2O_2 was determined according to Velikova et al. (2000). Aliquots of the supernatant were added to the incubation buffer, prepared with 10 nM potassium phosphate, pH 7.0, and 1 M potassium iodide. The samples were pipetted in duplicates into ELISA plates and were read on a spectrophotometer at 390 nm. The concentration of H_2O_2 was calculated according to a standard curve, and the results were expressed as mmol H_2O_2 mg^{-1} FW.

The activity of CAT was analyzed following the protocol of Havir and McHake (1987). The samples were incubated in a buffer solution of 50 nm potassium phosphate, pH 7.6, and ethylenediamine tetraacetic acid (Na_2EDTA), in a water bath at 30°C. Hydrogen peroxide was prepared as a stock solution (200 mM) and added at the time of reading. The samples were read in spectrophotometer at 240 nm every 15 seconds for 3 minutes. Decrease in absorbance occurs at a molar extinction coefficient of 36 nM^{-1} cm^{-1}, and the determination of catalase activity was calculated as CAT μmol CAT min^{-1} mg FW^{-1}.

2.7 Laboratory experiment

Treatments (Control, Neem, MeJA, and Neem + MeJA) were applied to V2-stage soybeans as previously described, using 10 plants per treatment as replicates. Next, the uppermost trifoliate of the plants were collected from V3-plants in the greenhouse and brought to the laboratory. Three 0.9-mm leaf discs were cut out from each trifoliate using a metallic puncher, being one disc per leaflet, and transferred to Petri dishes. Each replicate was composed of three individual Petri dishes with the leaf discs prepared from the same trifoliate.

The performance of FAW third-instar larvae was evaluated in Petri dishes (5 cm diameter) lined with moist filter paper. The larvae were obtained from the rearing colony, weighed in analytical scale, and individually transferred to the dishes. The bioassay was installed in a completely randomized design in a laboratory climatized room (25 ± 2 °C, 60 ± 10% RH, and 14L:10D h). Leaf discs were exchanged after 4 days, and the bioassay was ended after seven days.

The effects of treatments were evaluated on FAW survival, weight gain, and leaf consumption after the bioassay was ended. Larval mortality was inspected visually and with the aid of a soft touch by a fine paintbrush. Leaf consumption was recorded on a dry weight basis; for this, 15 aliquots of leaf discs were prepared with extra plants that were maintained in the same greenhouse conditions and collected from the same leaf position and stage of soybean. All leaf discs were dried in an oven (EL402, Electrolab, Sao Paulo, Brazil) at 50^{o}C for 72h, and the weight of leaf material after larvae feeding was subtracted from the weight of intact leaf discs (aliquots), obtaining the leaf dry weight consumed. The weight of larvae was obtained by weighing them on analytical scale (ATY 224R, Shimadzu, Sao Paulo, Brazil), and the fresh weight gain was calculated based on the final weight minus the initial weight of larvae.

2.8 Statistical analysis

Data obtained in experiments were checked for the normality of residuals and homogeneity of variances. Data did not show normal distribution and were analyzed using generalized linear models. For the greenhouse experiment, both treatment and herbivory were used as fixed effects, and their interaction was also analyzed. For the laboratory experiment, treatment was used as fixed effect, while replicate was used as random effect. When significant differences were found, means of treatments were compared by Tukey's test (α=0.05). Statistical analysis was performed in R software (R Core Team, 2014).

3. Results

3.1 Greenhouse experiment

3.1.1 FAW larval performance and soybean plant growth

In the greenhouse experiment, the mortality ($F_{3, 36} = 2.31$, $P = 0.09$) and weight gain ($F_{3, 36} = 0.44$, $P = 0.72$) of FAW larvae were not affected by treatments application (Table 1).

Table 1. Effects of treatments on FAW larval mortality (%) and weight gain (mg).

Treatments	Mortality (%)	Weight gain (mg)
Control	40.0 ± 8.32 A	1.76 ± 0.38A
Neem	43.3 ± 10.00 A	2.94 ± 0.82 A
MeJA	30.0 ± 7.78 A	0.77 ± 0.27 A
Neem + MeJA	40.0 ± 8.32 A	1.79 ± 0.69 A

Means followed by the same letters in columns do not differ by Tukey's test ($P>0.05$).

For the plant growth parameters evaluated, only the root length showed a significant interactive effect of treatment x herbivory ($F_{3, 72} = 2.97$, $P = 0.03$). Untreated soybean plants exhibited greater root growth than those treated with Neem + MeJA in the condition without herbivory. When plants were FAW-infested, only control plants showed lower root growth relative to uninfested plants. The other plant growth parameters, namely plant height ($F_{3, 72} = 0.46$, $P = 0.70$), foliage dry weight ($F_{3, 72}= 2.30$, $P = 0.08$), root dry weight ($F_{3, 72}= 0.19$, $P = 0.90$), number of root nodules ($F_{3, 72} =1.06$, $P = 0.36$), and relative chlorophyll index ($F_{3, 72} = 1.25$, $P= 0.29$) were not influenced by treatments x herbivory (Table 2).

Considering the effects of treatment, there was a significant difference only in foliage dry weight ($F_{3, 72} =3.86$, $P = 0.01$), in which untreated plants showed greater biomass than those treated with Neem + MeJA; Neem and MeJA were intermediate, and did not differ from control and Neem + MeJA (Fig. 1). The other plant growth parameters were not affected ($P>0.05$) by treatments application.

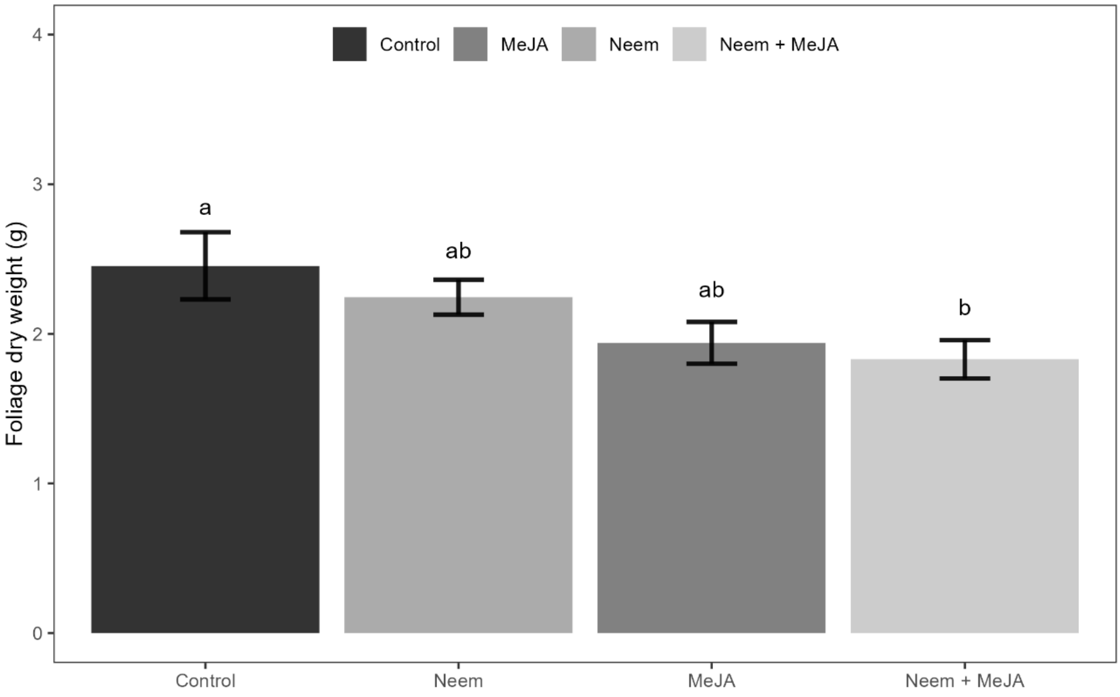

Fig. 1

Means (g ± SE) of soybean foliage dry weight in function of treatment applicaton. Different letters on bars denote significant difference by Tukey’s test (*P*<0.05).

For the effects of herbivory, there was a significant difference ($F_{3, 72}$ =11.15, *P* = 0.001) only on the foliage dry weight of soybean plants. The other plant growth parameters did not show significant differences in function of FAW herbivory (Table 3).

Table 2. Values of statistical analysis for the effects of treatment, herbivory and treatment x herbivory for soybean plant growth parameters.

Factors	RL		RDW		NRN		PH		FDW		RCI	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Treatements (T)	1.60	0.19 ns	1.29	0.28 ns	1.42	0.21 ns	0.11	0.94 ns	3.86	0.01 *	1.53	0.21 ns
Herbivory (H)	1.47	0.22 ns	1.43	0.23 ns	0.42	0.51 ns	0.02	0.87 ns	11.15	0.00 *	1.40	0.23 ns
T x H	2.97	0.03 *	0.19	0.90 ns	1.06	0.36 ns	0.46	0.70ns	2.30	0.08 ns	1.25	0.29 ns

RL - Root length after infestation; RDW – Root dry weight; NRN – Number of root nodules; PH - Plant height; FDW - foliage dry weight; RCI - Relative chlorophyll index; *$P<0.05$; ns: non-significant

Table 3. Effects of treatments x herbivory interaction on the plant parameters root length (cm), root dry weight (g), number of root nodules, plant height (cm), foliage dry weight (g), and relative chlorophyll index.

Treatments	RL		RDW		NRN	
	- H	+ H	- H	+ H	- H	+ H
Control	42.8 ± 2.27Aa	34.1 ± 2.56 Ab	1.8 ± 0.14 Aa	3.0 ± 0.34 Aa	5.6 ± 2.05 Aa	3.3 ± 1.13 Aa
Neem	40.8 ± 1.93 ABa	39.5 ± 1.82 Aa	2.1 ± 0.13 Aa	2.3 ± 0.19 Aa	2.7 ± 0.66 Aa	1.5 ± 0.47 Aa
MeJA	39.7 ± 2.49 ABa	38.1 ± 2.13 Aa	1.7 ± 0.22 Aa	2.1 ± 0.16 Aa	4.0 ± 1.17 Aa	3.5 ± 0.94 Aa
Neem + MeJA	33.5 ± 1.36 Ba	37.6 ± 2.43 Aa	1.6 ± 0.10 Aa	2.0 ± 0.22 Aa	2.9 ± 0.82 Aa	4.7 ± 1.46 Aa

Treatments	PH		FDW		RCI	
	- H	+ H	- H	+ H	- H	+ H
Control	23.9 ± 0.93 Aa	22.4 ± 1.08 Aa	0.7 ± 0.06 Aa	0.8 ± 0.04 Aa	30.4 ± 1.20 Aa	30.1 ± 1.34 Aa
Neem	23.6 ± 0.79 Aa	23.1 ± 1.11 Aa	0.7 ± 0.04 Aa	0.7 ± 0.04 Aa	29.2 ± 0.90 Aa	29.9 ± 0.47Aa
MeJA	23.6 ± 1.27 Aa	23.3 ± 0.77 Aa	0.7 ± 0.07 Aa	0.8 ± 0.06 Aa	33.3 ± 1.49 Aa	30.1 ± 1.29 Aa
Neem + MeJA	23.8 ± 0.78 Aa	23.6 ± 0.96 Aa	0.6 ± 0.05 Aa	0.7 ± 0.06 Aa	30.4 ± 0.67 Aa	29.6 ± 0.43 Aa

RL - Root length; RDW – Root dry weight; NRN – Number of root nodules; PH - Plant height after infestation; FDW - Foliage dry weight; RCI - Relative chlorophyll index. Means followed by the same uppercase letters in columns to compare treatments, and lowercase letters in rows to compare herbivory do not differ by Tukey's test ($P>0.05$).

The dry weight of soybean foliage was significantly greater when the plants were infested by FAW larvae (Fig. 2). This indicates that, insetad of reducing leaf area, FAW herbivory stimulated the increase of soybean plant biomass.

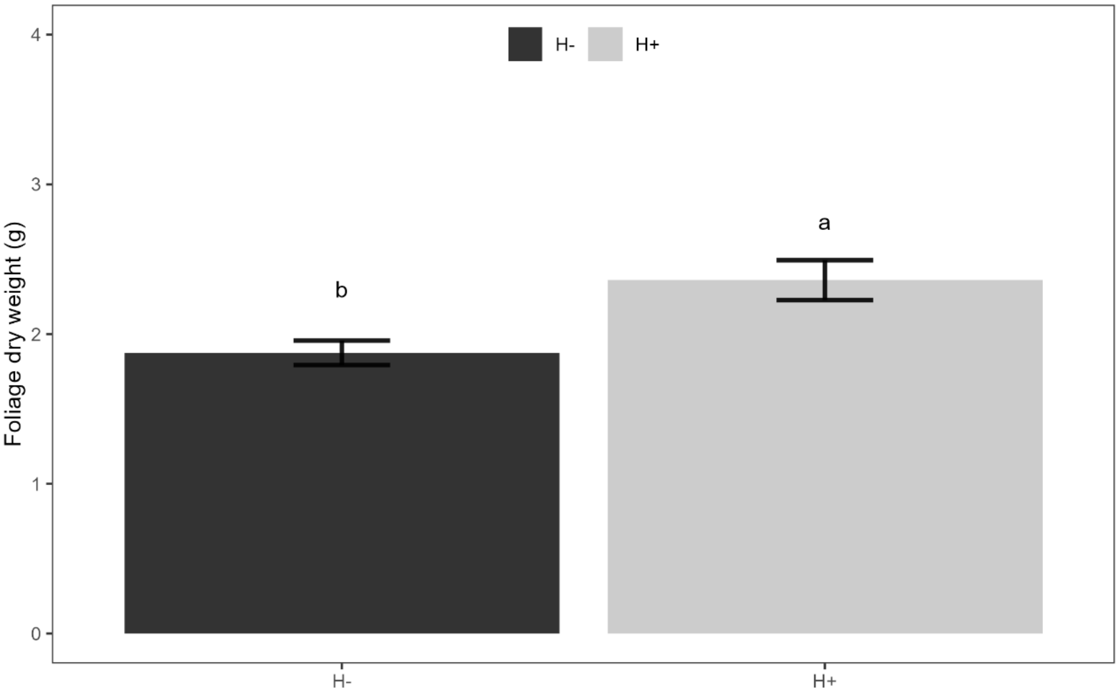

Fig. 2 Means (mg ± SE) of soybean foliage dry weight in function of FAW herbivory. Different letters on bars denote significant difference by Tukey's test (*P*<0.05).

3.1.2 Hydrogen peroxide concentration and antioxidant enzyme activity

The concentrations of H_2O_2 were not influenced by the interaction of treatment x herbivory ($F_{3, 32} = 0.219$, $P = 0.881$) (Table 4). The main effect of treatment was not significant for H_2O_2 concentration ($F_{3, 32} = 1.233$, $P = 0.330$), but herbivory significantly affected its concentration ($F_{3, 32} = 6.263$, $P = 0.023$).

Table 4. Values of statistical analysis for the effects of treatment, herbivory, and treatment x herbibory interaction on oxidative stress and enzymatic activity and of soybean plants.

Factors	H_2O_2		CAT	
	F	*P*	*F*	*P*
Treatment (T)	1.23	0.33 ns	0.81	0.50 ns
Herbivory (H)	6.26	0.02 *	0.01	0.89 ns
T x H	0.21	0.88 ns	0.49	0.69 ns

H2O2 - hydrogen peroxide; CAT - activity catalase; **P*<0.05; ns: non-significant

Soybean plants infested by FAW larvae showed significantly greater H_2O_2 concentrations than uninfested plants (Fig. 3). This result indicates that herbivory caused stress to the plants, whose concentrations of H_2O_2 were systemically accumulated on the leaves that were not directly infested by FAW.

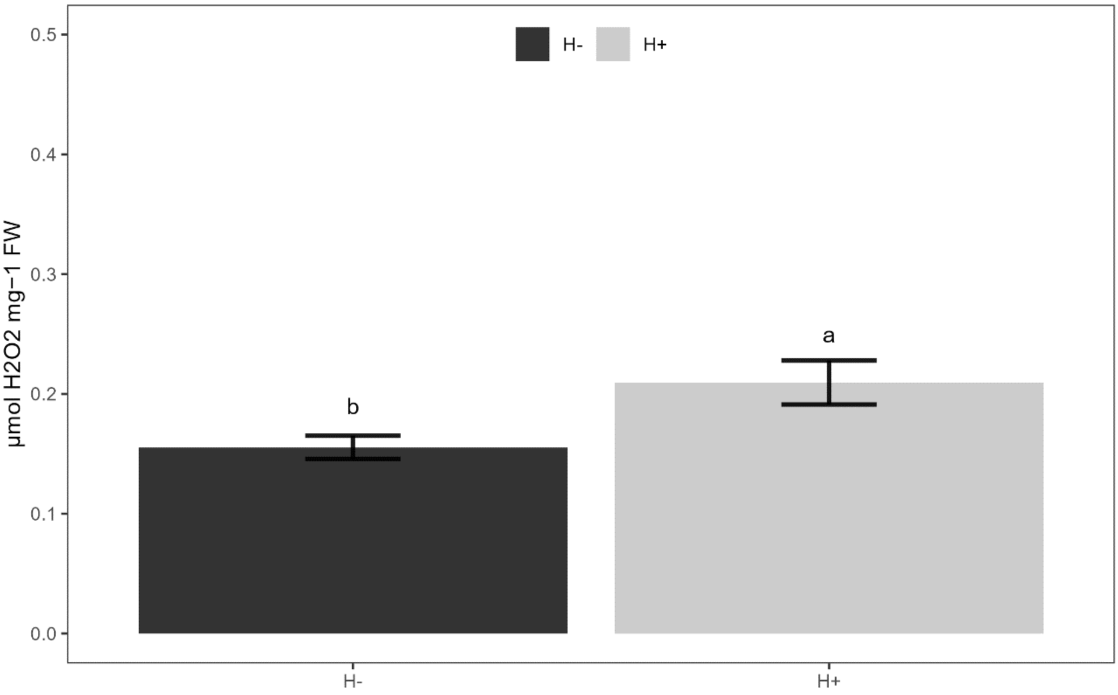

Fig. 3 Means (g ± SE) of concentrations of hydrogen peroxide (µmol H_2O_2 mg^{-1} FW) in function of FAW herbivory. Different letters on bars denote significant difference by Tukey's test (*P*<0.05).

On the other hand, there were no significant differences in the enzymatic activity of CAT considering the interaction of treatment x herbivory ($F_{3, 32}$ = 0.492, *P*= 0.692), or for the main effects of treatment ($F_{3, 32}$= 0.817, *P*= 0.502) and herbivory ($F_{3, 32}$ = 0.017, *P* = 0.897) (Table 4).

The concentrations of H_2O_2 in soybean plants across treatments varied from 0.133 and 0.229 µmol mg^{-1} FW of leaf tissue, whereas CAT enzymatic activity varied from 0.821 to 1.064 µM min^{-1} mg^{-1} of leaf tissue (Table 5).

Table 5. Effects of treatment x herbivory on hydrogen peroxide concentrations (µmol H_2O_2 mg^{-1} FW) and catalase enzymatic activity (µM CAT min^{-1} mg^{-1}).

Treatments	H_2O_2 (µmol mg^{-1} FW)		CAT (µM min^{-1} mg^{-1}).	
	- H	+ H	- H	+ H
Control	0.177 ± 0.06 Aa	0.229 ± 0.00 Aa	1.064 ± 0.02 Aa	0.955 ± 0.18 Aa
Neem	0.174 ± 0.02 Aa	0.227 ± 0.02 Aa	0.953 ± 0.03 Aa	0.919 ± 0.13 Aa
MeJA	0.133 ± 0.04 Aa	0.214 ± 0.00 Aa	0.821 ± 0.08 Aa	0.869 ± 0.05 Aa
Neem + MeJA	0.137 ± 0.00 Aa	0.168 ± 0.02 Aa	0.842 ± 0.13 Aa	0.977 ± 0.08 Aa

H_2O_2 – hydrogen peroxide; CAT – activity catalase. Means followed by the same uppercase letters in columns to compare treatments, and lowercase letters in rows to compare herbivory do not differ by Tukey's test (*P*>0.05).

4. Laboratory experiments

4.1 Evaluation of FAW larval performance

There were no significant differences of treatments on FAW larval mortality ($F_{3, 36}$ = 0.81, *P* = 0.49) and weight gain ($F_{3, 36}$ = 0.91, *P* = 0.44). For leaf consumption, there was a significant difference ($F_{3, 36}$= 9.46, *P* < 0.001), such that soybean plants treated with Neem + MeJA were more consumed by FAW, followed by MeJA (Table 6).

The mortality of FAW larvae varied from 17.7 and 24.4% across treatments, whereas their fresh weight gain from 0.233 to 0.663 mg (Table 6), which did not differ.

Table 6. Effects of treatments on FAW larval mortality (%), fresh weight gain (mg), and dry leaf consumed (mg).

Treatments	Mortality (%)	Weight gain (mg)	Leaf consumption (mg)
Control	17.7 ± 3.395 A	0.427 ± 0.093 A	0.071 ± 0.003 C
Neem	20.0 ± 3.229 A	0.480 ± 0.133 A	0.079 ± 0.002 BC
MeJA	22.2 ± 2.342 A	0.663 ± 0.298 A	0.094 ± 0.005 AB
Neem + MeJA	24.4 ± 3.629 A	0.233 ± 0.146 A	0.098 ± 0.004 A

Means followed by the same letters do not differ by Tukey's test (*P*<0.05).

5. Discussion

This study evaluated the singly and combined application of the phytohormone-derivative methyl jasmonate and neem-based biostimulant to soybeans on the biological performance of FAW and growth of plants in the presence and absence of herbivory. The results of the greenhouse experiment indicated the mortality and weight gain of FAW larvae were not altered regardless of whether Neem and MeJA were applied alone or in combination compared to control. However, there were differences of elicitors application and FAW herbivory on the soybean plant growth in the greenhouse experiment and on the larval consumption in the laboratory. Collectively, these results may suggest a negative trade-off between antibiosis and tolerance types of resistance when Neem + MeJA was applied in function of FAW herbivory.

There was an interactive effect of treatment and herbivory only on roots length regarding soybean plant growth. Uninfested plants showed lower roots length when treated with Neem + MeJA, while their individual application were intermediate, not differing from the control and the combined application. On the other hand, the treatments did not differ for root length in the presence of FAW herbivory, and the control plants had lower root growth in the presence of herbivory compared to uninfested plants. Additionally, Neem + MeJA reduced the biomass of soybean foliage, indicating that the combined application of elicitor and biostimulant can negatively affect the roots growth.

Similar outcomes were observed across three plants species when MeJA was applied, including a 15% reduction in root dry mass, 22% decrease in shoot dry mass, 26% decline in root length, and 17% decrease in the height of treated soybean plants (Li et al., 2018). Another study on rice indicated that MeJA applied as seed treatment caused a 32% reduction in root biomass compared to untreated plants (Kraus; Stout, 2019). The suppression of plant growth induced by MeJA seems to be associated with changes in mechanisms of cell division (Swiatek et al., 2002; 2004). In another study, soybean plants induced with JA generated 10.1% fewer seeds that were 9.0% lighter, with 19.2% lower germination rates. In contrast, MeJA applied to soybean plants did not alter seed production in the study of Chen et al. (2018). These results indicate that induced resistance may be costly without herbivory (Accamando; Cronin, 2012), emphasizing the importance of examining the costs and benefits of defense elicitors in the absence and presence of insect pests, which can give contrasting outcomes depending on the plant fitness costs.

Contrasting with the reduced soybean root length and foliage biomass with Neem + MeJA application, FAW herbivory improved foliage biomass relative to uninfested plants, resulting in overcompensation of leaf injury. This effect was related to the higher concentrations of H_2O_2 in the FAW-infested plants, which systemically accumulated in the uppermost leaves that were not directly attacked by the larvae. Hydrogen peroxide stands out as a primary reactive oxygen species (ROS) generated during plant stress, triggering the expression of defense-related genes when accumulated at lower concentrations (Torres, 2010). This ROS also functions as a signaling molecule that plays a role in many plant biological processes like cell proliferation, differentiation, and induced defense responses (Zhang et al., 2019). Therefore, the stress caused by FAW herbivory to the soybean plants likely triggered physiological changes that stimulated the growth of plants foliage to tolerate leaf tissue loss.

Tolerance is a resistance category defined as the ability of plants to recover from or compensate for the injury caused by herbivory (Painter, 1951; Peterson et al. 2017; Smith 2005; Stenberg; Muola 2017), not affecting insect behavior and development. The mechanisms of tolerance include alterations in photosynthesis and other physiological plant processes such as growth, phenology, reallocation, and use of stored nutrients and photoassimilates that make plants better able to mitigate insect herbivory (Chen et al., 2015; Strauss; Agrawal 1999; Stowe et al. 2000; Tiffin 2000). Although in our study the H_2O_2 concentrations were related to soybean tolerance to FAW herbivory, the enzymatic activity of catalase did not explain this effect. Other enzymes that were not evaluated herein also play a role in the antioxidant metabolism of plants to stress conditions, such as superoxide dismutase and ascorbate peroxidase, helping the plant cells to maintain the balance of ROS and homeostasis (Sousa et al., 2021).

In the laboratory experiment, there was greater leaf consumption of FAW larvae in the Neem + MeJA treatment, followed by MeJA. Because there was no difference in the larval weight gain among treatments, we assume that application of Neem + MeJA, followed by MeJA alone, reduced the suitability of leaves for FAW feeding, in which the larvae had to compensate for the detrimental effects by consuming more leaf tissue to gain the same weight. Therefore, there is evidence that application of MeJA especially mixed with Neem affected the food conversion into biomass in FAW. A study with similar results using different types of elicitors in soybean and cotton showed that jasmonate derivatives consistently reduced the weight gain and growth of FAW larvae (Gordy et al., 2015). In another study, larval weight gain of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) was reduced by 55-75% on four tomato genotypes treated with 2.5 mM methyl jasmonate (Tian et al., 2014).

We hypothesize that application of Neem + MeJA and MeJA may have induced in soybean plants the increased production of compounds acting as digestibility reducers against FAW. There are reports in the literature on the increased concentrations of phytoalexins and other phytochemicals that made larvae to compensate for the poor host-plant quality by increasing the amount of leaf tissue they consumed (Slansky and Wheeler 1992, Lavoie and Oberhauser 2004). Previous studies have attributed soybean resistance induction on herbivores to changes in leaf phytoalexin and proteinase inhibitor contents after herbivory (Accamando; Cronin, 2012; Kogan; Fischer 1991; Gatehouse, 2011; Pinheiro et al., 2024). Proteinase inhibitors are known to act as defensive proteins against insects by affecting their larval nutrition and developmental delay (Gatehouse, 2011; Pinheiro et al., 2024; Underwood et al. 2002; Wasternack et al. 2006).

The present investigation suggests that the application of the elicitor MeJA combined with the neem-based biostimulant altered the food conversion into larval weight gain of FAW. This change in food digestibility can be considered an induced plant defense effect by the application of MeJA that syngergized with the neem extract. However, there was a negative trade-off in the root and foliage growth of soybean plants when plants were not subjected to FAW herbivory.

Future studies are encouraged to better investigate other factors that may be related to the soybean induced resistance to FAW, such as different concentrations of the elicitors, application methods, frequency of application, as well as the fitness costs to the growth and reproduction of treated plants. This knowledge will be important to gain insights into the costs and benefits of integrating resistance elicitors and biostimulants in integrated pest management in soybean production.

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TERCEIRA PARTE

CONSIDERAÇÕES FINAIS

A presente tese explorou os entraves e possíveis novas ferramentas para o manejo integrado de pragas com ênfase principalmente na resistência de plantas a insetos relacionado a cultura da soja e as espécies de lagartas a *C. includens* e *S. frugiperda* com a utilização plantas transgênicas e uso de formulados a base de Nim isolado ou associados a um fitormônio sintético. No primeiro capítulo do estudo foi abordado a efetividade de cultivares transgênicas e não transgênicas em regiões de expansão da cultura de soja em Minas Gerais no controle de duas populações de espécies diferentes de lagartas pragas. Os resultados indicaram que tanto a diferença de idade quanto a espécie de lagarta impactaram a eficácia das cultivares com proteína Bt no controle, além de terem sido observados efeitos antinutricionais sobre os insetos na conversão alimentar. De acordo com o nível de resistência e suscetibilidade das cultivares Bt e não Bt, as cultivares destacadas podem ser recomendadas para plantio como culturas principais e áreas de refúgio, respectivamente. Essas descobertas são cruciais para a definição de estratégias regionais de manejo integrado e de resistência de *C. includens* e *S. frugiperda* em áreas de expansão do cultivo de soja no Brasil.

No segundo capítulo decidimos verificar e investigar os efeitos da aplicação da bioestimulante de extrato de nim, aplicado isoladamente e em combinação com o derivado de fitormônio metil jasmonato, pode induzir resistência em plantas de soja à *S. frugiperda.* Mediante ao que foi encontrado no estudo aplicação de formulado a base de Nim juntamente com um jasmonato de metila demonstrou uma indução de resistência para a lagarta devido a não conversão de alimento em ganho de peso e crescimento das mesmas, como também foi observado alterações nos parâmetros das plantas como crescimento de raiz podendo afetar a produção da cultura.

Portanto, os resultados obtidos nos dois trabalhos da tese são importantes para o manejo integrado de pragas em excepcional no manejo de resistência de planta a insetos seja induzida e constitutivas na cultura da soja.