UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS RURAIS
PROGRAMA DE PÓS-GRADUAÇÃO EM AGRONOMIA

Eduardo Perkovski Machado

SOBREVIVÊNCIA E DESENVOLVIMENTO DE ESPÉCIES DE *Spodoptera* (LEPIDOPTERA: NOCTUIDAE) EM SOJA EXPRESSANDO Cry1Ac E Cry1F E IMPLICAÇÕES NO MANEJO DA RESISTÊNCIA

Santa Maria, RS
2020

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Dissertação apresentada ao Curso de Mestrado do Programa de Pós-graduação em Agronomia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Mestre em Agronomia**.

Orientador: Prof. Dr. Oderlei Bernardi

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This study was financied in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Finance Code 001

Machado, Eduardo Perkovski
Sobrevivência e desenvolvimento de espécies de Spodoptera (Lepidoptera: Noctuidae) em soja expressando Cry1Ac e Cry1F e implicações no manejo da resistência / Eduardo Perkovski Machado.- 2020.
63 p.; 30 cm

Orientador: Oderlei Bernardi
Dissertação (mestrado) - Universidade Federal de Santa Maria, Centro de Ciências Rurais, Programa de Pós Graduação em Agronomia, RS, 2020

1. Lagarta-do-cartucho 2. Soja Bt 3. Tabela de vida de fertilidade I. Bernardi, Oderlei II. Título.

Sistema de geração automática de ficha catalográfica da UFSM. Dados fornecidos pelo autor(a). Sob supervisão da Direção da Divisão de Processos Técnicos da Biblioteca Central. Bibliotecária responsável Paula Schoenfeldt Patta CRB 10/1728.

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Aprovado em 07 de fevereiro de 2020:

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Santa Maria, RS
2020

DEDICATÓRIA

A Deus, pelo dom da vida e por estar sempre ao meu lado em todos os momentos.

AGRADEÇO

A minha mãe Rosângela Salete Perkovski e minha irmã Andressa Perkovski Machado Follmann, aos meus avós Niraci Machado e Ildo Bittencourt Machado, ao meu pai José Ariosto Machado e ao meu padrasto Marcos Melo Neves pelo afeto, incentivo e apoio em minha formação pessoal e profissional.

DEDICO E AGRADEÇO

AGRADECIMENTOS

À Universidade Federal de Santa Maria e ao Programa de Pós-Graduação em Agronomia pela oportunidade de realizar mestrado e pelo conhecimento adquirido.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela concessão da bolsa de estudo.

Ao Prof. Dr. Oderlei Bernardi pela orientação, ensinamentos, confiança, amizade e oportunidade para realização deste trabalho.

À Corteva Agriscience™ pelo apoio para a realização deste estudo, em especial à Luiz Henrique Marques e Antonio Cesar Santos.

A todos os professores do Programa de Pós-Graduação em Agronomia da Universidade Federal de Santa Maria pelos conhecimentos transmitidos.

Ao Professor Dr. Enio Marchesan pelo apoio e incentivo durante a realização dos trabalhos.

Aos colegas e amigos de pós-graduação Júnior Cesar Somavilla, Dionei Schmidt Muraro, Regis Felipe Stacke, Bruna Hettwer, Leonardo Burtet, Maiquel Pizzutti, Gabriel Donato, Vagner Ludwig, Regis Trentin Piovesan, Hector dos Santos Facco e Raí Ferreira pela companhia, troca de conhecimento e amizade.

Aos bolsistas e estagiários que são ou foram do Grupo de Pesquisa em Resistência de Insetos (G-PRI), Cínthia Gabriela Garlet, Daniela Neves Godoy, Gisele Elisa Cossa, Gerson Luis dos Santos Rodrigues Júnior, Manoela Hanich, Stefan Luis Zago, Fábio Miguel Fuhr, Alexandre Castro Reis e Rafaella Pretto Moreira, pela amizade, ajuda na coleta de dados e condução deste trabalho.

À minha namorada Cínthia Gabriela Garlet, pelo apoio, companheirismo desde a graduação e compreensão pelos meus momentos de ausência, principalmente durante os meses de aperfeiçoamento profissional.

A todos os funcionários do Departamento de Defesa Fitossanitária da UFSM, pela ajuda e serviços prestados.

A todos aqueles que direta ou indiretamente contribuíram para o êxito deste trabalho, o meu sincero agradecimento.

Muito Obrigado!

"A primeira condição para modificar a realidade consiste em conhecê-la."
Eduardo Galeano

RESUMO

SOBREVIVÊNCIA E DESENVOLVIMENTO DE ESPÉCIES DE *Spodoptera* (LEPIDOPTERA: NOCTUIDAE) EM SOJA EXPRESSANDO Cry1Ac E Cry1F E IMPLICAÇÕES NO MANEJO DA RESISTÊNCIA

AUTOR: Eduardo Perkovski Machado
ORIENTADOR: Oderlei Bernardi

As espécies do gênero *Spodoptera* (Lepidoptera: Noctuidae) têm causado danos significativos na cultura da soja, *Glycine max* (L.) Merril no Brasil. Dentre essas espécies destacam-se *Spodoptera frugiperda* (Smith, 1797), *Spodoptera eridania* (Stoll, 1782), *Spodoptera cosmioides* (Walker, 1858) e *Spodoptera albula* (Walker, 1857). Recentemente, uma nova tecnologia de soja geneticamente modificada que expressa as proteínas inseticidas Cry1Ac e Cry1F (evento DAS-81419-2) de *Bacillus thuringiensis* (Berliner) foi liberada para uso comercial no Brasil, a qual pode ser uma opção para o manejo dessas espécies. Nesse sentido, foram realizados estudos para avaliar a sobrevivência e o desenvolvimento de linhagens resistentes de *S. frugiperda* ao milho Bt expressando Cry1F e Cry1F/Cry1A.105/Cry2Ab2, híbridos F_1 das linhagens selecionadas (heterozigotos) e suscetíveis em soja Cry1Ac/Cry1F. Também avaliou-se a sobrevivência e desenvolvimento de populações de *S. eridania*, *S. cosmioides* e *S. albula* na soja Cry1Ac/Cry1F. No primeiro estudo, neonatas de uma linhagem suscetível de referência de *S. frugiperda* não sobreviveram na soja Cry1Ac/Cry1F. No entanto, neonatas das linhagens resistentes e heterozigotos foram capazes de sobreviver e originar adultos férteis quando alimentados com folhas de soja Cry1Ac/Cry1F. Estudos da tabela de vida de fertilidade revelaram que os insetos resistentes apresentaram similar sobrevivência, desenvolvimento e crescimento populacional em soja Cry1Ac/Cry1F e não-Bt. Em contraste, os heterozigotos apresentaram menor sobrevivência e crescimento populacional em soja Cry1Ac/Cry1F. No segundo estudo, populações de *S. eridania* e *S. cosmioides* apresentaram menor sobrevivência larval e maior duração da fase larval e do período ovo-adulto em soja Cry1Ac/Cry1F. Isso reduziu o crescimento populacional destas espécies em soja Cry1Ac/Cry1F em laboratório. *Spodoptera albula* também apresentou uma menor sobrevivência larval e número de insetos que atingiram a fase adulta na soja Cry1Ac/Cry1F. No entanto, não foram detectados efeitos significativos da soja Cry1Ac/Cry1F em parâmetros de crescimento populacional desta espécie. Em resumo, a resistência de *S. frugiperda* ao milho Cry1F e Cry1F/Cry1A.105/Cry2Ab2 afeta negativamente a eficácia da soja Cry1Ac/Cry1F, permitindo a sobrevivência dos resistentes e heterozigotos. Portanto, a resistência de *S. frugiperda* a proteínas Cry1 expressas em milho Bt resulta em resistência cruzada com as proteínas Bt expressas na soja Cry1Ac/Cry1F. Ainda, a soja Cry1Ac/Cry1F reduz a sobrevivência e o desenvolvimento de *S. eridania* e *S. cosmioides*, causando supressão do crescimento populacional. Entretanto, a soja Bt não tem efeitos relevantes na sobrevivência e crescimento populacional de *S. albula*.

Palavras-chave: Lagarta-do-cartucho. Soja Bt. Tabela de vida de fertilidade.

ABSTRACT

SURVIVAL AND DEVELOPMENT OF SPECIES OF *Spodoptera* (LEPIDOPTERA: NOCTUIDAE) ON SOYBEAN EXPRESSING Cry1Ac AND Cry1F AND IMPLICATIONS FOR RESITANCE MANAGEMENT

AUTHOR: Eduardo Perkovski Machado
SUPERVISOR: Oderlei Bernardi

Species of the genus *Spodoptera* have caused significant damage to soybean, *Glycine max* (L.) Merril in Brazil. Among these species, stand out *Spodoptera frugiperda* (Smith, 1797), *Spodoptera eridania* (Stoll, 1782), *Spodoptera cosmioides* (Walker, 1858) and *Spodoptera albula* (Walker, 1857). Recently, a new genetically-modified soybean technology that expresses Cry1Ac and Cry1F (event DAS-81419-2) proteins has been released for commercial use in Brazil, which may be an option for the management of these species. Here, were conducted studies to evaluate the survival and development of resistant *S. frugiperda* strains selected on Bt-maize single (Cry1F) and pyramided (Cry1F/Cry1A.105/Cry2Ab2) events and F_1 hybrids (heterozygotes) of the selected and susceptible strains on genetically-modified soybeans (event DAS-81419-2) expressing Cry1Ac and Cry1F. Were also evaluate the survival, development and population growth of *S. eridania*, *S. cosmioides* and *S. albula* on Cry1Ac/Cry1F-soybean. In the first study, susceptible insects of *S. frugiperda* did not survive on Cry1Ac/Cry1F-soybean. However, homozygous-resistant and heterozygous insects were able to survive and emerge as fertile adults when fed on Cry1Ac/Cry1F-soybean. Life history studies revealed that homozygous-resistant insects had similar development, reproductive performance and population increases on Cry1Ac/Cry1F-soybean and non-Bt soybean. In contrast, heterozygotes had their fertility life table parameters significantly reduced on Cry1Ac/Cry1F-soybean. In the second study, *Spodoptera eridania* and *S. cosmioides* showed long development time and low larval and egg-to-adult survival when developed on Cry1Ac/Cry1F-soybean. This negatively affected the population growth of these species. *S. albula* also had low larval survival and number of insects that reached adulthood on Cry1Ac/Cry1F-soybean. However, no significant effects of Cry1Ac/Cry1F-soybean on population growth parameters of this species were detected. In summary, *S. frugiperda* resistance to Cry1F (single) and Cry1F/Cry1A.105/Cry2Ab2 (pyramided) maize negatively affects the efficacy of Cry1Ac/Cry1F-soybean. Therefore, the selection of *S. frugiperda* for resistance to single and pyramided Bt maize can result in cross-crop resistance to Cry1Ac/Cry1F-soybean. The Cry1Ac/Cry1F-soybean also reduces the survivorship and population growth of *S. eridania* and *S. cosmioides* causing population suppression of these species. However, this Bt soybean has no relevant effect on the survival and population growth of *S. albula*.

Keywords: Fall armyworm. Bt soybean. Fertility life table.

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1 INTRODUÇÃO

A soja, *Glycine max* (L.) Merril (Fabaceae: Phaseoleae) é a cultura agrícola de maior importância para o agronegócio brasileiro, com uma área estimada de aproximadamente 36 milhões de hectares na safra 2019/2020 (CONAB, 2020). A capacidade produtiva dessa cultura é limitada por fatores ambientais, dentre eles destacam-se os danos causados pelo ataque de insetos-praga, os quais podem causar danos desde a emergência até a fase reprodutiva da cultura. Os insetos da Ordem Lepidoptera (lagartas) representam um grupo de pragas da soja que podem causar danos significativos à cultura, como redução da população de plantas, desfolha e danos em estruturas reprodutivas (BUENO et al., 2011; HOFFMANN-CAMPO et al., 2012).

A lagarta falsa-medideira, *Chrysodeixis includens* (Walker, 1858) e a lagarta-da-soja, *Anticarsia gemmatalis* Hübner (1818) são considerados lepidópteros-praga primários que atacam a soja no Brasil (BERNARDI, O. et al., 2012; GONÇALVES et al., 2019, JUSTINIANO et al., 2014; LUZ et al., 2018; STACKE et al., 2018). No entanto, tem sido observado um aumento na ocorrência de lepidópteros considerados secundários, como as espécies do gênero *Spodoptera* (BLANCO et al., 2016; BUENO et al., 2011; CONTE et al., 2019; JUSTINIANO et al., 2014). As principais espécies de *Spodoptera* que tem causado danos à cultura da soja no Brasil são: *Spodoptera frugiperda* (Smith, 1797), *Spodoptera eridania* (Stoll, 1782), *Spodoptera cosmioides* (Walker, 1858) e *Spodoptera albula* (Walker, 1857). *Spodoptera frugiperda* tem causado danos na soja em diversas regiões do Brasil, desde o período vegetativo até o reprodutivo, reduzindo a população de plantas, causando desfolha e danos nas inflorescências e legumes (BARROS et al., 2010; HOFFMANN-CAMPO et al., 2012; SILVA et al., 2017a). Por outro lado, *S. eridania* ocorre predominantemente em áreas de produção no cerrado brasileiro, podendo ocasionar danos significativos nos legumes em formação (BORTOLOTTO et al., 2014; SILVA et al., 2017b; SOUZA et al., 2019). *Spodoptera cosmioides* tem sido documentada em diversas regiões do Brasil, podendo consumir quase o dobro da área foliar da soja do que outras espécies do gênero *Spodoptera* (BUENO et al., 2011). No entanto, dentre as espécies de *Spodoptera* que atacam a soja, *S. albula* é a que apresenta menor ocorrência na cultura (LUZ et al., 2018).

No contexto do Manejo Integrado de Pragas (MIP), a principal estratégia de controle de espécies de *Spodoptera* é por meio do uso de inseticidas químicos (BERNARDI, O. et al., 2014; LUZ et al., 2018). No entanto, o uso de outras estratégias de MIP vem ganhando espaço nas últimas décadas, como o uso de inseticidas biológicos e inimigos naturais (BORTOLOTTO et al., 2014; POMARI et al., 2012; SOUZA et al., 2019). Além destas estratégias de MIP, a transgenia de plantas mediante inserção de genes da bactéria *Bacillus thuringiensis* Berliner (Bt) para o controle dos insetos-praga tem disponibilizado novas tecnologias para o manejo de lagartas em soja. O uso das plantas Bt tornou mais eficiente o controle desses insetos em soja, milho e algodão no Brasil (BERNARDI, O. et al., 2012; BLANCO et al., 2016; YANO et al., 2016). Isso contribuiu para a redução no uso de inseticidas químicos, aumento na preservação de inimigos naturais e a integração com o controle biológico, permitindo um refinamento nas estratégias de MIP normalmente utilizadas para o controle de pragas agrícolas (BROOKES, 2018; CIB, 2018).

No Brasil, a liberação de plantas Bt iniciou-se em 2005, com o algodão Bt expressando a proteína inseticida Cry1Ac, o qual tem como alvo de controle *Alabama argillacea* (Hübner, 1818), *Pectinophora gossypiella* (Saunders, 1844) e *Chloridea virescens* (Fabricius, 1781). Em 2007 houve a liberação comercial do primeiro evento de milho Bt expressando Cry1Ab, tendo como principal alvo de controle a lagarta-do-cartucho do milho, *S. frugiperda* (BLANCO et al., 2016). Contudo, somente em 2010 houve a liberação do primeiro evento de soja Bt, a qual expressa Cry1Ac (evento MON 87701 × MON 89788) (CTNBio, 2010). A soja Cry1Ac foi primeiramente cultivada na safra 2013/2014 e apresentou eficácia contra *C. includens* (BERNARDI, O. et al., 2012; YANO et al., 2016), *A. gemmatalis* (BERNARDI, O. et al., 2012), *C. virescens* (BERNARDI, O. et al, 2014a) e *Helicoverpa armigera* (Hübner, 1809) (DOURADO et al., 2016). No entanto, a soja Cry1Ac demonstrou baixa toxicidade para espécies do gênero *Spodoptera* (BERNARDI, O. et al., 2014b; BORTOLOTTO et al., 2017; SILVA et al., 2016). Recentemente, um novo evento de soja Bt (evento DAS-81419-2) foi liberado para comercialização no Brasil (CTNBio, 2016). Essa tecnologia de soja Bt expressa duas proteínas inseticidas (Cry1Ac e Cry1F), cujo intuito foi ampliar a ação sobre o complexo de lepidópteros-praga que atacam a soja. Em condições de campo, a soja Cry1Ac/Cry1F apresentou eficácia para *C. includens*, *A. gemmatalis* e *H. armigera* (MARQUES et al., 2016, 2017). No entanto, não há dados do efeito desta soja Bt sobre as espécies do gênero *Spodoptera*.

No Brasil são cultivados aproximadamente 36 milhões de hectares com a tecnologia Bt, o que representa 62, 79 e 82% do total da área plantada com soja, milho e algodão, respectivamente (CIB, 2018). A grande adoção de plantas Bt para o manejo de insetos-praga e a baixa adoção de estratégias de Manejo da Resistência de Insetos (MRI) tem comprometido a eficácia de várias plantas Bt, devido à evolução da resistência (CARRYÈRE et al., 2019; STORER et al., 2010; TABASHNIK et al., 2009, 2014). No Brasil, *S. frugiperda* evoluiu para resistência às proteínas Cry1F e Cry1Ab expressas em milho Bt (FARIAS et al., 2014; OMOTO et al., 2016). Também foi reportada a resistência cruzada de proteínas do grupo Cry1 (Cry1F, Cry1Ab e Cry1A.105) em populações de *S. frugiperda* (BERNARDI, D. et al., 2015; SANTOS-AMAYA et al., 2015). A resistência de *S. frugiperda* a proteínas Cry1 expressas em milho Bt também afetou a eficácia de tecnologias de algodão Bt (HORIKOSHI et al., 2016). Atualmente, em áreas de produção de soja, milho e algodão no Brasil há uma predominância de populações de *S. frugiperda* portadoras de alelos de resistência às proteínas Cry1 (BERNARDI, D. et al., 2015; FARIAS et al., 2014, 2016; OMOTO et al., 2016; SANTOS-AMAYA et al., 2015, 2017).

Diante disso, além dos insetos-praga primários da soja, as espécies do gênero *Spodoptera* também serão expostas às proteínas Cry1Ac e Cry1F expressas no evento de soja Bt DAS-81419-2. Portanto, entender a sobrevivência e o desenvolvimento de espécies do gênero *Spodoptera* nesta nova tecnologia de soja Bt é particularmente importante para dar suporte aos programas de MIP e MRI. Nesse sentido, esse estudo teve como objetivos:

1) Avaliar a sobrevivência e o desenvolvimento de linhagens de *S. frugiperda* selecionadas para resistência ao milho Cry1F (single) e Cry1F/Cry1A.105/Cry2Ab2 (piramidado), em soja DAS-81419-2 que expressa as proteínas Cry1Ac e Cry1F.

2) Avaliar a sobrevivência e o desenvolvimento de populações de *S. eridania*, *S. cosmioides* e *S. albula* em soja Bt (evento DAS-81419-2) que expressa as proteínas Cry1Ac e Cry1F.

2 ARTIGO 1

Cross-crop resistance of *Spodoptera frugiperda* selected on Bt maize to genetically-modified soybean expressing Cry1Ac and Cry1F proteins in Brazil

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Spodoptera frugiperda is one the main pests of maize and cotton in Brazil and has increased its occurrence on soybean. The resistance of this species to *Bacillus thuringiensis* (Bt) proteins expressed in maize has been characterized in Brazil, Argentina, Puerto Rico and southeastern U.S. Here, we conducted the first studies to evaluate the survival and development of *S. frugiperda* strains that are susceptible, selected for resistance to Bt-maize single (Cry1F) or pyramided (Cry1F/Cry1A.105/Cry2Ab2) events and F_1 hybrids of the selected and susceptible strains (heterozygotes) on genetically-modified soybeans (event DAS-81419-2) expressing Cry1Ac and Cry1F. Susceptible insects of *S. frugiperda* did not survive on Cry1Ac/Cry1F-soybean. However, homozygous-resistant and heterozygous insects were able to survive and emerge as fertile adults when fed on Cry1Ac/Cry1F-soybean. Life history studies revealed that homozygous-resistant insects had similar development, reproductive performance and population increases on Cry1Ac/Cry1F-soybean and non-Bt soybean. In contrast, heterozygotes had their fertility life table parameters significantly reduced on Cry1Ac/Cry1F-soybean. Therefore, the selection of *S. frugiperda* for resistance to single and pyramided Bt maize can result in cross-crop resistance to Cry1Ac/Cry1F-soybean. The importance of these results to integrated pest management (IPM) and insect resistance management (IRM) programs are discussed.

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Introduction

Transgenic plants expressing insecticidal proteins from *Bacillus thuringiensis* Berliner (Bt) have significantly contributed to IPM programs worldwide in the last decades[1–4]. Brazil is one of the largest adopter of biotech crops that express Bt proteins in the world, with approximately 36 million hectares of cultivated area during the 2017/2018 season, representing 62, 79 and 82% of the total area planted with soybean, maize and cotton, respectively[4].

Brazil was also the first country in the world to approve the commercial release of Bt-soybean expressing the Cry1Ac protein (event MON 87701 × MON 89788)[5], which has been cultivated since 2013/2014 season. This biotech event provided control of important soybean pests, such as *Anticarsia gemmatalis* (Lepidoptera: Erebidae), *Chrysodeixis includens*, *Chloridea virescens* and *Helicoverpa armigera* (Lepidoptera: Noctuidae)[6–10]. Recently, a new Bt soybean (event DAS-81419-2) was released for commercialization in Brazil[11]. This soybean technology, known as Conkesta™ (Corteva Agriscience, Wilmington, DE), was developed using an *Agrobacterium* mediated transformation process to express Cry1Ac and Cry1F proteins and phosphinothricin acetyltransferase (PAT)[12]. Under field conditions, Cry1Ac/Cry1F-soybean shows efficacy against *A. gemmatalis, C. includens, C. virescens* and *H. armigera*[13,14].

In Brazilian soybean fields there is an increase in the occurrence of *Spodoptera* species, as *Spodoptera frugiperda* (Lepidoptera: Noctuidae)[15–17] — the main lepidopteran pest of maize (*Zea ma*ys L.) and cotton (*Gossypium hirsutum* L.)[18,19]. Their occurrence in soybean can be explained by their ability to develop in several cultivated plants[20,21], adult dispersion[22], reproductive capacity, multiple generations per year[23] and the Brazilian crop production system where there is an overlap of cultivated host plants (i.e. maize, cotton, sorghum, rice and soybean)[24]. These biological characteristics associated with the crop production landscapes favors the infestation of this pest on distinct cultivated host plants throughout the seasons.

Spodoptera frugiperda field-evolved resistance to Cry1F[25] and Cry1Ab[26] proteins in Brazil, and this also resulted in high survival rates on maize and cotton plants expressing pyramided Bt proteins[27,28]. Field-evolved resistance to Cry1F maize has also been documented in Puerto Rico[29], some areas of the southeastern region of the mainland United States[30] and Argentina[31]. The resistance of *S. frugiperda* to Cry1 proteins expressed in maize negatively affected the performance of Bt cotton technologies[32,33], due to cross-resistance between Bt proteins[34–37]. Therefore, the resistance of *S. frugiperda* to Bt proteins is the main threat to the sustainability of current and future Bt plants used in IPM programs in Brazil.

In the Brazilian crop production landscapes, *S. frugiperda* resistant to Bt proteins will also be exposed to Cry1Ac/Cry1F-soybean. Therefore, evaluating the ability of *S. frugiperda* strains that have been selected for resistance to Bt maize to survive and develop on Cry1Ac/Cry1F-soybean is essential to support IPM and IRM programs. Here, we present data from the first study evaluating the survival and development of *S. frugiperda* strains selected for resistance to single and pyramided maize, as well as F_1 hybrids between these and a susceptible strain (assumed to be heterozygous for Bt resistance), on DAS-81419-2 soybeans that express Cry1Ac and Cry1F proteins.

Results

Plant tissue bioassays

Larval mortality, stunting and weight of the *S. frugiperda* strains fed on leaves of Cry1Ac/Cry1F-soybean (event DAS-81419-2) and non-Bt soybean (isoline) are presented in Table 1. Homozygous-resistant *S. frugiperda* from P-R (selected for resistance to Cry1F/Cry1A.105/Cry2Ab2) and H-R (selected for resistance to Cry1F) strains had similar mortality, stunting and larval weights when fed on Cry1Ac/Cry1F-soybean and non-Bt soybean. In contrast, heterozygous larvae from the crosses (P-R♀ × Sus♂ and H-R♀ × Sus♂) showed a significant higher mortality on Cry1Ac/Cry1F-soybean (68 and 67%) than on non-Bt soybean (< 7% mortality). More than 70% of heterozygous larvae on Cry1Ac/Cry1F-soybean did not reach third instar at 5 days and larval weights were reduced by more than 50% compared to the same strains on non-Bt soybean. The susceptible strain (Sus) had higher mortality (97 and 82%), stunting and weight reduction on Cry1Ac/Cry1F-soybean than on non-Bt soybean (2 and 8% mortality and stunting, respectively).

Life history traits of *S. frugiperda* strains on Cry1Ac/Cry1F soybean

No significant differences in the duration and survival of egg and pupal stages of homozygous-resistant (P-R and H-R) and heterozygous insects on Cry1Ac/Cry1F-soybean and non-Bt soybean were detected (Fig. 1). However, larval stage duration of P-R and H-R strains were significantly shorter (~2 days) on Cry1Ac/Cry1F-soybean, and this also reduced the egg-to-adult period when compared to the same strains developing on non-Bt soybean. Larval and egg-to-adult survival of P-R strain were significantly lower on Cry1Ac/Cry1F-soybean, while H-R strain showed higher survival on non-Bt (Fig. 1). For heterozygotes, the duration of larval (25 and 24 days) and egg-to-adult (43 and 38 days) periods was longer on Cry1Ac/Cry1F-soybean than non-Bt (18 and 33 days, respectively) (Fig. 1). However, the survival of heterozygous larvae on Cry1Ac/Cry1F-soybean was lower than 35%, while on non-Bt was higher than 95%. There was also a reduction in the number of heterozygous insects that completed the life cycle on Cry1Ac/Cry1F-soybean (24% reaching the adult stage), when compared to non-Bt (more than 80% originated adults). In contrast, susceptible insects did not survive until adult stage on Cry1Ac/Cry1F-soybean, while on non-Bt more than 60% developed into adults in 35 days. When fed on the same plant (Fig. S1), P-R insects had higher survival from neonate to adult on Cry1Ac/Cry1F-soybean (82%) than H-R (67%), heterozygous (28%) and Sus (no survival) insects. On non-Bt soybean, the survival of P-R and heterozygous insects (higher than 88%) was similar, while H-R and Sus insects had a lower survival (58% and 75%, respectively).

No significant differences were detected in the larval weights of P-R and H-R insects on Cry1Ac/Cry1F-soybean and non-Bt soybean (Table 2). By contrast, pupal weight of resistant insects was significantly heavier on Cry1Ac/Cry1F-soybean than on non-Bt. Heterozygous larvae from both resistant lines presented lower weight on Cry1Ac/Cry1F-soybean, but the progeny from P-R♀ × Sus♂ had similar pupal weight on Bt and non-Bt soybean, while the insects from H-R♀ × Sus♂ had higher pupal weight on non-Bt. P-R females produced similar number of eggs when larvae developed on Cry1Ac/Cry1F-soybean or non-Bt, while H-R females produced more eggs when their development occurred on Bt-soybean. Females from P-R♀ × Sus♂ fed on Cry1Ac/Cry1F-soybean oviposited low eggs than non-Bt. On the other hand, females from H-R♀ × Sus♂ presented similar number of eggs on Bt and non-Bt soybean. On the same host plant, larval weight was higher for resistant insects on Cry1Ac/Cry1F-soybean than other strains (Fig. 3). In contrast, on non-Bt soybean heterozygotes had higher larval weight. Pupae were heavier for the H-R insects on Cry1Ac/Cry1F-soybean and H-R♀ × Sus♂ on non-Bt. Resistant and heterozygous females on Cry1Ac/Cry1F-soybean produced a similar number of eggs. However, on non-Bt heterozygous females produced more eggs.

Fertility life table parameters of P-R strain on the mean generation time, net reproductive rate, intrinsic and finite rate of population increase was similar on Cry1Ac/Cry1F-soybean and non-Bt (Table 3). Based on this, after ∼40 days, 257 and 326 females from each P-R female are expected on Cry1Ac/Cry1F-soybean and non-Bt, respectively. However, H-R females presented higher fertility life table parameters on Bt soybean. For this strain, after ∼40 days, 297 females/female are expected when feeding on Cry1Ac/Cry1F-soybean, while on non-Bt only 157 females/female in 45 days. By contrast, heterozygotes on Cry1Ac/Cry1F-soybean had their life history parameters negatively affected. For these insects, 94 and 105 females are expected from each female on Cry1Ac/Cry1F-soybean in 44 to 49 days, while on non-Bt soybean more than 540 females/female would be produced in 39 days. This represents a reduction of 80% in the number of females produced per generation on Cry1Ac/Cry1F-soybean. When fertility life table parameters were compared in a same host plant, the homozygous-resistant insects on Cry1Ac/Cry1F-soybean presented shortest generation time, better reproductive performance and population increase (Table 4).

Discussion

The colonies of *S. frugiperda* selected for resistance to single- and pyramided-maize technologies showed high survival on Cry1Ac/Cry1F-soybean. These expected results can be explained by the cross-resistance between Cry1 proteins expressed in Bt plants[34–37] and their low natural susceptibility to Cry1Ac protein as reported in studies with Cry1Ac-cotton[32,38–40], Cry1Ac-soybean[41,42], and diet bioassays containing Cry1Ac[41,43]. The cross-resistance among Cry1 proteins is attributed to their similar amino acid sequence[36] and also their same binding sites in the midgut of *S. frugiperda*[35]. Previous studies also showed that *S. frugiperda* resistant to Bt maize survived on single and pyramided Bt cotton[32,33], indicating cross-crop resistance. Our results also revealed that homozygous-resistant insects had similar development and reproductive performance on Cry1Ac/Cry1F-soybean and non-Bt soybean. These finding indicate that resistant insects have no adaptive disadvantage in the absence of the selection agent, maintaining the resistance frequency in the field[44].

By contrast, heterozygous insects showed lower survival than homozygous-resistant insects on Cry1Ac/Cry1F-soybean, but produced fertile adults. This demonstrates that the Cry1Ac/Cry1F-soybean does not meet the high-dose definition (Bt protein expression that cause more than 95% mortality of heterozygotes)[45] for *S. frugiperda*. The survival of heterozygous insects on Cry1Ac/Cry1F-soybean also contributes to maintaining the resistance allele to Cry1 proteins in field populations. On the other hand, heterozygotes on Cry1Ac/Cry1F-soybean had lower larval weight and longer development time until adults. This feature could be exploited in IPM programs by increasing the exposure on the plant to beneficial arthropods or entomopathogenic agents. Unlike to previous results, the susceptible *S. frugiperda* had complete mortality on Cry1Ac/Cry1F-soybean, due to its high susceptibility to Cry1F protein, as previous reported before the field-evolved resistance of this species to Cry1F-maize[25,27–34].

In the current Brazilian crop production landscapes, with successive cultivation of maize, cotton and soybean, *S. frugiperda* populations are exposed to high selection pressure for resistance to Bt proteins. Resistance has been observed in *S. frugiperda* field populations to several Bt proteins expressed in maize (i.e. Cry1F, Cry1Ab and Cry1A.105) in Brazil[25-28]. Currently, field populations of *S. frugiperda* are composed predominantly by insects carrying Cry1Ab, Cry1F, and Cry1A.105 resistance alleles[26,28,46–48], reflected by the increasing of insecticide applications in fields cultivated with crops expressing these proteins[49]. For example, on single or pyramided maize technologies expressing Cry1 and Cry2 proteins up to four insecticidal sprays may be needed to manage *S. frugiperda*, under extreme infestation[49,50]. Based on this, it is expected that Cry1Ac/Cry1F-soybean may not provide stand-alone protection against *S. frugiperda* under Brazilian field conditions, making this species a non-target pest of this Bt technology. However, Cry1Ac/Cry1F-soybean was developed and does provide high efficacy against the key soybean pests (*A. gemmatalis*, *C. includens* and *H. armigera*) under field conditions in Brazil[13,14], which are the driver pests for the development of Bt traits in soybean. Therefore, in order to maintain the effectiveness of Cry1Ac/Cry1F-soybean over time it is essential the adoption of structured refuge (20% of cultivated area should be planted with non-Bt soybean) to delay or prevent resistance evolution[36,45].

According to our results, alternative IPM strategies will be necessary to control *S. frugiperda* on Cry1Ac/Cry1F-soybean. Therefore, monitoring the presence of larvae and the damage to Cry1Ac/Cry1F-soybean are essential for supporting decision making regarding the use of other IPM tactics. The use of chemical insecticides probably will be the main tactic against *S. frugiperda* on Cry1Ac/Cry1F-soybean. However, Cry1Ac/Cry1F-soybean could also be integrated with biological control agents as baculovirus-based insecticides (e.g. *Spodoptera frugiperda* multiple nucleopolyhedrovirus - SfMNPV)[51] and natural enemies[52]. In summary, IPM and IRM programs that integrate multiple control tactics with diverse mortality factors, rather than just relying on wide scale use of single control tactics like Bt crops, are needed to ensure the sustainability of Bt crops[53] in Brazil, where the resistance of *S. frugiperda* to Bt proteins is already widespread.

Methods

Description of *S. frugiperda* strains

Two putative *S. frugiperda* resistant colonies were selected from a field population collected in maize in Paulínia, São Paulo, Brazil (22°42'38"S and 47°06'26"W) using the F_2 screen method developed by Andow and Alstad[54]. The selection and rearing of resistant colonies was described in detail by Muraro et al.[50]. The homozygous-resistant strains used in this study were H-R (selected for resistance to Cry1F-maize) and P-R (selected for resistance to Cry1F/Cry1A.105/Cry2Ab2-maize). We also used a strain of *S. frugiperda* that has been maintained in the laboratory since 2012 without exposure to Bt proteins. We refer to this colony as a susceptible (Sus). To evaluate putative heterozygous insects, the crossing between resistant♀ × susceptible♂ were performed. We only used heterozygotes from this cross because the resistance is autosomally inherited and heterozygous larvae have demonstrated similar mortality-response to Bt proteins in diet and leaf bioassays[25,27-29,33,50].

Soybean plants

Seeds from Cry1Ac/Cry1F-soybean (event DAS-81419-2) and non-Bt soybean (isoline) were sown in 12-liter plastic pots (4 seeds/pot) in a greenhouse. Before the bioassays, all plants were tested for Bt protein expression using detection kits for Cry1Ac and Cry1F (Envirologix, QuickStix™).

Plant tissue bioassays

Bioassays were performed with soybean leaves from Cry1Ac/Cry1F-soybean (event DAS-81419-2) and non-Bt soybean (isoline) in V_{5}-$_{6}$ and R_{4}-$_{5}$ growth stages. Leaves were removed from the upper third part of the plants and, in the laboratory, were placed on a gelled mixture of agar-water at 2.5% in 100 ml plastic cups. Subsequently, neonates from resistant or susceptible strains or their F_1 hybrid were placed on each cup. Cups were sealed and maintained in a room at 25 ± 2°C, 60 ± 10% RH, and a photophase of 14 h. The experimental design was completely randomized with 10 replicates of 10 neonates/strain/growth stage. Mortality, stunting (larvae that did not reach the 3rd instar) and weight were assessed after 5 days.

Life history traits of *S. frugiperda* strains on Cry1Ac/Cry1F-soybean

Homozygous-resistant, heterozygous or susceptible neonates were reared on leaves of Cry1Ac/Cry1F-soybean (event DAS-81419-2) and non-Bt soybean (isoline) excised from greenhouse-grown plants at the R_1 growth stage. In the laboratory, leaves were cut into pieces and placed on a gelled mixture of agar-water at 2.5% in 50 ml plastic cups. Then, a single neonate (6 replicates of 10 neonates/strain/treatment) was placed in each cup. Leaves were replaced every 48 h and cups were maintained in the same environmental conditions described above. The following life history traits were evaluated: duration and survival of egg, larva, pupa and total cycle periods (egg-to-adult); larval weight at 14 days; pupae weight

24 h after pupal formation; and number of eggs per female. The number of eggs were assessed daily from 18 couples kept in PVC cages (23-cm height × 10-cm diameter) internally coated with a paper towel and closed at the top with a voile-type fabric. To determine the embryonic period and survival, 100 eggs of the 2nd oviposition were obtained from each couple. The eggs were observed daily and the number of hatched larvae was counted.

Data analysis

The number of insects tested, dead and those did not develop to 3rd instar on Cry1Ac/Cry1F-soybean (event DAS-81419-2) and non-Bt soybean (isoline) were used to estimate 95% confidence intervals for the probability of mortality and stunting, according to a binomial distribution. For these analyses, the function *binom.probit* from the package *binom* in R 3.1.0 (R Development Core Team, 2014)[55] was used. Percent mortality and stunting were considered significantly different when the 95% confidence intervals on Cry1Ac/Cry1F-soybean did not overlap the 95% confidence intervals on non-Bt soybean. The life history data of *S. frugiperda* strains on Bt and non-Bt soybean were compared by *t*-test using the PROC TTEST procedure in SAS® 9.1[56]. A fertility life table was also calculated by estimating the mean generation time (T), the net reproductive rate (R_o), and the intrinsic (r_m) and finite (λ) rate of increases by the jackknife technique using "*lifetable.sas*" procedure developed by Maia et al.[57] in SAS® 9.1[56].

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Acknowledgments

This work was supported by National Council for the Improvement of Higher Education (CAPES) that provide a scholarship to the first author. The field population of *S. frugiperda* used in this study were registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen number AF999FD).

Author Contributions

EPM LHM ACS CO and OB conceived and designed the studies; EPM GLSRJ FMF SLZ selected the resistant colonies, performed experiments and collected data; EPM and OB analyzed the data; EPM LHM ACS CO TN MLD and OB interpreted the results, discussed and wrote the manuscript; EPM, OB and LHM coordinated the project. All authors read and approved the final manuscript.

Competing Financial Interests

The authors declare no competing financial interests

Table 1. Percent mortality, stunting (larvae did not reach third instar) and mean weight (mg/larvae) of *S. frugiperda* genotypes after five days on leaves of Bt soybean (DAS-81419-2 event) and non-Bt soybean (isoline) in laboratory trials.

S. frugiperda genotype	% mortality (95% CI)[a]		% stunting (95% CI)[a]		Mean weight ± SE[b]	
	V_{5-6}	R_{4-5}	V_{5-6}	R_{4-5}	V_{5-6}	R_{4-5}
P-R						
DAS-81419-2 soybean	7.0 (3.0 – 13.0) a	10. 0 (5.0 – 17.0) a	19. 0 (12.0 – 27.0) a	14.0 (8.0 – 21.0) a	3.5 ± 0.4 a	5.2 ± 0.3 a
Non-Bt soybean	7.0 (3.0 - 13.0) a	2.0 (0.4 – 6.0) a	14.0 (8.0 – 21.0) a	5.0 (1.9 – 10.0) a	4.0 ± 0.3 a	5.3 ± 0.2 a
H-R						
DAS-81419-2 soybean	9.0 (4.0 – 15.0) a	5.0 (1.9 – 10.0) a	14.0 (8.0 – 21.0) a	12.0 (6.0 – 19.0) a	7.3 ± 0.5 a	3.8 ± 0.2 a
Non-Bt soybean	3.0 (0.8 – 8.0) a	3.0 (0.8 – 8.0) a	9.0 (4.0 – 15.0) a	8.0 (3.0 – 14.0) a	7.5 ± 0.6 a	3.3 ± 0.1 a
P-R♀ × Sus♂						
DAS-81419-2 soybean	68.0 (58.4 – 76.5) a	35.0 (26.2 – 44.7) a	97.0 (91.7 – 99.1) a	70.0 (60.5 – 78.3) a	0.7 ± 0.2 b	1.2 ± 0.2 b
Non-Bt soybean	2.0 (0.4 – 6.8) b	5.0 (1.9 – 10.9) b	3.0 (0.9 – 8.2) b	11.0 (6.0 – 18.4) b	7.0 ± 0.8 a	7.3 ± 0.8 a
H-R♀ × Sus♂						
DAS-81419-2 soybean	67.0 (57.3 – 75.6) a	49.0 (39.3 – 58.7) a	94.0 (87.8 – 97.4) a	76.0 (66.9 – 83.5) a	0.6 ± 0.2 b	1.2 ± 0.2 b
Non-Bt soybean	1.0 (0.1 – 5.5) b	7.0 (3.2 – 13.5) b	3.0 (0.9 – 8.2) b	12.0 (6.7 – 19.6) b	6.2 ± 0.5 a	2.4 ± 0.3 a
Sus						
DAS-81419-2 soybean	97.0 (91.7 – 99.1) a	82.0 (69.5 – 90.7) a	100.0 (96.4 – 100.0) a	100.0 (92.9 – 100.0) a	0.2 ± 0.02 b	0.3 ± 0.1 b
Non-Bt soybean	2.0 (0.4 – 6.8) b	2.0 (0.2 – 10.5) b	8.0 (3.9 – 14.7) b	3.0 (0.8 – 8.2) b	4.0 ± 0.3 a	5.9 ± 0.6 a

a) Values represent means and respective 95% confidence intervals (CIs) or standard error (SE). For each pair of means, those followed by the same letter in each column for each *S. frugiperda* genotype are not significantly different due to non-overlap of 95% CIs. b) A separate *t*-test ($P < 0.05$) was conducted between Bt soybean (DAS-81419-2 event) and the non-Bt soybean (isoline) for each growth stage and *S. frugiperda* genotype (for each pair of means, those followed by different letter in each column for each *S. frugiperda* genotype are significantly different).

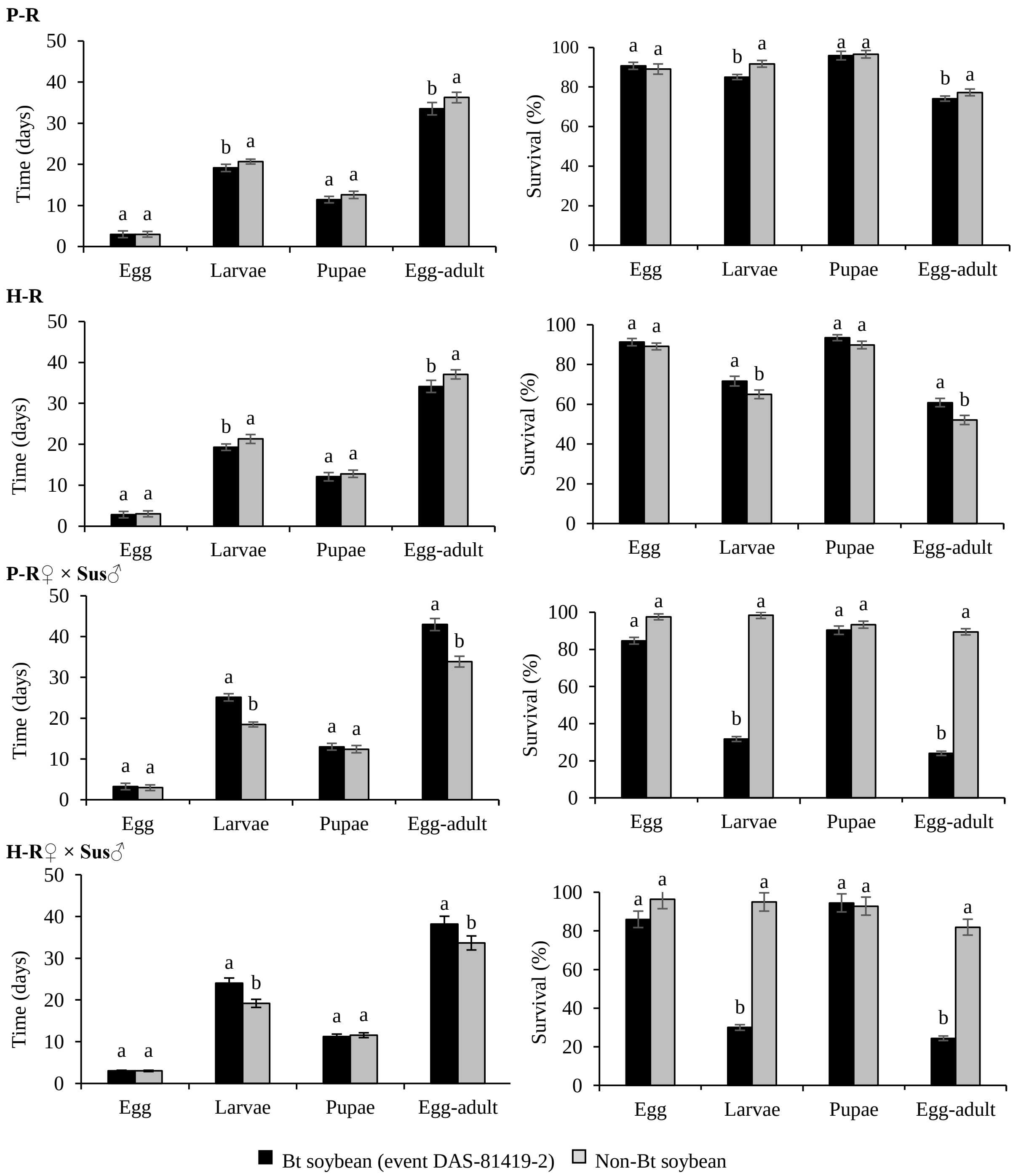

Figure 1. Life history traits of *S. frugiperda* genotypes on leaves of Bt soybean (event DAS-81419-2) expressing Cry1Ac + Cry1F proteins and non-Bt soybean (isoline). Pairs of bars (± SE) with different letters differ significantly by *t*-test (P < 0.05).

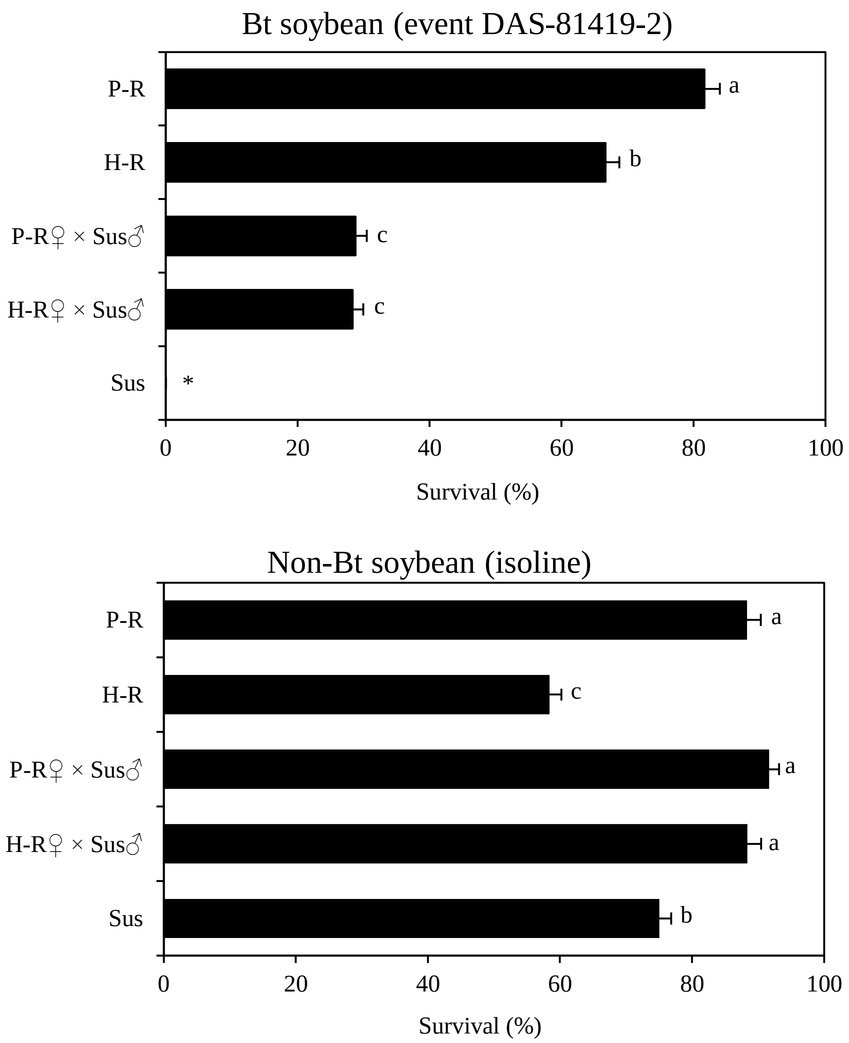

Figure 2. Survival from neonate to adult of *S. frugiperda* genotypes on leaves of Bt soybean (event DAS-81419-2) expressing Cry1Ac + Cry1F proteins and non-Bt soybean (isoline). Bars (± SE) with different letters differ significantly by *t*-test ($P < 0.05$). An asterisk (*) indicates that confidence intervals were not estimated because no variability existed.

Table 2. Biological parameters of *S. frugiperda* genotypes on leaves of Bt soybean (event DAS-81419-2 event) expressing Cry1Ac + Cry1F proteins and non-Bt soybean (isoline).

Biological parameter[a]	Bt soybean (event DAS-81419-2)	Non-Bt soybean (isoline)	*P* value
P-R			
Larval weight at 14 d (mg)	250.0 ± 9.5	231.2 ± 20.2	0.4349
Pupae weight (mg)	154.8 ± 2.8	141.0 ± 4.2	0.0204
Mean eggs/female	742.0 ± 73.3	916.5 ± 75.3	0.1089
H-R			
Larval weight at 14 d (mg)	233.3 ± 19.3	202.4 ± 20.2	0.2942
Pupae weight (mg)	187.5 ± 4.2	157.0 ± 3.9	0.0003
Mean eggs/female	987.7 ± 120.4	636.5 ± 51.2	0.0143
P-R♀ × Sus♂			
Larval weight at 14 d (mg)	72.7 ± 12.2	382.1 ± 17.9	<0.0001
Pupae weight (mg)	169.3 ± 8.2	157.8 ± 9.8	0.1940
Mean eggs/female	859.3 ± 121.0	1318.5 ± 53.8	0.0007
H-R♀ × Sus♂			
Larval weight at 14 d (mg)	73.2 ± 11.8	355.1 ± 12.9	<0.0001
Pupae weight (mg)	146.1 ± 4.3	180.8 ± 4.0	0.0002
Mean eggs/female	953.6 ± 145.4	1511.6 ± 74.8	0.0011

a) Values represent means ± SE. A separate *t*-test ($P < 0.05$) was conducted between Bt soybean (event DAS-81419-2) and the non-Bt soybean (isoline) for each biological parameter.

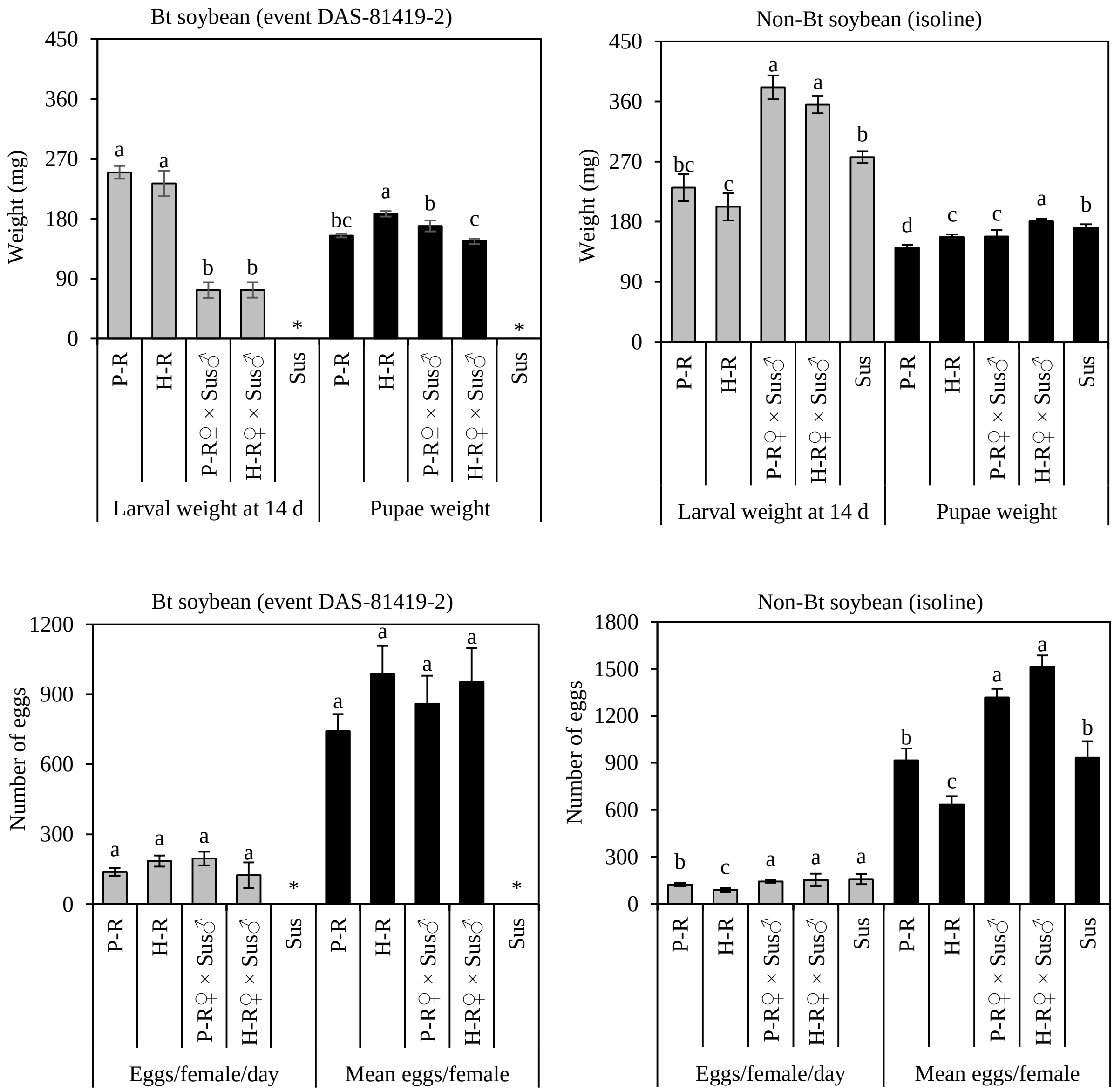

Figure 3. Average weight of larvae and pupae and number of eggs of *S. frugiperda* genotypes on leaves of Bt soybean (event DAS-81419-2) expressing Cry1Ac + Cry1F proteins and non-Bt soybean (isoline). Bars (± SE) with different letters for each variable differ significantly by *t*-test ($P < 0.05$). An asterisk (*) indicates that standard errors were not estimated because no variability existed.

Table 3. Fertility life table parameters of *S. frugiperda* genotypes on leaves of Bt soybean (event DAS-81419-2) expressing Cry1Ac + Cry1F proteins and non-Bt soybean (isoline).

S. frugiperda genotype	Fertility life table parameter[a,b]			
	T (days)	R_o (♀ / ♀)	r_m (♀ / ♀*day)	λ
P-R				
Bt soybean (event DAS-81419-2)	39.37 ± 0.12 a	257.80 ± 24.80 a	0.14 ± 0.003 a	1.15 ± 0.003 a
Non-Bt soybean	40.55 ± 0.29 a	326.45 ± 27.78 a	0.14 ± 0.004 a	1.15 ± 0.002 a
H-R				
Bt soybean (event DAS-81419-2)	40.59 ± 0.51 b	297.97 ± 37.07 a	0.14 ± 0.003 a	1.15 ± 0.003 a
Non-Bt soybean	45.80 ± 0.36 a	156.98 ± 12.56 b	0.11 ± 0.002 b	1.12 ± 0.003 b
P-R♀ × Sus♂				
Bt soybean (event DAS-81419-2)	49.50 ± 0.29 a	94.87 ± 13.36 b	0.09 ± 0.003 b	1.09 ± 0.003 b
Non-Bt soybean	39.77 ± 0.13 b	539.79 ± 22.04 a	0.16 ± 0.001 a	1.17 ± 0.001 a
H-R♀ × Sus♂				
Bt soybean (event DAS-81419-2)	44.83 ± 0.41 a	105.27 ± 16.04 b	0.10 ± 0.003 b	1.11 ± 0.003 b
Non-Bt soybean	39.81 ± 0.14 b	570.16 ± 28.21 a	0.16 ± 0.002 a	1.17 ± 0.001 a

a) T = mean length of a generation (days); R_o = net reproductive rate (females per female per generation); r_m = intrinsic rate of population increase (per day); λ = finite rate of population increase (per day). b) Means within a column followed by the same letter in each *S. frugiperda* genotype are not significantly different (*t*-tests for pairwise group comparisons, $P > 0.05$).

Table 4. Life history traits of *S. frugiperda* genotypes on leaves of Bt soybean (event DAS-81419-2) expressing Cry1Ac + Cry1F proteins and non-Bt soybean (isoline).

S. frugiperda genotype	Fertility life table parameter[a,b]			
	T (days)	R_o (♀ / ♀)	r_m (♀ / ♀*day)	λ
Bt soybean (event DAS-81419-2)				
P-R	39.37 ± 0.12 c	257.80 ± 24.80 a	0.14 ± 0.003 a	1.15 ± 0.003 a
H-R	40.59 ± 0.51 c	297.97 ± 37.07 a	0.14 ± 0.003 a	1.15 ± 0.003 a
P-R♀ × Sus♂	49.50 ± 0.29 a	94.87 ± 13.36 b	0.09 ± 0.003 b	1.09 ± 0.003 b
H-R♀ × Sus♂	44.83 ± 0.41 b	105.27 ± 16.04 b	0.10 ± 0.003 b	1.11 ± 0.003 b
Sus	–[c]	–	–	–
Non-Bt soybean				
P-R	40.55 ± 0.29 c	326.45 ± 27.78 b	0.14 ± 0.004 b	1.15 ± 0.002 b
H-R	45.80 ± 0.36 a	156.98 ± 12.56 c	0.11 ± 0.002 c	1.12 ± 0.003 c
P-R♀ × Sus♂	39.77 ± 0.13 c	539.79 ± 22.04 a	0.16 ± 0.001 a	1.17 ± 0.001 a
H-R♀ × Sus♂	39.81 ± 0.14 c	570.16 ± 28.21 a	0.16 ± 0.002 a	1.17 ± 0.001 a
Sus	42.90 ± 0.39 b	277.84 ± 13.80 b	0.13 ± 0.002 b	1.14 ± 0.002 b

a) *T* = mean length of a generation (days); R_o = net reproductive rate (females per female per generation); r_m = intrinsic rate of population increase (per day); λ = finite rate of population increase (per day). b) Means within a column followed by the same letter in each *S. frugiperda* genotype are not significantly different (*t*-tests for pairwise group comparisons, $P > 0.05$). c) There is no survival insects from Sus genotype on Bt soybean (event DAS-81419-2).

3 ARTIGO 2

Survival and development of *Spodoptera eridania*, *Spodoptera cosmioides* and *Spodoptera albula* (Lepidoptera: Noctuidae) on genetically-modified soybean expressing Cry1Ac and Cry1F proteins

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Running title: Survival and development of *Spodoptera* species on Cry1Ac/Cry1F-soybean

Abstract

BACKGROUND: *Spodoptera eridania*, *Spodoptera cosmioides* and *Spodoptera albula* are considered secondary pests of soybean in Brazil. The genetically-modified soybean (event DAS-81419-2) expressing Cry1Ac and Cry1F provides a potential tool for integrated pest

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management of these species in Brazilian soybean fields. We conducted bioassays to evaluate the survival, development, female fecundity, progeny egg viability and population growth of *S. eridania*, *S. cosmioides* and *S. albula* fed on Cry1Ac/Cry1F-soybean leaf tissue.

RESULTS: *Spodoptera eridania* and *S. cosmioides* exposed to Cry1Ac/Cry1F-soybean showed longer development time, lower larval survival, and lower egg to adult survival compared with those exposed to non-Bt soybean, reducing the population growth of these species. *S. albula* also had lower larval survival and number of insects that reached adulthood on Cry1Ac/Cry1F-soybean. However, no significant effects of Cry1Ac/Cry1F-soybean on population growth parameters were detected in this species.

CONCLUSIONS: Soybean event DAS-81419-2 expressing Cry1Ac/Cry1F proteins reduced the survivorship and population growth of *S. eridania* and *S. cosmioides*. Therefore, it is expected that Cry1Ac/Cry1F-soybean may provide population suppression of *S. eridania* and *S. cosmioides* under Brazilian soybean fields. However, this Bt soybean had smaller effects on *S. albula*, and is unlikely to have population-level effects on this species under field conditions.

Keywords: transgenic soybean; secondary pest species; life history traits; integrated pest management.

1 INTRODUCTION

Soybean, *Glycine max* (L.) Merrill (Fabaceae: Phaseoleae), is the most important agricultural crops in Brazil, being cultivated on approximately 36 million ha during 2019/2020 season.[1] Velvetbean caterpillar, *Anticarsia gemmatalis* (Hübner, 1818) and soybean looper, *Chrysodeixis includens* (Walker, 1858), are considered primary soybean pests.[2-4] However, outbreaks of *Spodoptera* species are being reported more frequently in Brazilian soybean fields, including *Spodoptera frugiperda* (Smith, 1797), *Spodoptera eridania* (Stoll), *Spodoptera cosmioides* (Walker, 1858) and *Spodoptera albula* (Walker, 1857) .[4–6] From these species, some Brazilian researchers and consultants also consider *S. frugiperda* as primary soybean pest in Brazil. *Spodoptera eridania* is considered an important pest on soybean in the Cerrado region[6,7], while *S. cosmioides* occurs in several regions in Brazil and it can consume almost twice the leaf area of soybeans than other species of this genus.[8] In contrast, among these *Spodoptera* species, *S. albula* has relatively lower occurrence.[6]

Spodoptera species are considered secondary soybean pests in Brazil.[4,5] However, infestations of these species can cause high damage on soybean due to their defoliation capacity[6] and attacks on reproductive structures (flowers and pods).[8-11] Outbreaks of *Spodoptera* species on Brazilian fields soybean are influenced by the current crop production landscapes where there is an overlap or succession of cultivated host plants including soybean, cotton, and beans in some regions (specially Central Brazil). This overlap provides food sources for their survival and development throughout the seasons.[9]

The main control tactic against *Spodoptera* species in soybean is carried out with chemical insecticides.[10] Alternative control strategies such as biological insecticides and natural enemies are available, but are rarely used.[7,11,12] Another control tactic used against lepidopteran pests in soybean is the adoption of transgenic soybean plants expressing *Bacillus thuringiensis* Berliner (Bt) proteins; this has proven to be an effective control strategy against key defoliators (*A. gemmatalis*, *C. includens* and *H. armigera*) of soybean in Brazil.[2,13–17]

Currently, Bt soybean expressing Cry1Ac protein is the main control tactic for management of lepidopteran pests in Brazil, grown on more than 60% of the total area (approximately 22 million hectares) during 2017/2018 season.[18] However, Cry1Ac-soybean does not provide stand-alone protection against *Spodoptera* species.[3,6–8,12] The increase in the occurrence of *Spodoptera* species also may be associated with the widespread use of Bt soybeans (Cry1Ac-soybean), which is highly effective against other important key pests, which provides opportunities for secondary pests, such as *Spodoptera* species become more prevalent. Recently, a new Bt soybean (event DAS-81419-2), known as Conkesta™ technology (Corteva Agriscience, Wilmington, DE) was approved for commercial use in Brazil.[19] This biotech event combines two Bt proteins, Cry1Ac and Cry1F, derived from the *Bacillus thuringiensis kurstaki* and *Bacillus thuringiensis aizawai* subspecies, respectively.[20] Under field conditions, Cry1Ac/Cry1F-soybean shows efficacy against *A. gemmatalis*, *C. includes* and *H. armigera*,[21,22] which are important pests species considered for the development of Bt soybean events.

The effects of Cry1Ac/Cry1F-soybean against *Spodoptera* species have not been previously reported. Understanding the survival and development of *Spodoptera* species on this Bt soybean technology is particularly important to support integrated pest management (IPM), insect resistance management (IRM) programs and best agricultural practices to manage these species in Brazilian soybean fields. Here, we present data from the first studies that evaluate the survival and development of *S. eridania*, *S. cosmioides* and *S. albula* on Bt soybeans expressing both Cry1Ac and Cry1F proteins (event DAS-81419-2).

2 MATERIAL AND METHODS

2.1 Populations of *Spodoptera* species

Two populations of *S. eridania* and *S. cosmioides* and a single population of *S. albula* were collected in commercial plantings of non-Bt soybean (more than 200 larvae per species per location) in distinct soybean growing regions of Brazil (Table 1). After collection, larvae were taken to the laboratory and placed on artificial diet based on white bean, wheat germ and yeast (adapted from Greene et al. 1976)[23], and maintained at 25 ± 2°C with a 14:10 h light:dark photoperiod. Neonates from the F_1 generation of each species and population were used to perform these studies.

2.2 Soybean plants

Seeds from Cry1Ac/Cry1F-soybean (event DAS-81419-2) and non-Bt soybean (isoline) were sown in 12-liter plastic pots (4 seeds/pot) in a greenhouse. Before the bioassays, all plants were checked for Bt protein expression using detection kits for Cry1Ac and Cry1F (Envirologix, QuickStix™).

2.3 Biological parameters of *Spodoptera* species on Cry1Ac/Cry1F-soybean

Bioassays were performed with soybean leaves from Cry1A/Cry1F-soybean (event DAS-81419-2) and non-Bt soybean (isoline) from R_1 to R_5 growth stages. Leaves were removed from the middle third and top of the plants and placed on a gelled mixture of agar-water at 2.5% in 50 ml plastic cups. Each cup was infested with a single neonate and then sealed and maintained in a room at 25 ± 2°C with a 14:10 h light:dark photoperiod. The experimental design was completely randomized with 10 replicates (10 larvae/species/replicate). Leaves were replaced every 48 h. The following biological parameters were evaluated: duration and survival of egg, larva, pupa and total life cycle (egg to adult); larval weight at 14 days; pupae weight 24 hours after pupal formation and number of eggs per female. The number of eggs were assessed daily from 18 couples kept in PVC cages (23-cm height × 10-cm diameter) internally coated with a paper towel and closed at the top with a voile-type fabric. To determine the embryonic period and survival, 50 to 100 eggs of the second oviposition were obtained from each couple. The biological parameters of each *Spodoptera* species on

Cry1Ac/Cry1F-soybean and non-Bt soybean (isoline) were compared by *t*-test ($P < 0.05$) using the PROC TTEST procedure in SAS® 9.1.[24]

2.4 Population growth parameters of *Spodoptera* species on Cry1Ac/Cry1F-soybean

To estimate parameters related to population growth potential, fertility life tables were generated for *Spodoptera* species on Cry1Ac/Cry1F-soybean (event DAS-81419-2) and non-Bt soybean (isoline). The net reproductive rate (R_o), the intrinsic rate of increase (r_m), the mean generation time (T) and the finite rate of increase (λ) were estimated by the jackknife technique using "*lifetable.sas*" protocol developed by Maia et al. in SAS® 9.1.[25] This protocol allows the estimation of confidence intervals for all estimated parameters, and conducted one-sided and two-sided *t*-tests to perform pairwise comparisons between groups ($P < 0.05$).

3 RESULTS

3.1 Biological parameters of *Spodoptera* species on Cry1Ac/Cry1F-soybean

No significant differences in the duration and survival of egg and pupal stages of *S. eridania* and *S. cosmioides* populations fed on Cry1Ac/Cry1F-soybean and non-Bt soybean were detected (Fig. 1). However, larval development was significantly longer for *S. eridania* and *S. cosmioides* on Cry1Ac/Cry1F soybean (31 and 32 days, respectively) than on non-Bt (20 and 18 days, respectively). These species took 10 days longer to develop into adults on Cry1Ac/Cry1F-soybean. Larval survival of *S. eridania* and *S. cosmioides* feeding on Cry1Ac/Cry1F soybean (< 45% survival) compared with those feeding on non-Bt soybean (> 70% survival). This significantly reduced the number of insects that completed the life cycle on Cry1Ac/Cry1F-soybean (10 and 39% survival for *S. eridania* and *S. cosmioides*), compared with those non-Bt soybean (31% and 65% survival for *S. eridania* and *S. cosmioides*) (Fig. 1). In contrast, durations of egg, larval, pupal and egg to adult periods were similar on Bt and non-Bt soybean. However, survival of larvae, pupae and insects that complete the life cycle were reduced for insects fed on Cry1Ac/Cry1F-soybean compared with those fed on non-Bt soybean.

When fed on Cry1Ac/Cry1F-soybean, significant reductions in larval weight of *S. eridania*, *S. cosmioides* and *S. albula* (from 52 to 71%, respectively) were observed (Table 2). Similar effects were observed for pupal weight of *S. eridania* and *S. cosmioides*, which weighed 16 and 25% less than pupae from larvae fed on non-Bt soybean. On the other hand, *S. albula* had similar pupae weight (165.7 *vs* 167.5 mg) when larvae were fed on Bt and non-Bt soybean. Females from *S. albula* and *S. cosmioides* produced similar numbers of eggs when their larval development occured on Bt and non-Bt soybean. By contrast, *S. eridania* females oviposited 55% fewer eggs when larvae developed on Cry1Ac/Cry1F-soybean (Table 2).

3.2 Population growth parameters of *Spodoptera* species on Cry1Ac/Cry1F-soybean

A summary of the life table statistics for each *Spodoptera* species evaluated are shown in Table 3. The estimated population growth parameters indicated that *S. eridania* and *S. cosmioides* populations had higher development time and lower population increases when fed on Cry1Ac/Cry1F soybean than when fed on non-Bt soybean. In contrast, *S. albula* presented similar development time and population increases on Bt and non-Bt soybean. Based on these results, females of *S. eridania* and *S. cosmioides* originated from larvae fed on Cry1Ac/Cry1F soybean produced less than 34 and 415 females per generation (*Ro*), respectively, in an average generation time (*T*) up to 58 days, while females of these species produced more than 178 and 607 females, respectively, in less than 49 days when fed on non-Bt soybean. In a same period, *S. albula* produced a similar number of females on Cry1Ac/Cry1F soybean and non-Bt soybean (156 *vs* 217 females.female^{-1}, respectively).

These results indicate that females from *S. eridania* and *S. cosmioides* produced 93 and 51% less females per generation, respectively, on Cry1Ac/Cry1F soybean. When exposed to Cry1Ac/Cry1F soybean, *S. eridania* and *S. cosmioides* populations also presented an intrinsic rate of population increase lower than 0.07 and 0.10, while finite rate of population increases ranged from 1.04 to 1.11 (Table 3). In other words, for every *S. eridania* and *S. cosmioides* female present on a given day, from 1.04 to 1.11 individuals will be expected on the next day if feeding on Bt soybean, while 1.14 to 1.17 individuals will be expected on non-Bt. These parameters indicated a reduction in the intrinsic and finite rate of population increases of these species on Cry1Ac/Cry1F soybean. In contrast to the other *Spodoptera* species, *S. albula* had similar intrinsic and finite rate of population increase on Bt and non-Bt soybean.

4. DISCUSSION

The Cry1Ac/Cry1F-soybean variety tested in this study (containing event DAS-81419-2) reduced the survivorship, development and population growth of *S. eridania* and *S. cosmioides*. However, this Bt soybean had poor activity against *S. albula*. These results also indicate that *Spodoptera* species evaluated had lower susceptibility to Cry1Ac/Cry1F-soybean than other lepidopteran species, such as *A. gemmatalis, C. includens* and, *H. armigera*,[21,22] which are the main target pests of this biotech event. The relatively low susceptibility of *Spdoptera* species to Cry1Ac and Cry1F proteins expressed on soybean reflects their inherentlyer low sensitivity to these Bt proteins. Similar results were observed for *S. eridania* fed on Cry1Ac/Cry1F-cotton.[26] Previous studies also reported that *S. eridania* and *S. cosmioides* had low susceptibility to Cry1Ac and Cry1F proteins in diet bioassays[12,27–29] and to Cry1Ac-soybean[12,13,30] and Cry1Ac-cotton.[31,32] Consistent with these findings, other species from the *Spodoptera* genus including *Spodoptera frugiperda* (Smith, 1797), *Spodoptera exigua* (Hübner, 1808) and *Spodoptera litura* (Fabricius, 1775) exhibited low susceptibility to

Cry1Ac-soybean,[12,33] Cry1Ac-[34–37] and Cry1Ac/Cry1F-cotton.[36–38] The low susceptibility of *S. eridania, S. cosmioides* and *S. albula* to Cry1Ac and Cry1F also may be associated with low affinity of these Bt proteins to midgut receptors, and faster protein degradation in the larval midgut.[39,40]

Our findings also indicate that distinct populations of *S. eridania* and *S. cosmioides* show similar biological parameters when exposed to Cry1Ac/Cry1F-soybean. Similar effects on survivorship and development were also observed in *S. frugiperda* fed on Cry1Ac-soybean[12], Cry1Ac-cotton[41] and Cry1Ab-maize.[42,43] In contrast, no differences in biological parameters were observed in *S. eridania* and *S. cosmioides* fed on Cry1Ac-soybean,[12,13,30] Cry1Ac-cotton[32] and respective non-Bt plants. The effects of Cry1Ac/Cry1F-soybean on immature stages of *S. eridania* and *S. cosmioides* negatively affected population growth parameters, causing population suppression of these species. In contrast, Cry1Ac/Cry1F-soybean did not significantly affect *S. albula* biological parameters compared to non-Bt plants.

Within the Insect Resistance Management (IRM) context, it is expected that coefficients of selection for resistance will be lower than for the key target pests, and selection pressure for resistance in these *Spodoptera* species to Cry1Ac/Cry1F-soybean will also be lower. However, these species will also be exposed to Cry1Ac and Cry1F expressed in single and pyramided soybean and cotton events, that can reduce their susceptibility to Bt proteins and the lifetime of future Bt technologies. To maintain the effectiveness of Cry1Ac/Cry1F-soybean against key soybean pests it is essential the adoption of an appropriate non-Bt refuge to reduce selection pressure and delay resistance evolution.[44,45] The refuge areas can also assist in resistance management of the secondary pests, as *Spodoptera* species, because it will serve a source of susceptible insects.[43] In the context of Integrated Pest Management (IPM), the effects of Cry1Ac/Cry1F-soybean on *S. eridania* and *S. cosmioides* reducing their larval weight and increasing the developmental time until adulthood, may make the insects more susceptible to natural enemies[14] and entomopathogenic agents[46], which consequently would further reduce population growth and may reduce the population density and outbreaks of *S. eridania* and *S. cosmioides* in Brazilian agricultural landscapes.

Therefore, it is expected that Cry1Ac/Cry1F-soybean may provide population suppression of *S. eridania* and *S. cosmioides* but no significant protection against outbreaks of *S. albula* under Brazilian soybean fields. Thus, scouting for larvae and the damage of these species on Cry1Ac/Cry1F-soybean will help decision making regarding the use of other IPM tactics. Chemical insecticides will likely be the main control tactic against *Spodoptera* species on this Bt soybean. Accordingly, if outbreaks of these species are verified it is recommended that insecticides be applied when the action thresholds are reached.[6] In summary, to extend the benefits of Cry1Ac/Cry1F-soybean against lepidopteran pests that attack soybean in Brazil, this biotech event must be combined with other IPM tools and robust IRM plans to extend the benefits and prolong the durability of this soybean technology.

ACKNOWLEDGMENTS

This work was supported by National Council for the Improvement of Higher Education (CAPES) that provide a scholarship to the first author.

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Table 1. Field populations of *Spodoptera* species used to evaluate the survival and development on Cry1Ac/Cry1F-soybean (event DAS-81419-2).

***Spodoptera* species**	**City, State**	**Farm**	**Latitude**	**Longitude**	**Date**
Spodoptera eridania	Campo Verde, MT	Capão da Onça	15º22'59.91"S	55º09'09"W	January 2019
Spodoptera eridania	Quevedos, RS	Bom Retiro	29°16'18.04"S	53°59'29"W	February 2019
Spodoptera cosmioides	Santa Maria, RS	Estação de Pesquisa	29°42'57.79"S	53°44'03"W	February 2019
Spodoptera comioides	São Pedro do Sul, RS	Ramiro Ebling	29°35'54.23"S	54°14'36"W	February 2019
Spodoptera albula	Sapezal, MT	Céu Azul	13°29'29.29"S	58º26'52"W	February 2019

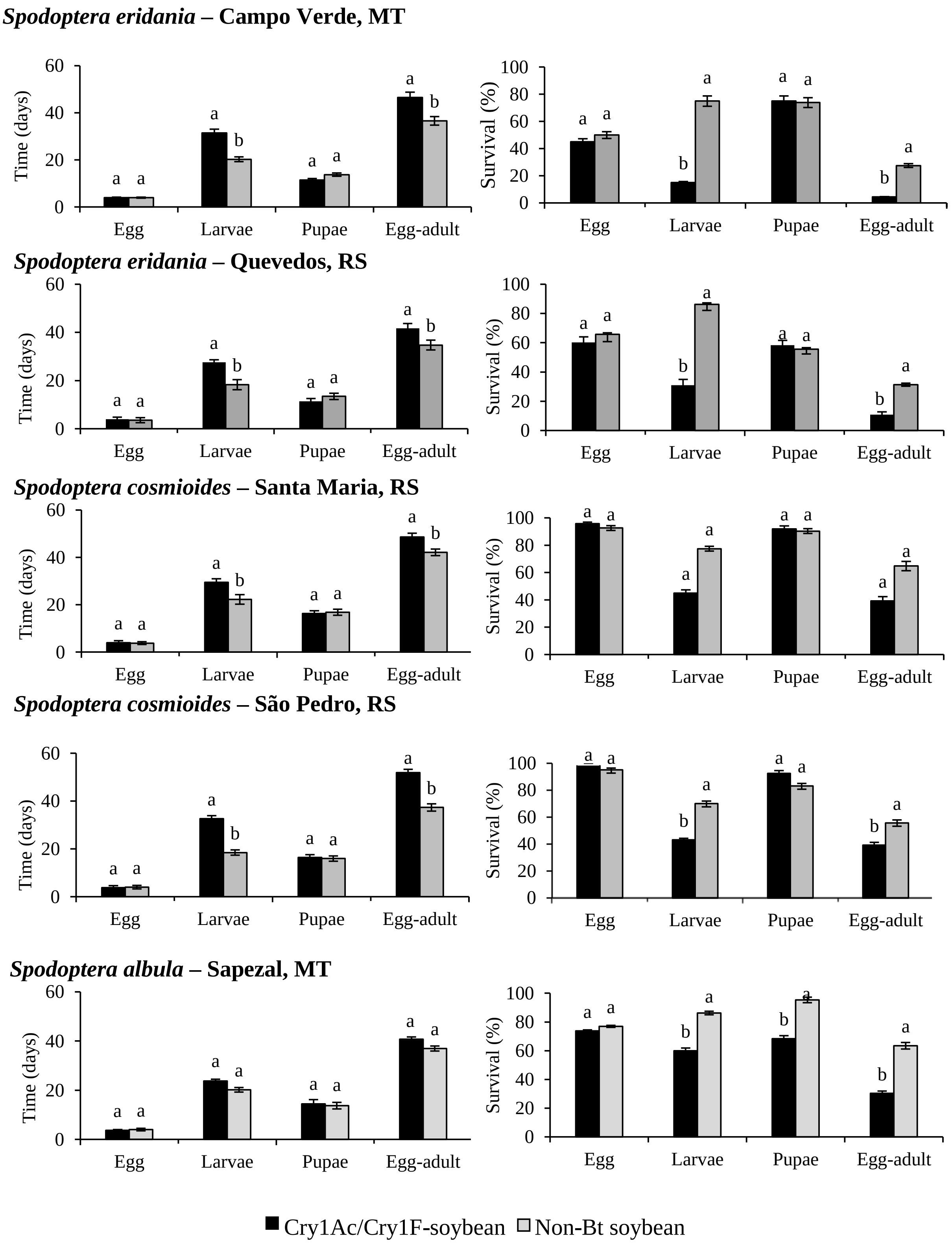

Figure 1. Duration and survival rates of *Spodoptera* species fed on leaves of Cry1Ac/Cry1F-soybean (event DAS-81419-2) and non-Bt soybean (isoline). Pairs of bars (± SE) with different letters differ significantly by *t*-test (P < 0.05).

Table 2. Biological parameters of *Spodoptera* species fed on leaves of Cry1Ac/Cry1F-soybean (event DAS-81419-2) and non-Bt soybean (isoline).

Biological parameter[a]	Cry1Ac/Cry1F-soybean	Non-Bt soybean	*P* value
***Spodoptera eridania* – Campo Verde, MT**			
Larval weight at 14 d (mg)	111.7 ± 8.4	315.3 ± 30.2	<0.0001
Pupae weight (mg)	195.5 ± 12.4	229.9 ± 2.9	0.0275
Mean eggs/female	845.3 ± 152.2	1440.8 ± 95.1	0.0099
***Spodoptera eridania* – Quevedos, RS**			
Larval weight at 14 d (mg)	145.0 ± 9.5	504.7 ± 36.5	<0.0001
Pupae weight (mg)	176.8 ± 5.2	210.1 ± 2.2	0.0004
Mean eggs/female	522.60 ± 91.9	1159.0 ± 91.2	0.0007
***Spodoptera cosmioides* – Santa Maria, RS**			
Larval weight at 14 d (mg)	196.0 ± 6.8	403.1 ± 30.7	<0.0001
Pupae weight (mg)	315.8 ± 6.8	347.9 ± 5.0	0.0023
Mean eggs/female	1852.7 ± 153.4	2284.1 ± 148.9	0.0547
***Spodoptera cosmioides* – São Pedro, RS**			
Larval weight at 14 d (mg)	138.8 ± 18.2	447.0 ± 31.7	<0.0001
Pupae weight (mg)	309.2 ± 8.4	413.1 ± 10.2	<0.0001
Mean eggs/female	2157.2 ± 102.7	2399.4 ± 235.7	0.2990
***Spodoptera albula* – Sapezal, MT**			
Larval weight at 14 d (mg)	159.7 ± 13.1	452.3 ± 9.7	<0.0001
Pupae weight (mg)	165.7 ± 2.4	167.5 ± 2.0	0.5924
Mean eggs/female	990.3 ± 177.5	738.4 ± 87.4	0.1661

[a]Values represent means ± SE. A separate *t*-test ($P < 0.05$) was conducted between Cry1Ac/Cry1F-soybean and the non-Bt soybean for each biological parameter.

Table 3. Comparison of population growth parameters of *Spodoptera* species fed on leaves of Cry1Ac/Cry1F-soybean (event DAS-81419-2) and non-Bt soybean (isoline).

Spodoptera species	Population growth parameter[a,b]			
	T (days)	R_o (♀ / ♀)	r_m (♀ / ♀*day)	λ
***Spodoptera eridania* – Campo Verde, MT**				
Cry1Ac/Cry1F-soybean	52.38 ± 0.25 a	12.64 ± 3.20 b	0.05 ± 0.004 b	1.04 ± 0.001 b
Non-Bt soybean	43.85 ± 0.26 b	178.94 ± 11.81 a	0.14 ± 0.005 a	1.17 ± 0.006 a
***Spodoptera eridania* – Quevedos, RS**				
Cry1Ac/Cry1F-soybean	48.78 ± 0.24 a	33.89 ± 5.94 b	0.07 ± 0.004 b	1.07 ± 0.004 b
Non-Bt soybean	41.61 ± 0.99 b	191.78 ± 35.01 a	0.13 ± 0.002 a	1.14 ± 0.002 a
***Spodoptera cosmioides* – Santa Maria, RS**				
Cry1Ac/Cry1F-soybean	56.43 ± 0.22 a	332.37 ± 27.52 b	0.10 ± 0.002 b	1.11 ± 0.002 b
Non-Bt soybean	49.09 ± 0.22 b	684.77 ± 45.66 a	0.13 ± 0.001 a	1.14 ± 0.001 a
***Spodoptera cosmioides* – São Pedro, RS**				
Cry1Ac/Cry1F-soybean	58.09 ± 0.56 a	415.47 ± 39.63 b	0.10 ± 0.001 b	1.11 ± 0.001 b
Non-Bt soybean	42.60 ± 0.25 b	607.05 ± 59.64 a	0.15 ± 0.003 a	1.16 ± 0.003 a
***Spodoptera albula* – Sapezal, MT**				
Cry1Ac/Cry1F-soybean	47.18 ± 0.41 a	156.64 ± 24.49 a	0.11 ± 0.004 a	1.11 ± 0.005 a
Non-Bt soybean	46.73 ± 0.39 a	217.81 ± 33.83 a	0.12 ± 0.003 a	1.12 ± 0.004 a

[a]T = mean length of a generation (days); R_o = net reproductive rate (females per female per generation); r_m = intrinsic rate of population increase (per day); λ = finite rate of population increase (per day).

[b]Means ± SE within a column followed by the same letter in each *Spodoptera* species are not significantly different (t-tests for pairwise group comparisons, P > 0.05).

4 DISCUSSÃO

A soja geneticamente modificada que expressa as proteínas inseticidas Cry1Ac e Cry1F (evento DAS-81419-2) demonstrou não afetar a sobrevivência e o desenvolvimento de linhagens de *S. frugiperda* com resistência as proteínas Cry1F e Cry1F, Cry1A.105 e Cry2Ab2 expressas em milho Bt. As linhagens resistentes ainda apresentaram similar crescimento populacional em soja Bt e não-Bt. Por outro lado, os heterozigotos apresentaram redução na sobrevivência e inibição no seu desenvolvimento quando alimentados com folhas da soja Cry1Ac/Cry1F. No entanto, os heterozigotos completaram o ciclo biológico em soja Bt e originaram adultos viáveis. Em contraste, não houve sobreviventes da linhagem suscetível de referência de *S. frugiperda* quando exposta à soja Cry1Ac/Cry1F. A mortalidade completa da linhagem suscetível em soja Cry1Ac/Cry1F indica que essa tecnologia apresentaria boa eficácia no controle de *S. frugiperda* se as populações de campo dessa espécie não tivessem evoluído para resistência as proteínas Cry1 expressas em milho Bt.

Quando outras espécies do gênero *Spodoptera* foram alimentadas com folhas da soja Cry1Ac/Cry1F, observou-se que populações de *S. eridania* e *S. cosmioides* apresentaram uma menor sobrevivência e desenvolvimento em soja Bt quando comparado à soja não-Bt. Para ambas as espécies houve uma menor sobrevivência larval, aumento na duração da fase larval e redução no número de insetos que completaram o ciclo biológico. Isso afetou negativamente os parâmetros de crescimento populacional de ambas as espécies em soja Cry1Ac/Cry1F. Diante disso, espera-se que em condições de campo a soja Cry1Ac/Cry1F cause supressão no crescimento populacional de *S. eridania* e *S. cosmioides*, podendo reduzir as infestações e os danos a essa tecnologia Bt. No entanto, essas espécies também estão sendo expostas às proteínas Cry1Ac e Cry1F expressas em eventos de algodão Bt, o que pode reduzir a sua suscetibilidade a essas proteínas Bt e, consequentemente, a vida útil de futuras tecnologias. Nesse cenário, para a sustentabilidade da soja Cry1Ac/Cry1F para o manejo de lepidópteros-praga primários e secundários é essencial a adoção de áreas de refúgio (20% da área cultivada com soja não-Bt) para evitar ou retardar a evolução da resistência. Em contraste, a soja Cry1Ac/Cry1F não afetou a sobrevivência, desenvolvimento e crescimento populacional de *S. albula*. Esses resultados indicam que os coeficientes de seleção para resistência para essa espécie em soja Cry1Ac/Cry1F serão relativamente baixos. Em resumo, em condições de campo, quando ocorrer surtos populacionais de espécies de *Spodoptera* em soja Cry1Ac/Cry1F, outras estratégias de MIP devem ser consideradas para o manejo efetivo destas espécies.

5 CONCLUSÕES

A resistência de *S. frugiperda* ao milho Bt com expressão de Cry1F e Cry1F/Cry1A.105/Cry2Ab2 afeta negativamente a eficácia da soja Cry1Ac/Cry1F, devido à presença de resistência cruzada entre as proteínas Bt.

A soja Cry1Ac/Cry1F reduz a sobrevivência larval e causa supressão no crescimento populacional de *S. eridania* e *S. cosmioides*. No entanto, a soja Cry1Ac/Cry1F não tem efeitos relevantes na sobrevivência larval e no crescimento populacional de *S. albula*.

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