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

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

Rafael Paz Marques

DANOS DE *Melanagromyza sojae* (DIPTERA: AGROMYZIDAE) EM SOJA

Santa Maria, RS
2021

Rafael Paz Marques

Danos de *Melanagromyza sojae* (Diptera: Agromyzidae) em soja

Dissertação apresentada ao Curso 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. PhD. Jonas Andre Arnemann

Santa Maria, RS
2021

Marques, Rafael
Danos de Melanagromyza sojae (Diptera: Agromyzidae) em soja / Rafael Marques.- 2021.
75 p.; 30 cm

Orientador: Jonas André Arnemann
Coorientador: Alberto Cargnelutti Filho
Dissertação (mestrado) - Universidade Federal de Santa Maria, Centro de Ciências Rurais, Programa de Pós Graduação em Agronomia, RS, 2021

1. Redução de produtividade de grãos 2. Manejo 3. Mosca-da-haste da soja 4. Glycine max I. Arnemann, Jonas André II. Cargnelutti Filho, Alberto III. 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.

Endereço: Rua Floresta, n. 212, Camobi, Santa Maria, RS. CEP: 97110-290
Fone: (55) 999144714; E-mail: rafael_pazmarques@hotmail.com

Rafael Paz Marques

DANOS DE *Melanagromyza sojae* (DIPTERA: AGROMYZIDAE) EM SOJA

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

Aprovado em 06 de novembro de 2021:

____

Jonas André Arnemann, Prof. PhD. (UFSM) - Videoconferência
(Presidente/Orientador)

____

Jerson Vanderlei Carús Guedes, Prof. Dr. (UFSM) - Videoconferência

____

Janine Palma, Drª. (Palmagri Pesquisa e Desenvolvimento) - Videoconferência

Santa Maria, RS
2021

AGRADECIMENTOS

Agradeço primeiramente à Deus por ter me dado saúde, força e coragem, sempre me iluminando para o melhor.

À minha mãe, Ana Cláudia Paz, e avó, Iracema Paz, que com amor nunca mediram esforços e sempre apoiaram e confiaram na minha capacidade.

Aos meus tios, Luiz Fernando Paz e Nádia Casarotto Paz por todo o apoio ao longo da jornada.

À minha namorada Júlia Guimarães Bevilaqua por todo o amor, dedicação e companheirismo.

Ao amigo e orientador, PhD. Jonas Arnemann, por ter me recebido em seu grupo de pesquisa, pelas oportunidades de crescimento pessoal e profissional, confiança depositada e conselhos durante o nosso convívio.

Aos colegas e professores integrantes dos grupos de pesquisa Manejo e Genética de Pragas e LabMIP da UFSM, e em especial aos amigos Henrique Pozebon e Guilherme Padilha, pelo companheirismo, incentivo e aprendizado.

A todos os colegas e amigos pelo incentivo e pelos momentos de descontração e aprendizado.

A todos que de alguma forma contribuíram e estiveram comigo durante esta jornada, Muito Obrigado!

RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Agronomia
Universidade Federal de Santa Maria

DANOS DE *Melanagromyza sojae* (DIPTERA: AGROMYZIDAE) EM SOJA

AUTOR: Rafael Paz Marques
ORIENTADOR: Prof. PhD. Jonas André Arnemann
Santa Maria, 06 de novembro de 2021.

A mosca-da-haste da soja, *Melanagromyza sojae* Zehntner (Diptera: Agromyzidae), um importante inseto-praga nativo da Ásia, invadiu e colonizou os principais países produtores de soja na América do Sul. Por ser uma praga invasiva cuja presença foi confirmada recentemente, as estratégias de manejo permanecem em grande parte pouco desenvolvidas devido à falta de informações sobre o seu potencial de ocasionar danos à produtividade da cultura no Brasil e países vizinhos. O objetivo deste trabalho foi quantificar a redução na produtividade ocasionada pelos danos de *M. sojae*. Dois experimentos foram conduzidos durante as safras 2019/2020 (experimento A) e 2020/2021 (experimento B). O delineamento experimental foi inteiramente casualizado com oito tratamentos e dez repetições para o experimento A e sete tratamentos e dez repetições para o experimento B, representando diferentes níveis de convivência entre a praga alvo e as plantas de soja. O número de legumes, número de grãos, massa de 1000 grãos e produtividade de grãos de soja foram quantificados para os terços inferior, médio e superior do dossel das plantas. No experimento A, cada ponto percentual de haste danificada reduziu nos terço inferior, médio e superior ($p<0,0001$), respectivamente: 1,41, 0,71 e 0,75 grãos planta^{-1}; 2,45, 3,10 e 3,36 g na massa de 1000 grãos; e 0,43, 0,24 e 0,23 g planta^{-1} na produtividade de grãos. Considerando os três terços, a redução de produtividade foi de 0,9 g planta^{-1} ($p<0,0001$) para cada ponto percentual da haste danificada. Os resultados deste estudo apontam para um elevado potencial de dano de *M. sojae* nas condições de cultivo brasileiras e destacam a necessidade de desenvolver estratégias de manejo para esta praga na cultura da soja.

Palavras-chave: Redução de produtividade de grãos. Manejo. Mosca-da-haste da soja. *Glycine max.*

ABSTRACT

Master's Thesis
Agronomy Postgraduate Program
Federal University of Santa Maria

Melanagromyza sojae (DIPTERA: AGROMYZIDAE) DAMAGE ON SOYBEAN

AUTHOR: Rafael Paz Marques
ADVISOR: Prof. PhD. Jonas André Arnemann
Santa Maria, November 06th, 2021.

The soybean stem fly, *Melanagromyza sojae* Zehntner (Diptera: Agromyzidae), an important soybean pest in East Asia, has recently invaded and colonized the major soybean producing countries of South America. Management strategies for this pest remain largely undeveloped due to lack of information regarding its damaging potential in Brazil and neighbouring countries. The objective of this study was to quantify soybean yield reduction caused by *M. sojae* damage. Two experiments were carried out during the 2019/2020 (experiment A) and 2020/2021 (experiment B) cropping seasons. The experimental design was completely randomized with eight treatments and ten replicates for experiment A and seven treatments and ten replicates for experiment B, representing different levels of coexistence between the target pest and soybean plants. Number of grains, 1000-grain weight, grain yield and number of pods were quantified for lower, middle and upper canopy segments of the plants. In experiment A, each 1% of injured stem in the lower, middle and upper canopy segments reduced ($p<0.0001$), respectively: 1.41, 0.71 and 0.75 grains/plant; 2.45, 3.10 and 3.36 g of 1000-grain weight; and 0.43, 0.24 and 0.23 g of yield/plant. Considering the three segments, yield reduction reached 0.9 g/plant ($p<0.0001$) for each 1% of injured stem. The findings from this study point to a high damaging potential of *M. sojae* under Brazilian growing conditions and highlight the need to develop management strategies for this pest in soybean crops.

Key-words: Yield losses. Management. Soybean stem fly. *Glycine max.*

LISTA DE ILUSTRAÇÕES

ARTIGO: ***Melanagromyza sojae*** **(Diptera: Agromyzidae) damage on soybean: high yield losses in the New World**

Figura 1 Relationship between percentage of stem length injured by *Melanagromyza sojae* and number of grains/plant in lower ($R^2 = 0.6504$; $P<0.0001$), middle ($R^2 = 0.6011$; $P<0.0001$) and upper ($R^2 = 0.8758$; $P<0.0001$) canopy segments of soybean plants. Data points in the chart represent mean values from experiment A.

Figura 2 Relationship between percentage of stem length injured by *Melanagromyza sojae* and 1000-grain weight in lower ($R^2 = 0.8525$; $P<0.0001$), middle ($R^2 = 0.7678$; $P<0.0001$) and upper ($R^2 = 0.7903$; $P<0.0001$) canopy segments of soybean plants. Data points in the chart represent mean values from experiment A.

Figura 3 Relationship between percentage of stem length injured by *Melanagromyza sojae* and grain yield/plant in lower ($R^2 = 0.8032$; $P<0.0001$), middle ($R^2 = 0.7448$; $P<0.0001$) and upper ($R^2 = 0.8473$; $P<0.0001$) canopy segments of soybean plants. Data points in the chart represent mean values from experiment A.

LISTA DE TABELAS

LISTA DE ABREVIATURAS

AGROFIT	Sistema de Agrotóxicos Fitossanitários
Bt	*Bacillus thuringiensis*
CONAB	Companhia Nacional de Abastecimento
DNA	Ácido desoxirribonucleico
FAOSTAT	FAO Statistical Database
UFSM	Universidade Federal de Santa Maria

SUMÁRIO

1 INTRODUÇÃO

A soja (*Glycine max* (L.) Merril) é uma das culturas de grãos de maior destaque no cenário mundial. O Brasil é o maior produtor e o maior exportador mundial de soja, sendo que a área cultivada na safra 2020/21 foi de 38,5 milhões de hectares (CONAB, 2021). Por ser um ambiente com intenso sistema agro-produtivo, o Brasil apresenta condições ideais para manutenção e desenvolvimento de insetos-praga nativos, que causam injúrias e reduções na produtividade de grãos da cultura, além de favorecer as pragas invasivas (TAY et al., 2017).

Dentre as pragas invasivas detectadas no Brasil e na América do Sul, a mosca-da-haste, *Melanagromyza sojae* (Diptera: Agromyzidae), tem se destacado pela expansão na sua ocorrência na América do Sul, tendo confirmada a sua presença no Brasil em 2015 (ARNEMANN et al., 2016), no Paraguai em 2016 (GUEDES et al., 2017), na Bolívia em 2019 (VITORIO et al., 2019) e na Argentina em 2020 (TROSSERO et al., 2020). As maiores ocorrências de *M. sojae* tem sido observadas em cultivos de soja de segunda safra (semeada no sul do Brasil a partir de 31 de dezembro (FOLLMANN et al., 2017). No cultivo de segunda safra do ano agrícola 2018/2019 no Rio Grande do Sul, foram constatados níveis de infestação entre 97 e 100% (POZEBON et al., 2020).

As larvas dessa espécie formam galerias no interior das hastes das plantas de soja, comprometendo a translocação de água e nutrientes nos vasos do xilema (CHIANG; NORRIS, 1983), o que pode levar a redução da produção de matéria seca das plantas de soja (TALEKAR; CHEN, 1985). Os danos ocasionados pela fase larval de *M. sojae* podem causar em níveis elevados de infestação a murcha das folhas e maturação precoce, o que pode afetar negativamente componentes de rendimento como número de legumes e número de grãos (CZEPAK et al., 2018; TALEKAR, 1989; VAN DEN BERG; SHEPARD; NASIKIN, 1998).

Como os danos ocorrem no interior das hastes das plantas e estas não apresentam sintomas externos facilmente perceptíveis, a ocorrência de *M. sojae* geralmente passa despercebida pelos agricultores. Estudos realizados no continente asiático (região de onde *M. sojae* é endêmica), apontam para reduções de produtividade de 30% na China (DU E

HONG, 1982), 21% no Taiwan (TALEKAR, 1989) e 40% na Índia (JADHAV, 2011). Além disso, há indícios de que o impacto dos danos na produtividade varia conforme o percentual da haste é danificada pelo inseto. Bhattacharjee (1980) indica que a cada ponto percentual da haste consumida pela larva há a redução de 0,18 cm na estatura e de 0,11 g planta^{-1}. Em outro estudo foram observadas reduções de 1,65 e 2,74 g planta^{-1} para cada 10% de haste danificada (VENKATESAN; KUNDU, 1994). No entanto, por ser uma praga invasiva cuja presença foi confirmada recentemente, ainda não foram mensurados os impactos dos seus danos na produtividade de soja na América do Sul. Frente a isso, o objetivo deste trabalho foi quantificar e correlacionar os danos ocasionados por *M. sojae* e os seus efeitos sobre a produtividade de soja sob as condições brasileiras de cultivo.

2 REFERENCIAL TEÓRICO

2.1 A MOSCA-DA-HASTE DA SOJA *Melanagromyza sojae* (ZEHNTNER)

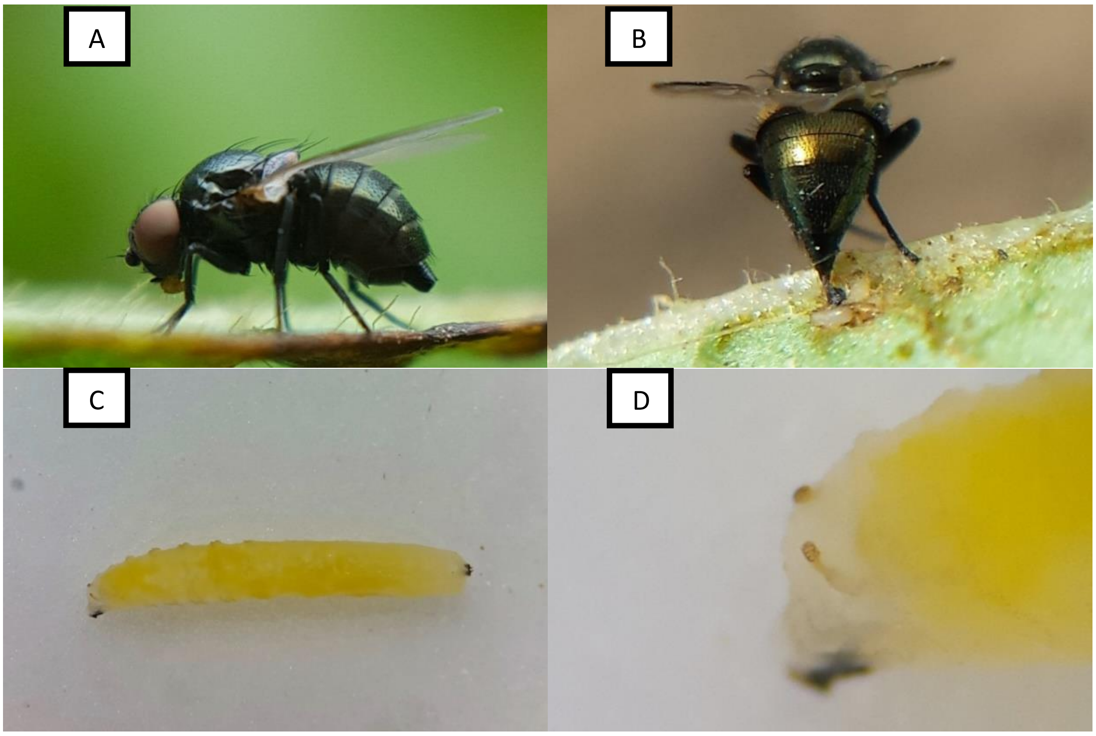
2.2.1 Ocorrência na América do Sul

A soja (*Glycine max* (L.) Merril) é atacada por insetos-praga da família Agromyzidae, como *Melanagromyza sojae*, *Melanagromyza dolichostigma*, *Ophiomyia phaseoli* e *Ophiomyia centrosematis* (SPENCER, 1990; TALEKAR; CHEN, 1985). Dentre estas espécies de dípteros, *M. sojae* destaca-se como um dos principais insetos-praga de países como a Indonésia (VAN DEN BERG et al., 1998), China (WANG; GAI, 2001), Índia (THAPA, 2012), Austrália (BRIER; CHARLESTON, 2013) e em algumas regiões da Rússia (STRAKHOVA et al., 2013).

O primeiro registro da ocorrência do gênero *Melanagromyza* em lavouras de soja na América do Sul foi realizado em 1983, onde foram encontrados espécimes nos municípios de Passo Fundo e Santa Maria, Rio Grande do Sul (GASSEN E SCHNEIDER, 1985). Recentemente, foi confirmada por meio de identificação taxonômica e caracterização do DNA mitocondrial a presença de *M. sojae* na região Sul do Brasil em 2015 (ARNEMANN et al., 2016) e Centro-oeste em 2018 (CZEPAK et al., 2018), no Paraguai em 2016 (GUEDES et al., 2017), na Bolívia em 2019 (VITORIO et al., 2019) e na Argentina em 2020 (TROSSERO et al., 2020). A caracterização do DNA mitocondrial de 79 indivíduos oriundos destes países revelou a presença de 22 haplótipos, o que indica elevada variabilidade genética, em um tamanho de amostra relativamente pequeno, e também pode ser considerada evidência da ocorrência de múltiplas introduções desta praga nestes países (POZEBON, et al., 2021 (a)).

2.2.2 Morfologia e bioecologia

Os adultos desta espécie são moscas com mesonoto de coloração preta e abdômen verde metálico. A envergadura das asas dos machos mede de 1,68 a 2,28 mm (THAPA, 2012). As pupas são cilíndricas e medem cerca de 2 mm, possuem coloração amarelada, passando gradualmente para o marrom ao longo do desenvolvimento. As larvas são de coloração amarelo-claro, translúcidas e medem até 4 mm. Apresentam espiráculos anteriores com forma de botão, os quais possuem oito poros minúsculos. Os espiráculos posteriores são separados e apresentam seis poros elevados no entorno de uma estrutura em forma de chifre (SPENCER, 1973; TALEKAR, 1990).



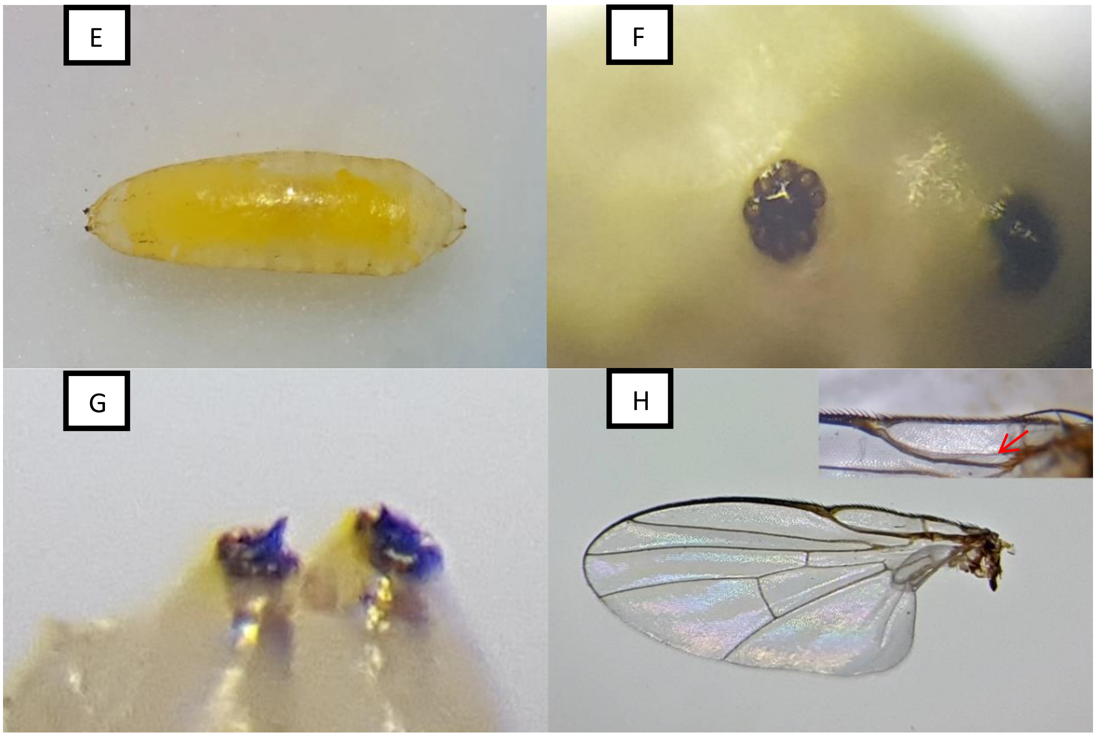


Figura 4 Características morfológicas de Melanagromyza sojae: fêmea adulta (A), fêmea adulta ovipositando (B), larva (C), espiráculos anteriores da larva (D), pupa (E), espiráculos posteriores da pupa (F, G), Detalhe da asa e inserção subcostal (H).
Fonte: (VITORIO et al., 2019)

O ciclo de vida completo da mosca-da-haste compreende as fases de ovo (dois a sete dias), larva (sete a doze dias), pupa (seis a onze dias) e adulta (dez a quarenta e seis dias) (WANG, 1979). O intervalo da fase de ovo até a fase adulta varia de 16 a 26 dias, o que permite que esta espécie complete de três a cinco gerações por ciclo de cultivo da soja (GANGADRE; KOGAN, 1980; SPENCER, 1973; VAN DER GOOT, 1930). As fêmeas de *M. sojae* produzem com o seu aparelho ovipositor uma fenda na epiderme, próxima às nervuras na face adaxial das folhas mais jovens e tenras, onde depositam de um a dois ovos no mesofilo foliar (LEE, 1962). Cada fêmea oviposita em média 170 ovos ao longo da sua vida, podendo variar de 41 a 270 ovos (JADHAV, 2011; WANG, 1979).

Após a eclosão, a larva de *M. sojae* alimenta-se dos tecidos do parênquima foliar em um ritmo médio de 1,4 mm por hora (LEE, 1962) e avança em direção à nervura mais próxima, a qual penetra e forma uma galeria através do pecíolo e chega até a haste principal da planta de soja em até dois dias após a eclosão (TALEKAR; CHEN, 1985). Ao chegar na haste principal, a larva alimenta-se da medula da planta, podendo danificar os tecidos vasculares, consequentemente, podendo comprometer o transporte de água e nutrientes e o fluxo de fotoassimilados (CHIANG; NORRIS, 1983).

Com frequência, são encontradas mais de uma galeria na mesma planta. Geralmente, as galerias no terço inferior das hastes das plantas são mais velhas e de coloração mais escura, iniciando-se na junção do pecíolo das folhas opostas com a haste principal e se estendendo até a região do colo das plantas, indicando que a larva que causou esse dano é oriunda de uma oviposição realizada nessas folhas. Em situações que a oviposição ocorreu em algum trifólio, as galerias podem se estender da junção do pecíolo do trifólio com a haste até a superfície do solo, ou, quando confrontado com a presença de uma galeria mais antiga, até o início desta e, posteriormente, invertendo o seu sentido de alimentação e indo em direção ao ápice da planta, podendo causar a morte do ponteiro (TALEKAR E CHEN, 1985).

Após completar quatro ínstares, a larva atravessa as paredes da haste, danificando o xilema e floema e abre um orifício de saída, o qual fica protegido por detritos para a sua proteção durante a fase de pupa. Por fim, os insetos adultos emergem no interior da haste e saem pelo orifício aberto pela larva (VAN DER GOOT, 1930; WANG, 1979). As fêmeas adultas copulam cinco dias após a emergência e vivem de 15 a 36 dias, enquanto os machos vivem de 10 a 46 dias (SPENCER, 1973).

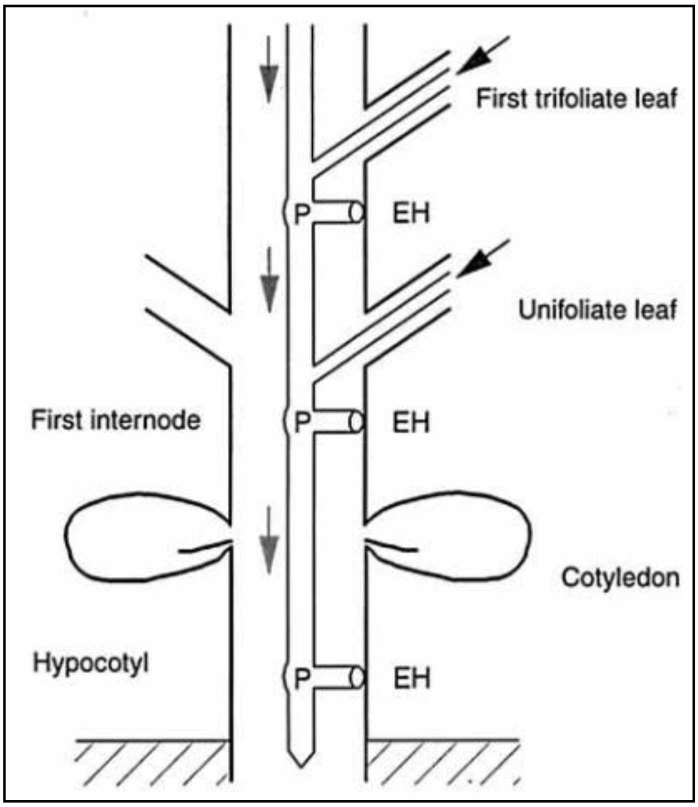


Figura 5 Diagrama representando o terço inferior da haste da planta de soja atacado por *Melanagromyza sojae*, mostrando a galeria formada na medula da planta em decorrência da sua alimentação, a posição da pupa (P) e o orifício de saída (EH).
Fonte: (VAN DEN BERG; SHEPARD; NASIKIN, 1998)

Ciclo de vida curto e alta taxa reprodutiva permitem rápida colonização de lavouras de soja por *M. sojae*, de modo que várias larvas e pupas podem ser encontradas simultaneamente na mesma planta, tanto nas hastes principais quanto nas secundárias (GUEDES et al., 2015). Uma curva de crescimento populacional a partir de dois indivíduos (uma fêmea e um macho) de *M. sojae* foi estimada por Pozebon et al. (2020). Foi considerado 50% de mortalidade natural (por fatores ambientais como clima desfavorável, inimigos naturais, patógenos, canibalismo e morte por idade), ciclo de vida de 21 dias e taxa média de oviposição de 85 ovos por fêmea. A curva demonstra que na ausência de medidas de controle, dois indivíduos precisariam de três a quatro gerações para colonizar todas as plantas de uma lavoura, em uma proporção de 1:1 insetos por planta (Figura 3). Esta é uma curva de crescimento populacional hipotética, que pode ser reduzida a zero devido à condições adversas, no entanto, não é o que têm sido observado em cultivos de soja de segunda safra.

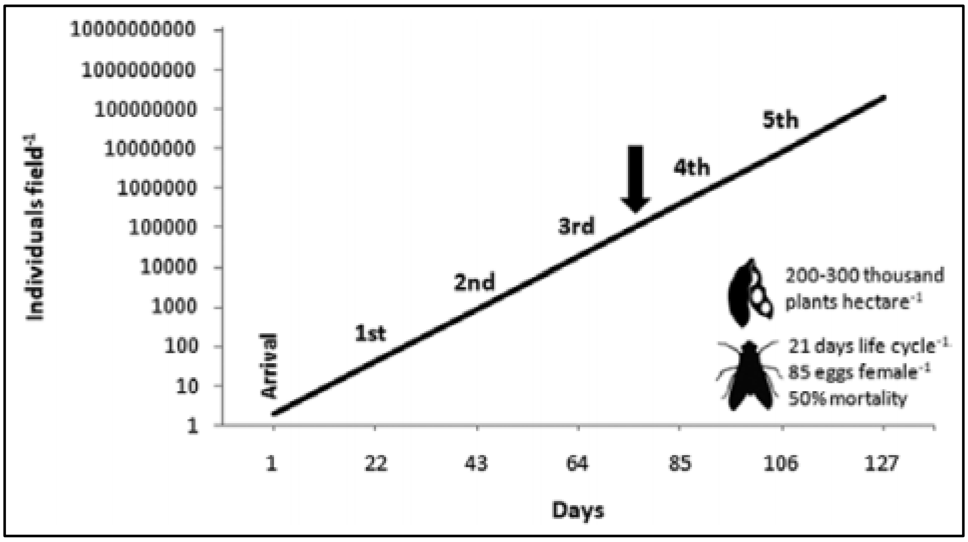


Figura 6 Curva de crescimento populacional de *M. sojae* considerando como população fundadora um macho e uma fêmea, ciclo de vida de 21 dias, 50% de mortalidade natural e taxa de oviposição de 85 ovos por fêmea.
Fonte: (POZEBON et al., 2020)

No continente asiático, a mosca-da-haste pode infestar a soja durante o ano inteiro, no entanto, a sua densidade populacional varia de acordo com a intensidade das chuvas e temperatura. Nas épocas do ano em que as temperaturas são mais amenas, as densidades populacionais de *M. sojae* reduzem consideravelmente. A maior incidência de *M. sojae* em soja ocorre nos períodos mais secos, pois as chuvas mais intensas impedem a alimentação e oviposição dos insetos adultos (TALEKAR E CHEN, 1983; YADAV et al., 2015). No Brasil, as maiores infestações de *M. sojae* têm sido observadas em cultivos de soja de segunda safra, semeados a partir de 31 de dezembro, após a colheita do milho (CÂMARA, 2015; FOLLMANN et al., 2017; POZEBON et al., 2020). No cultivo de segunda safra do ano agrícola 2018/2019, foram constatados níveis de infestação variando entre 97 e 100% em 28 municípios do Rio Grande do Sul (POZEBON et al., 2020).

2.2.3 Danos e manejo de *Melanagromyza sojae* na soja

A mosca-da-haste pode ser considerada uma ‘praga silenciosa’, visto que sua ocorrência frequentemente passa despercebida pelos agricultores. Para detectar a sua presença, é necessária a abertura das hastes das plantas por meio de um corte longitudinal afim de visualizar a presença do inseto ou do dano, tendo em vista que as plantas atacadas podem apresentar poucos ou nenhum sintoma externo (GANGRADE; KOGAN, 1980). Encurtamento de entrenós, ramificação excessiva e o engrossamento do caule podem ser respostas fisiológicas das plantas ao ataque de *M. sojae*, no entanto, nem sempre as plantas expressam esses sintomas. Alguns sinais expressos pelas plantas são apresentados na figura 4.

Os danos ocasionados pelas larvas de *M. sojae* na haste das plantas são resultantes do seu processo de alimentação, onde danificam os tecidos vasculares e, consequentemente, comprometem o transporte de água, nutrientes e o fluxo de assimilados (CHIANG; NORRIS, 1983), o que pode levar a redução da produção de matéria seca das plantas de soja (TALEKAR; CHEN, 1985). Altos níveis de infestação podem causar murcha das folhas e maturação precoce (CZEPAK, 2018). O rendimento de grãos pode ser diretamente afetado, pois as hastes das plantas armazenam a maior parte dos fotoassimilados que são translocados para os grãos durante a fase de enchimento de grãos (STREETER; JEFFERS, 1979). Parâmetros como a estatura da planta, área foliar, número de legumes, número de grãos e a nodulação por *Rhizobium* são afetados negativamente pelos danos ocasionados por *M. sojae* (TALEKAR, 1989; VAN DEN BERG; SHEPARD; NASIKIN, 1998).

Durante a fase adulta, as fêmeas de *M. sojae* perfuram as folhas para fins de oviposição e alimentação, formando pequenas lesões translúcidas na epiderme foliar; no entanto, essas lesões não causam danos significativos às plantas (TALEKAR; CHEN, 1985).

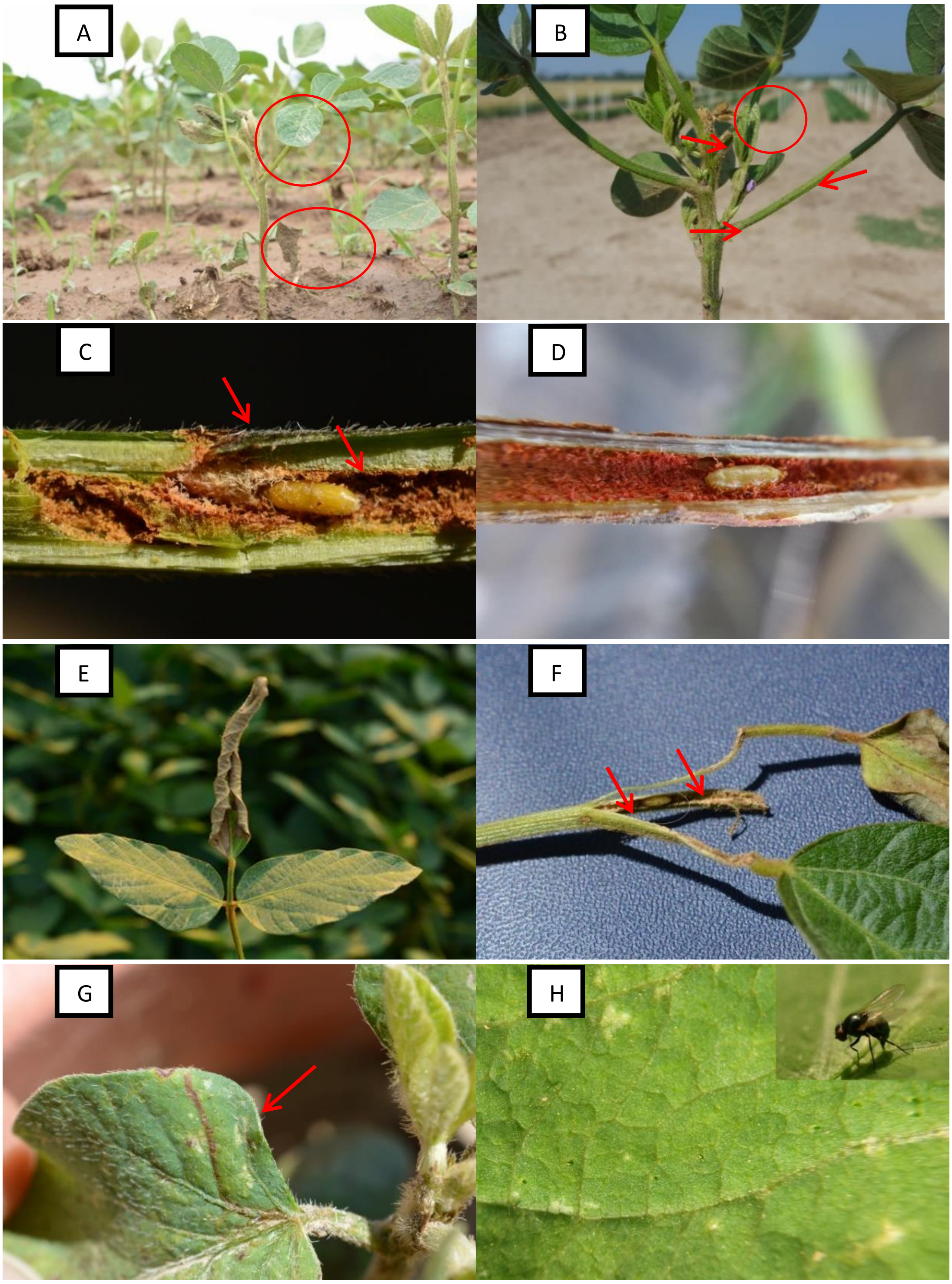


Figura 7 Plantas de soja com injúrias ocasionadas por *Melanagromyza sojae*: Murcha das folhas opostas (A), Brotos mumificados (B), pupa e orifício de saída na haste principal (C), haste danificada (D), folíolo seco (E), pupa e orifício de saída no pecíolo (F), nervura danificada na folha oposto (G), puncturas de alimentação dos adultos (H).

Fonte: (VITORIO et al., 2019)

As estimativas de redução de produtividade variam conforme a região de cultivo, estado nutricional das plantas, genótipo de soja utilizado, data de semeadura, estádio de crescimento das plantas no momento da infestação e as práticas de manejo adotadas (JADHAV et al., 2013; SAVAJJI, 2006; TALEKAR; CHEN, 1985; VAN DEN BERG; SHEPPARD; NASIKIN, 1998). Características morfo-fisiológicas das cultivares de soja como a densidade de tricomas, área foliar, diâmetro da haste e conteúdo de água também influem nos danos ocasionados (TALEKAR; CHEN, 1985). Embora *M. sojae* possa ocorrer ao longo de todo o ciclo da cultura, as perdas mais significativas de produtividade são observadas quando as plantas de soja são atacadas nos estádios iniciais de desenvolvimento (TALEKAR, 1989). O intervalo crítico para infestação de *M. sojae* é de quatro a cinco semanas após a emergência da planta; quanto mais tarde ocorre o dano, menos o rendimento de grãos é afetado, pois as galerias das larvas não atinge os tecidos do xilema (CABI, 2020; SPENCER, 1973; TALEKAR; CHEN, 1983).

As galerias formadas pelas larvas de *M. sojae* podem ocupar até 70% do comprimento da haste e reduzir a produtividade em até 36% (SINGH E SINGH, 1990). Bhattacharjee (1980) aponta que a cada ponto percentual da haste injuriada, há a redução de 0,18 cm na estatura e de 0,11 g planta^{-1} na produtividade de grãos (Figura 5). Em outro estudo, foram observadas reduções de 1,65 e 2,74 g planta^{-1} para cada 10% de haste danificada (VENKATESAN; KUNDU, 1994). Além disso, foram observadas reduções na produtividade de 30% na China (DU E HONG, 1982), 21% no Taiwan (TALEKAR, 1989) e 40% na Índia (JADHAV, 2011).

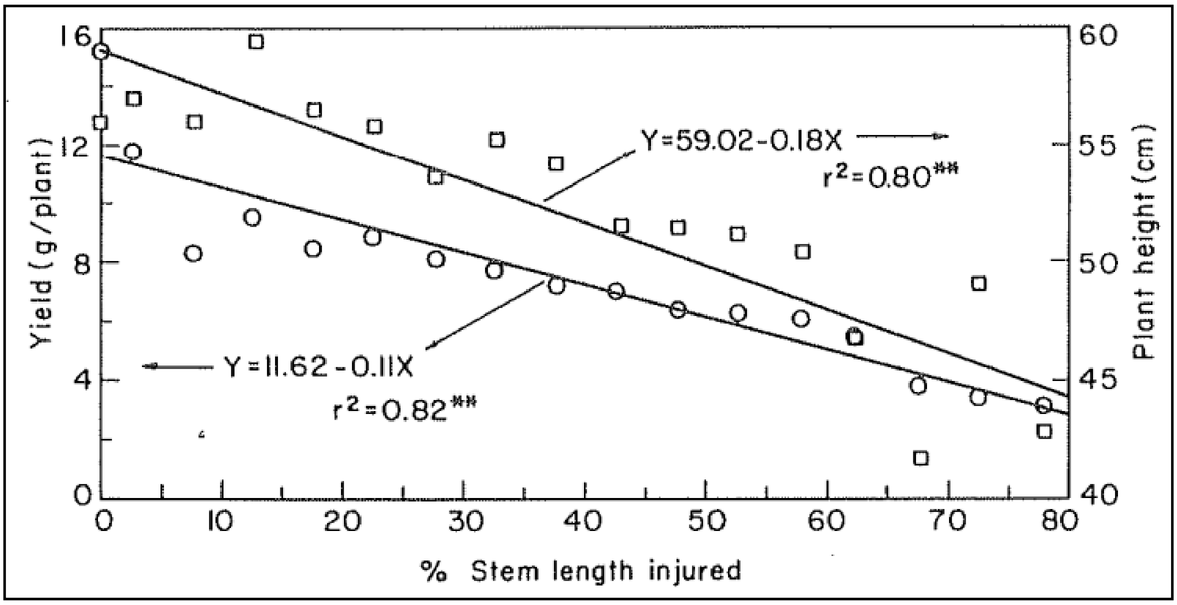


Figura 8 Estatura (cm) e produtividade de grãos (g plant^{-1}) de plantas de soja em resposta à percentagem de haste danificada por *Melanagromyza sojae*.
Source: (BHATTACHARJEE, 1980).

Normalmente costumam ocorrer diferentes estágios de vida de *M. sojae* simultaneamente nas lavouras de soja, e até mesmo na mesma planta, o que exige a adoção de mais de uma estratégia de controle (POZEBON, 2021b). Nos países asiáticos, região endêmica da *M. sojae*, adotam-se estratégias de manejo como a utilização de cultivares tolerantes (TALEKAR, 1980), semeaduras no início da estação de cultivo (TALEKAR; CHEN, 1983), controle biológico com parasitoides (TALEKAR, 1990; VAN DEN BERG et al., 1995), pulverização de inseticidas na semeadura e pós-emergência (ADAK, 2021; JADHAV et al., 2013).

No Brasil, a utilização de plantas geneticamente modificadas que expressam proteínas Bt e o controle químico com inseticidas são os métodos mais utilizados para o manejo de insetos na cultura da soja. A soja geneticamente modificada MON 87701 x MON 89788 que codifica a proteína Cry1Ac foi liberada para uso comercial no Brasil em 2010 (BERNARDI, 2012). Yu et al. (2014), ao estudarem a exposição de artrópodes não-alvo à Cry1Ac em lavouras de soja mencionaram a presença de níveis detectáveis da proteína Bt em adultos de *M. sojae*, no entanto, não foram relatados possíveis efeitos sobre o desenvolvimento destes insetos. As principais proteínas Bt que afetam os dípteros são Cry4Aa, Cry4Ba, Cry11Aa, Cyt1Aa, Cry10Aa e Cyt2Ba (BEN-DOV, 2014), porém, ainda não há cultivares comerciais que expressem essas proteínas.

Frente à escassez de informações a respeito da suscetibilidade de cultivares comerciais à *M. sojae*, além da inexistência de cultivares que expressem proteínas Bt com efeito sobre esses insetos, o manejo fica restrito à utilização de inseticidas. O uso de inseticidas na semeadura (via tratamento de sementes ou no sulco de semeadura) combinados com aplicações foliares logo após a emergência da cultura protegem as plantas durante a fase de desenvolvimento mais sensível ao ataque de *M. sojae* (CURIOLETTI et al., 2018). Embora os adultos de *M.* sojae não causem prejuízos econômicos às lavouras de soja, eles são facilmente controlados por pulverizações de inseticidas, ao passo que isso não ocorre para as larvas, pois estas permanecem protegidas dentro da haste das plantas. Independentemente do método de aplicação de inseticidas escolhido, eficácias de controle satisfatórias somente são obtidas antes da entrada da larva na haste da planta e após a emergência do adulto (POZEBON, 2021b). No Brasil, há 460 inseticidas formulados registrados para utilização na soja, no entanto, não há produtos registrados para o manejo de mosca-da-haste (AGROFIT, 2021). Apesar disso, alguns produtos possuem boa eficácia no controle desta praga, como por exemplo clorantraniliprole, fipronil, imidacloprido e tiodicarbe via tratamento de sementes, e imidacloprido, bifentrina e tiametoxam via aplicação foliar (CURIOLETTI et al., 2018).

Em países asiáticos, a ocorrência de parasitoides como *Gronotoma* sp., *Eurytoma* sp. e *Bracon* sp. emprega em torno de 70% de parasitismo de *M. sojae* (TALEKAR, 1990). Como apenas as larvas são atacadas pelos parasitoides, os danos não são evitados, no entanto a densidade populacional de *M. sojae* diminui, ao passo que a densidade populacional dos parasitoides aumenta, diminuindo os danos que seriam causados pelas futuras gerações de *M. sojae* (VAN DEN BERG et al., 1995). No Brasil foi relatado a ocorrência de parasitismo de *M. sojae* por *Syntomopus parisii*, com perspectivas de sucesso no controle biológico em escala comercial (BECHE, 2018). Outras estratégias de manejo como a rotação de culturas, especialmente com monocotiledôneas, auxiliam no manejo desta praga, visto que ainda não foi reportada a ocorrência de *M. sojae* em plantas deste grupo (FERREIRA et al., 2020). Além disso, plantas de soja voluntárias (originadas de perdas de colheita) devem ser eliminadas para reduzir a densidade populacional de *M. sojae* (CZEPAK et al., 2018).

3 OBJETIVOS

3.1 Objetivo principal

Quantificar os danos ocasionados por *M.* sojae e identificar o seu efeito sobre os componentes de produtividade de soja cultivada em segunda safra no sul do Brasil.

3.2 Objetivos secundários

1. Quantificar os danos de *M.* sojae em função do período de convivência do inseto adulto com as plantas de soja.
2. Correlacionar a severidade dos danos com a variação nos componentes de produtividade.

4 ARTIGO: *Melanagromyza sojae* (DIPTERA: AGROMYZIDAE) DAMAGE ON SOYBEAN: HIGH YIELD LOSSES IN BRAZIL

Rafael P. Marques[1], Henrique Pozebon[1], Júlia G. Bevilaqua[1], Guilherme Padilha[1], Alberto Rohrig[1], Ivair Valmorbida[2], Rubens Fiorin[4], Alessandro Donatti[1], Lauren B. Castilhos[1], Jerson V. C. Guedes[1], Alberto Cargnelutti Filho[3], José Domingos Jacques Leão[1], Janine Palma[5], Jonas A. Arnemann[1]*.

[1]Departamento de Defesa Fitossanitária, Universidade Federal de Santa Maria (UFSM), Santa Maria, Rio Grande do Sul, Brazil, [2]Iowa State University, Ames, Iowa, USA.
[3]Departamento de Fitotecnia, Universidade Federal de Santa Maria (UFSM), Santa Maria, Rio Grande do Sul, Brazil,[4]Sumitomo Chemical Latin America, Santa Maria, Rio Grande do Sul, Brazil,[5]Palmagri Pesquisa e Desenvolvimento, Cruz Alta, Rio Grande do Sul, Brazil.

***Correspondence:** Jonas André Arnemann, Departamento de Defesa Fitossanitária, Universidade Federal de Santa Maria (UFSM), Avenida Roraima 1000, Santa Maria, Rio Grande do Sul 97105-900, Brazil. E-mail: jonasarnemann@gmail.com

4.1 Abstract

The soybean stem fly, *Melanagromyza sojae* Zehntner (Diptera: Agromyzidae), an important soybean pest in East Asia, has recently invaded and colonized the major soybean producing countries of South America. Management strategies for this pest remain largely undeveloped due to lack of information regarding its damaging potential in Brazil and neighbouring countries. The objective of this study was to quantify soybean yield reduction caused by *M. sojae* damage. Two experiments were carried out during the 2019/2020 (experiment A) and 2020/2021 (experiment B) cropping seasons. The experimental design was randomized blocks with eight treatments and ten replicates for experiment A and seven treatments and ten replicates for experiment B, representing different levels of coexistence between the target pest and soybean plants. Number of grains, 1000-grain weight, grain yield and number of pods were quantified for lower, middle and upper canopy segments of the plants. In experiment A, each 1% of injured stem in the lower, middle and upper canopy segments reduced ($p<0.0001$), respectively: 1.41, 0.71 and 0.75 grains/plant; 2.4, 3.10 and 3.36 g of 1000-grain weight; and 0.43, 0.24 and 0.23 g of yield/plant. Considering the three segments, yield reduction reached 0.9 g/plant ($p<0.0001$) for each 1% of injured stem. The findings from this study point to a high damaging potential of *M. sojae* under Brazilian growing conditions and highlight the need to develop management strategies for this pest in soybean crops.

Keywords: *Glycine max*; integrated pest management; stem fly; yield reduction

4.2 Introduction

Soybean is the main cash crop grown in South America. Approximately 57 million hectares of South American agricultural land are currently grown with the crop (FAOSTAT, 2020), 38.5 million of which were located within Brazil during the 2020/21 cropping season (CONAB, 2021). Extensive cultivation and variation in sowing dates make this soybean belt highly vulnerable to insect pests, including those not native to this region (Pozebon et al., 2020). *Melanagromyza sojae* (Diptera: Agromyzidae), the soybean stem fly, is native to East Asia and was recently detected in the New World (Arnemann et al., 2016). This insect is among the major invasive pests that currently threaten South American soybean production (Pozebon et al., 2021).

Melanagromyza sojae was officially detected in South America for the first time in 2015 (Arnemann et al., 2016) but it is suspected to have been present in Southern Brazil since the 1980s (Gassen & Schneider, 1985). Since its detection in Brazil, *M. sojae* continued to spread across the South American soybean belt, reaching Paraguay (Guedes et al., 2017), Bolivia (Vitorio et al., 2019), Argentina (Trossero et al., 2020), and the Center-west region of Brazil (Czepak et al., 2018). In Southern Brazil, population outbreaks of *M. sojae* have been repeatedly observed on second-season soybean, sowed after maize harvest from December 31th onwards (Folmann et al., 2017; Pozebon et al., 2020).

Soybean plants are damaged by *M. sojae* when the larva bores into the main stem to feed in the pith, tunnelling it upwards and downwards. Because the damage occurs within the plants, exterior symptoms are almost imperceptible and frequently go unnoticed by soybean growers. Being an invasive pest recently confirmed in South America, there is paucity of data regarding the potential of *M. sojae* to cause yield reduction in soybeans, and integrated management programs targeting this pest remain largely undeveloped. Yield losses due to *M. sojae* attack have been estimated at 30% in Indonesia (Du & Hong, 1982) and 42% in India (Jadhav, 2011), varying according to region, crop nutrition, soybean cultivar, sowing date and management strategies employed (Savajji, 2006); however, this information is still lacking in Brazil and South America. Thus, the objective of this work was to quantify *M. sojae* damage and its effects on soybean grain yield under Brazilian growing conditions.

4.3 Material and Methods

Experimental sites

Two experiments were carried out in the 2019/2020 (Experiment A) and 2020/2021 (Experiment B) cropping seasons, at Federal University of Santa Maria (29º42'48"S, 53º43'59"W), Santa Maria-RS, Brazil. Soybean cultivars TMG 7063 IPRO (sowed in January 27th, 2020) and 6968RSF (sowed in January 25th, 2021) were used in Experiments A and B, respectively. Weeds were controlled prior to sowing with application of 1,005 g a.i./ha of 2,4-D + 1,040 g a.i./ha of glyphosate, and at soybean growth stage V3 with application of 1,040 g a.i./ha of glyphosate. Soybean seeds were treated with 30 g a.i. of carbendazim+ 70 g a.i. of thiram per 100 kg of seeds. Foliar sprays of strobilurin and triazole fungicides were carried out at growth stages V3, V7, R1, R4 and R5.2 for disease control. Defoliating caterpillars were controlled by the expression of insecticide Bt proteins in the soybean plants of Experiment A, and did not occur in Experiment B. Sap-sucking pestswere monitored at the field borders and controlled with application of 60 g a.i./ha of acetamiprid + 30 g a.i./ha of pyriproxyfen and 970 g a.i./ha of acephate, before reaching the experimental plots.

Experimental design and treatments

The experimental design was completely randomized with eight treatments and ten replicates in experiment A, and seven treatments and ten replicates in experiment B, with each soybean plant sampled representing one experimental unity, for the variable damaged stem. For the other variables (yield components), 4 repetitions were considered, so that each repetitions consisted of 4 plants. Each plot was 6×20 m large (12 soybean rows spaced 0.5 m per plot) and contained approximately 2,400 soybean plants, from which 10 plants were sampled per evaluation. The treatments comprised different numbers of weekly insecticide sprays, starting at the *M. sojae* infestation onset, to obtain a range of injured stems (Table 1). Treatment 1 represented the lowest level of *M. sojae* injury in both experiments, with sprays starting at growth stages V3 and V5 for experiments A and B, respectively. The untreated control was represented by treatments 8 and 9 in experiments A and B, respectively. The insecticide used was 26.5 g a.i./ha of lambda-cyhalothrin + 35.2 g a.i./ha of thiamethoxam. Sprays were carried out using a CO_2-pressurized backpack sprayer, with a spray volume of 150 L/ha and six spray nozzles model XR 110 020, spaced 0.5 m from each other.

Evaluations

Evaluations were carried out weekly by randomly sampling 10 soybean plants from each treatment, prior to the insecticide application, at growth stages V3, V6, V8, R2, R3, R4, R5.2 and R5.5 (Fehr & Caviness, 1977) for experiment A and V5, V7, R1, R2, R3, R4 and R5.3 for experiment B . Plant height was measured from the soil line to the last node of the main stem, and the presence of *M. sojae* tunnels was assessed by opening the main stem longitudinally, from bottom to top. The percentage of injured stem was determined as the ratio between plant height and tunnel length in the main stem.

At the end of the crop cycle, 16 soybean plants (4 plants per replicate) were harvested per plot. Grain yield (g/plant) and yield components (number of pods, number of grains and 1000-grain weight) were quantified for each segment of the plants' canopy (lower, middle and upper) and for the whole soybean plants. Yield was estimated based on number of grains and 1000-grain weight.

Statistical analysis

All variables (injured stem, number of pods, number of grains, 1000-grain weight and yield) were submitted to variance analysis and F-test ($p<0.05$). The means were compared through Scott-Knott test and linear regression analysis, with the resulting models describing the correlation between the percentage of injured stem and soybean yield components (grains/plant, 1000-grain weight, pods/plant and yield/plant). The percentage of injured stem was analysed using the evaluations from growth stages R2, R3, R4 and R5.5 for experiment A and growth stages R4 and R5.3 for experiment B, which presented statistical difference among treatments. Statistical analyses were carried out using the softwares Microsoft Excel and SISVAR (Ferreira, 2019) at 5% level of significance.

4.4 Results

Experiment A

M. sojae attack in experiment A significantly affected ($P<0.05$) the number of pods, number of grains, 1000-grain weight and grain yield of the soybean plants. The percentage of injured stem did not differ significantly ($P\leq0.05$) among treatments for the evaluations from V3 to V8 and R5.2 (Table 2). The other evaluations presented statistical difference for this variable, resulting in reduced yield components and indicating that control efficiency for *M.*

sojae varies according to the growth stage at which soybean plants are sprayed. The adjusted regression models presented high coefficients of determination, attesting to their representativeness of the relationship between *M. sojae* damage and reduction in soybean yield components (Figures 1, 2 and 3).

Variance analysis indicated no significant effect of the treatments for number of pods in the lower (F = 0.347; df = 31; P = 0.9235) and middle (F = 1.875; df = 31; P = 0.1186) segments, and significant for the upper segment (F = 2.931; df = 31; P = 0.0229), although not differing among themselves by Scott-Knott test (P<0.05). However, all remaining yield components presented significant difference according to Scott-Knott test (P<0.05), except for number of grains in the lower segment (Supplementary Table 3). The highest number of grains in the middle segment (51.0 grains/plant) was observed in treatment 1 (first sprayed at V3), whereas for the upper segments the highest values were obtained in treatments 1 (first sprayed at V3), 2 (first sprayed at V6) and 3 (first sprayed at V8), with 43.4, 40.0 e 40.5 grains/plant, respectively (Supplementary Table 3). Similarly, treatments 1 (first sprayed at V3), 2 (first sprayed at V6) and 3 (first sprayed at V8) presented the highest 1000-grain weight values in the three segments, differing statistically from the remaining treatments. The values observed for this variable in the aforementioned treatments were: 198.2, 194.5 and 192.1 g in the lower segment; 199.7, 203.0 and 197.5 g in the middle segment; and 187.7, 188.4 and 174.1 g in the upper segment, respectively (Supplementary Table 3).

Regression analysis was not carried out for the variable number of pods, as no effect of the treatments was observed in the middle and lower segments, as well as no significant difference in the upper segment according to Scott-Knott test (P≤0.05).The regression model for the variable number of grains is shown on Figure 1, indicating reduction of 0.71 grains/plant (R^2 = 0.6011 P<0.0001) in the middle segment and 0.75 grains/plant (R^2 = 0.8758; P<0.0001) in the upper segment for each percentage point of stem length injured by *M. sojae*. Similarly, each percentage point of injured stem caused a reduction of 2.4 (R^2 = 0.8525; P<0.0001), 3.1 (R^2 = 0.7678; P<0.0001) and 3.3 g (R^2 = 0.7903; P<0.0001) in 1000-grain weight, for the lower, middle and upper segments, respectively (Figure 2).

The highest yield values were observed in treatments 1 (first sprayed at V3), 2 (first sprayed at V6), 3 (first sprayed at V8) and 4 (first sprayed at R2), which differed statistically from the remaining treatments (Scott-knott p≤0.05) and yielded 37.5, 31.9, 31.4 and 26.1 g/plant, respectively (Supplementary Table 5). The regression model for the variable grain yield, considering the three canopy segments, indicated that each percentage point of soybean stem length injured by *M. sojae* caused a reduction of 0.9 g/plant (p<0.0001).

Lower, middle and upper segments presented yield reduction of 0.4 (R^2 = 0.8032; P<0.0001), 0.2 (R^2 = 0.7448; P<0.0001) and 0.2 (R^2 = 0.8473; P<0.0001) g/plant, respectively (Figure 3).

Experiment B

The treatments evaluated on Experiment B presented no significant effect (p<0.05) upon the variable injured stem until the two last evaluations [R4 (F = 2.491; df = 69; P =0.0316) and R5.3 (F = 3.237; df = 69; P =0.0078)], when treatments 1 (first sprayed at V5), 2 (first sprayed at V7) and 3 (first sprayed at R1) showed the lowest percentage of stem length injured by *M. sojae*, not differing among themselves by Scott-Knott test (p≤0.05). The values observed for this variable in the aforementioned treatments were 39.0, 35.6 and 32.5% at growth stage R4 and 45.9, 44.9 and 33.6% at growth stage R5.3, respectively (Supplementary Table 2). The remaining treatments presented greater percentage values of injured stem length and did not differ among themselves, ranging from 46.4 to 52.6% at R4, and from 56.3 to 65.5% at R5.3.

Regarding yield components, the evaluated treatments presented significant effect for the variables number of pods in the lower segment (F = 4.839; df =27; P =0.0009), number of grains in the lower (F = 5.089; df = 27; P = 0.0006) and upper (F = 2.206; df = 27; P = 0.0594), 1000-grain weight in the lower (F = 34.871; df = 27; P<0.0001), middle (F = 17.440; df = 27; P<0.0001) and upper (F = 7.066; df = 27; P =0.0012) segments, and grain yield in the lower segment (F = 6.151; df = 27; P =0.0012). The highest values for number of pods in the lower segment were observed in treatments 1 (first sprayed at V5), 3 (first sprayed at V7), 4 (first sprayed at R2) and 5 (first sprayed at R3), with 10.4, 11.1, 9.9 and 12.3 pods/plant, respectively, not differing significantly among themselves.

The highest values for number of grains in the lower segment were found in treatments 1 (first sprayed at V5), 3 (first sprayed at R1), 4 (first sprayed at R2) and 5 (first sprayed at R3), with 19.3, 20.0, 18.7 and 21.7 grains/plant, respectively. As for 1000-grain weight in the lower segment, the highest values were observed in treatments 1 (first sprayed at V5), 4 (first sprayed at R2) and 5 (first sprayed at R3), with 201.2, 206.1 and 204.0 g, respectively, not differing significantly among themselves. In the middle segment, the highest values for this variable were observed in treatments1 (first sprayed at V5) and 4 (first sprayed at R2), with 208.2 g and 206.1 g, respectively, not differing from each other. In the upper segment, however, treatments 1 (first sprayed at V5), 2 (first sprayed at V7), 5 (first sprayed at R3) and 7 (untreated) presented the best results, with 179.3, 181.4, 181.4 and 183.4 g, respectively, not differing among themselves (Supplementary Table 4).

Finally, for the variable yield in the lower segment, the highest values were observed in treatments 1 (first sprayed at V5), 3 (first sprayed at R1), 4 (first sprayed at R2), 5 (first sprayed R3) and 7 (untreated), with4.0, 3.6, 3.8, 4.3 and 3.2 g/plant, respectively. Despite the differences observed among treatments, the resulting regression models between the aforementioned variables and the percentage of injured stem were significant only for 1000-grain weight in the upper segment (P=0.031).

4.5 Discussion

Number of grains and 1000-grain weight

Soybean yield decreased as *M. sojae* injury increased, mainly as a result from reduced number of grains and lower 1000-grain weight. Despite of receiving weekly insecticide sprays since growth stage V3 (experiment A) and V5 (experiment B), treatment 1 also presented plants injured by *M. sojae* and yield reduction (although lower than all other treatments), indicating that the potential for yield reduction can be even higher than observed in this study.

The results obtained in both experiments indicate that controlling *M. sojae* affects positively the number of grains, as observed for the middle and upper segments in experiment A and lower segment in experiment B. The treatment that was first sprayed at the *M. sojae* infestation onset presented the highest number of grains in the aforementioned segments (Supplementary Tables 1 and 2). The regression models for the middle and upper segments in experiment A indicate a reduction of 0.71 and 0.75 grains/plant for each percentage point of stem length injured by *M. sojae*, respectively (Figure 1), attesting to the potential of this pest to affect negatively one of the main yield components in soybean plants. In experiment A, the variable 1000-grain weight also decreased as injury by *M. sojae* increased. Means were grouped together according to a clear-cut pattern in the three canopy segments: treatments 1 (first sprayed at V3) to 3 (first sprayed at V8) did not differ significantly among themselves, and the lowest grain weight values were observed in the unsprayed control plot (Supplementary Table 3).In experiment B, treatment 1 (first sprayed at V5) presented the highest values for this variable in the lower and middle segments, not differing significantly from treatment 4 (first sprayed at R2) in both segments and from treatment 5 (first sprayed at R3) in the lower segment (Supplementary Table 4). All remaining treatments presented significant reduction in grain weight, which was expected as stem tunnelling by *M. sojae* larvae hinders xylem transport within soybean plants (Talekar, 1989), consequently affecting grain filling.

Grain yield

Each percentage point of soybean stem length injured by *M. sojae* reduced 0.9 g/plant (Figure 3) in experiment A. In comparison, studies carried out in East Asia during the 1980s estimated a yield reduction of 0.11 g/plant for each percentage point of stem length injured by *M. sojae* (Talekar & Chen, 1985). This value was estimated at 1.1 g/plant for the species *Melanagromyza obtusa* (Gangrade & Sing, 1976). Considering that modern soybean cultivars present lower leaf area index, lower height and less secondary stems than cultivars grown 40 years ago, each unity of injury was expected to produce a greater impact on yield components than previously reported.

Yield reductions as high as 63.7% were observed in treatment 8 (untreated) (Supplementary Table 6). Similarly, Jadhav (2011) observed soybean yield losses ranging from 33 to 41% in India. This high potential for yield reduction is explained by the feeding behaviour of *M. sojae* larvae within the soybean stem, which damages vascular tissues and impairs xylem transport (Chiang & Norris, 1983). As a consequence, transport of water and nutrients from roots to stems and leaves is hindered (Talekar, 1989). Furthermore, soybean stems store most of the photoassimilates that are translocated to grains during grain filling (Streeter & Jeffers, 1979). Thus, grain yield is directly affected by insect herbivory in this site of the plant.

Spray moment

Treatments where insecticide applications started during the vegetative phase [1 (first sprayed at V3), 2 (first sprayed at V6) and 3 (first sprayed at V8)] presented the lowest damage by *M. sojae* in all evaluations of experiment A, not differing among themselves and also from treatments 8 (untreated), 4 (first sprayed at R2) and 5 (first sprayed at R3) in the evaluations at R2, R4 and R5.2, respectively (Supplementary Table 1). Similarly, the lowest damage by *M. sojae* in experiment B was observed in treatments 1 (first sprayed at V5), 2 (first sprayed at V7) and 3 (first sprayed at R1), not differing among themselves according to Scott-Knott test ($p \leq 0.05$; Supplementary Table 2).

Throughout the experiments, treatments submitted to more sprays until evaluation moment presented lower percentage of stem length injured by *M. sojae.* The reduction in percentage of injured stem in these treatments is probably linked to an effective control of *M. sojae* adults, allied to control of larvae before they bored into the main stem of the soybean plants.

Short lifecycle and high reproductive rate allow *M. sojae* to complete as much as five generations per soybean cycle (Pozebon et al., 2020), facilitating crop reinfestation and the occurrence of distinct life stages of the insect within the same plant. Adult females lay their eggs on young leaves (including unifoliate leaves), piercing holes where one or two eggs are placed. After two to four days, the eggs hatch and the larvae bore into the leaf's main vein, reaching the main stem of the plant via petiole. Each larva consumes around 1.4 mm of leaf tissue per hour (Lee, 1962), taking two days to reach the main stem after its emergence, on average. After this short interval, control possibilities are drastically reduced, as the insect becomes virtually unreachable by insecticide sprays. Apart from this period of larvae mobility within the leaf, control possibilities are restricted to adult insects, which fly above the plants and are easily hit by insecticide sprays.

The high correlation found between *M. sojae* damage level (i.e. percentage of injured stem) and reduction in yield components (number of grains, 1000-grain weight and grain yield) points to a high damaging potential in soybean crops and highlights the need to develop efficient management measures for this pest. The longer spray is delayed, allowing the pest to survive in the crop, the more stem length is injured and grain yield reduced.

Management strategies

M. sojae can attack soybean plants throughout the whole crop cycle, but population densities vary according to environmental factors such as rainfall, temperature and availability of plant hosts. During periods of the year when mild temperatures predominate, *M. sojae* populations decrease drastically. The highest incidence is observed in the hottest and driest months, as intense rainfall restrains feeding and oviposition by adult females (Talekar & Chen, 1985; Yadav et al., 2015). In Asian countries, stem flies typically overwinter as pupae within dead soybean stems (Pozebon et al., 2020). In Brazil, winter survival has been facilitated by the presence of volunteer soybean plants in the fields (Czepak et al., 2018) and of alternative plant hosts, such as *Trifolium resupinatum* (Ferreira et al., 2020).

Due to aforementioned environmental factors, population outbreaks of *M. sojae* have so far remained restricted to second-season soybean (i.e. sowed from December onwards) in Southern Brazil. However, Pozebon et al. (2020) alerts that *M. sojae* infestations are likely to become frequent in main-season soybeans as well (sowed from October onwards), due to the pest's high adaptability to Brazilian growing conditions. Besides reducing grain yield in main-season soybeans, the materialization of such scenario would boost *M. sojae* population levels during late sowing, potentially compromising second-season soybean cultivation altogether.

Soybean growers in Southern Brazil typically cut off expenses during second-season cultivation, leading to lower use of insecticides. When Bt soybean cultivars are used, the crops are sprayed with insecticide only after growth stage R3, targeting phytophagous stinkbugs. This scenario is directly linked to *M. sojae*'s potential to damage soybean plants and reduce grain yield, as observed in this study. Treatment 5, where two weekly insecticide sprays were carried out after growth stage R3, presented yield loss as high as 36.53%, not differing statistically from the unsprayed control plot (Supplementary Table 6).

Soybean pest management in Brazil relies heavily on use of chemical insecticides and Bt cultivars. The transgenic cultivar MON 87701 × MON 89788, expressing insecticide protein Cry1Ac, was commercially released in Brazil in 2010 (Bernardi, 2012). Yu et al. (2014), studying the exposure of non-target arthropods to Cry1Ac in soybean fields, reported the presence of detectable Bt protein levels in *M. sojae* adults, with no apparent effect upon the insect's development. Bt proteins Cry4Aa, Cry4Ba, Cry11Aa, Cyt1Aa, Cry10Aa and Cyt2Ba knowingly affect dipteran insects (Ben-Dov, 2014), but no transgenic cultivar made available so far expresses these proteins. Soybean cultivars in India are classified according to their susceptibility to *M. sojae* attack, based on percentage of attacked plants (Savajji, 2006), but in Brazil this information is lacking (Curioletti, 2016).

Unavailability of Bt cultivars with control effect upon dipteran pests, allied to lack of information regarding susceptibility to *M. sojae* attack in current soybean cultivars, have constrained management measures to insecticide spraying. The use of insecticides at sowing, combined with foliar sprays shortly after plant emergence, protects soybeans plants during the growth stages most vulnerable to *M. sojae* attack (Curioletti et al., 2018). The findings from this study highlight the importance of reducing the coexistence between pest and crop by spraying soybean plants during vegetative phase, thus preventing yield losses and avoiding lost revenue. Further studies should assess *M. sojae* damage on main-season growing conditions and use the data to determine an economic injury level for this pest in soybean crops.

4.6 Conclusions

1. Each percentage point of soybean stem length injured by *M. sojae* caused a yield reduction of 0.9 g/plant.
2. Number of grains/plant and 1000-grain weight decreased linearly as percentage of injured stem increased.
3. Soybean plants sprayed with insecticides during vegetative phase presented less damage by *M. sojae* and lower yield reduction.

Acknowledgements

We thank the members from Pest Management and Genetics Lab – UFSM for their fundamental help on the execution of this work. We also thank Dr. Janine Palma and her team, which were crucial for the development of this study. We acknowledge Msc. Luís Eduardo Curioletti, Msc. Leonardo Moreira Burtet and Dr. Maiquel Pizutti Pes, for providing experiment materials and helping design the experiment.

4.7 References

Arnemann, J. A., W. T. Tay, T. Walsh, H. Brier, K. Gordon, F. Hickmann, G. Ugalde, and J. V. C. Guedes. 2016. Soybean Stem Fly, *Melanagromyza sojae* (Diptera: Agromyzidae), in the New World: detection of high genetic diversity from soybean fields in Brazil. Genet. Mol. Res. 15(2): 1–13. DOI: 10.4238/gmr.15028610

Ben-Dov, E. 2014. *Bacillus thurigiensis* subsp. *israelensis* and its dipteran-specific toxins. Toxins 6: 1222–1243.

Bernardi, O. 2012. Avaliação do risco de resistência de lepidópteros-praga (Lepidoptera: Noctuidae) à proteína Cry1Ac expressa em soja MON 87701 × MON89788 no Brasil. Ph.D. dissertation, Escola Superior de Agricultura Luiz de Queiroz, Piracicaba, Brazil.

Chiang, H. S. and D. M. Norris. 1983. Physiological and anatomical stem parameters of soybean resistance to Agromyzid beanflies. Entomol. Exp. Appl. 33: 203–212.

Companhia Nacional de Abastecimento (CONAB). 2020. Acompanhamento da safra brasileira: grãos, safra 2018/19. https://www.conab.gov.br/info-agro/safras/graos (accessed 10 aug 2020)

Curioletti, L. E. 2016. Ocorrência, distribuição espacial e métodos de controle de *Melanagromyza sojae* em soja. Tese de MS. Universidade Federal de Santa Maria, Santa Maria.

Curioletti, L. E., J. A. Arnemann, D. S. Muraro, A. A. Melo, C. R. Perini, L. A. Cavallin, and J. V. C. Guedes. 2018. First insights of soybean stem fly (SSF) *Melanagromyza sojae* control in South America. Aust. J. Crop Sci. 12: 841–848.

Czepak, C., M. L. S. Nunes, T. Carvalhais, M. V. Anjos, R. F. Silverio, P. B. S. O. Lima, K. C. A. Godinho, A. M. Lima Junior, R. M. S. Coelho and P. C. Gontijo. 2018. First record of the soybean stem fly *Melanagromyza sojae* (Diptera: Agromyzidae) in the Brazilian Savannah. Pesqui. Agropecu. Trop. 48: 200–203.

Du, J. R. and L. M. Hong. 1982. An initial report of an experiment on controlling the agromyzid fly *(Melanagromyza sojae)* of soybean with carbofuran and the loss of yield caused by the pest. J. of Nanjing Agricultural University. **5**: 56-61.

Fehr, W. R. and C. E. Caviness. 1977. Stages of Soybean Development. Special Report 87. Cooperative Extension Service, Agriculture and Home Economics Experiments Station. Iowa State University of Science and Technology, Ames, USA,

Ferreira, D. R., E. Marchesan and G. A. Ugalde. 2020. The soybean stem fly found on Persian clover as an alternative wintering host in the soybean belt of South America. Genet. Mol. Res. 19(2): 1-6. DOI: 10.4238/gmr185627

Ferreira, **D.F.** SISVAR: A computer analysis system to fixed effects split plt type designs. Revista Brasileira de Biometria. [S.l.], v. 37, n. 4, p. 529-535, dec. 2019. ISSN 1983-0823. Available at: <http://www.biometria.ufla.br/index.php/BBJ/article/view/450>. Date accessed: 10 feb. 2020. doi: https://doi.org/10.28951/rbb.v37i4.450.

Follmann, D. N., A. Cargnelutti Filho, V. Q. de Souza, M. Nardino, I. R. Carvalho, G. H. Demari, M. Ferrari, A. J. de Pelegrin, and V. J. Szareski. 2017. Relações lineares entre caracteres de soja safrinha. Revista de Ciências Agrárias 40: 213–221.

Food and Agriculture Organization of the United Nations Statistical Database (FAOSTAT). 2020. Food and agriculture organization of the United Nations statistical database. http://www.fao.org/faostat/en/#data (accessed 10 aug 2020)

Gangrade, G. A. and O. P. Singh. 1976. Effect of stemfly, *Melanagromyza phaseoli* (Tryon), on yield of pods and grains of soybean in India.J. Appl. Entomol. 1: 438-441.

Gassen, D. K. and S. Schneider. 1985. Ocorrência de *Melanagromyza* sp. (Dip. Agromizidae) danificando soja no sul do Brasil. In: Reunião De Pesquisa da Soja da Região Sul, 13, 1985, Porto Alegre*. Soja: resultados de pesquisa 1984-85*. Passo Fundo: EMBRAPA-CNPT, 108-109.

Guedes, J. V. C., J. A. Arnemann, L. E. Curioletti, L. M. Burtet, M. L. Ramãrez Paredes, D. Noschang, F. I. de Oliveira, and W. T. Tay. 2017. First record of soybean stem fly *Melanagromyza sojae* (Diptera: Agromyzidae) in Paraguay confirmed by molecular evidence. Genet. Mol. Res. 16(3): 1-8. DOI: 10.4238/gmr16039707

Jadhav, S. 2011. Bio-ecology and management of stem fly, *Melanagromyza sojae* (Zehntner) (Agromyzidae: Diptera) in soybean ecosystem. Ph.D. dissertation, University of Agricultural Sciences, Dharwad, India.

Lee, S. Y. 1962. The mode of action of Endrin on the Bean Stem Miner, *Melanagromyza sojae* with special reference to its translocation in soybean plants. J. Econ. Entomol. 55: 956-964.

Pozebon, H., R. P. Marques, G. Padilha, M. O'Neal, I. Valmorbida, J. G. Bevilaqua, W. T. Tay and J. A. Arnemann. 2020. Arthropod invasions versus soybean production in Brazil. J. Econ. Entomol. 113: 1591-1608.

Pozebon, H., G. A. Ugalde, G. Smagghe, W. T. Tay, K. Karut, A.F. C. Bazán, L. Vitorio, R. Peralta, A. Saluso, M. L. Ramírez-Paredes, M. G. Murúa, J. V. C. Guedes and J. A. Arnemann. 2021. Highly diverse and rapidly spreading: *Melanagromyza sojae* threatens the soybean belt of South America. Biol. Invasions 23:1405–1423. DOI: 10.1007/s10530-020-02447-7

Savajji K. 2006. Biology and management of soybean stem fly *Melanagromyza sojae (*Zehntner) (Diptera: Agromyzidae*).* M.S. thesis. University of Agricultural Sciences, Bangalore.

Stacke, R. F., J. A. Arnemann, J. Rogers, R. S. Stacke, T. T. Strahl, C. R. Perini, M. F. Dossin, H. Pozebon, L. A. Cavallin and J. V. C. Guedes. 2018. Damage assessment of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in soybean reproductive stages. Crop Prot. 112: 10–17.

Streeter, J. G. and D. L. Jeffers. 1979. Distribution of total non-structural carbohydrates in soybean plants having increased reproductive load. Crop Science. 19: 729-733, 1979.

Talekar, N. S. 1989. Characteristics of *Melanagromyza sojae* (Diptera: Agromyzidae) damage in soybean. J. Econ. Entomol. 82: 584–588.

Talekar, N. S., and B. S. Chen. 1985. The beanfly pest complex of tropical soybean, pp. 257–271. In S. Shanmugasundaram and E. W. Sulzberger (eds.), Symposium [on] soybean in tropical and subtropical cropping systems. AVRDC, Shanhua, Taiwan.

Trossero, M., S. Zuil and J. Frana. 2020. Mosca Del tallo de la soja, *Melanagromyza sojae* (Diptera: Agromyzidae), em el centro de Santa Fe. *Hoja informativa EEA Rafaela*, Instituto Nacional de Tecnologia Agropecuaria.

Vitorio, L., A. F. C. Bazan, J. A. Arnemann, H. Pozebon, G. A. Ugalde, R. P. Marques, J. G. Bevilaqua, L. Puntel, T. L. Colpo, C. R. Perini and J. V. C. Guedes. 2019. First record of the soybean stem fly *Melanagromyza sojae* (Diptera: Agromyzidae) in Bolivia. Genet. Mol. Res. 18(1): 1-10. DOI: 10.4238/gmr18222

Yadav, P., S. Banerjee, M. P. Gupta and V. K. Yadav. 2015. Effect of weather factors on seasonal incidence of insect-pests of soybean. Technofame – A Journal of Multidisciplinary Advance Research 4: 46-51.

Yu, H., J. Romeis, Y. Li, X. Li and K. Wu. 2014. Acquisition of Cry1Ac protein by non-target arthropods in Bt soybean fields. PLos One, 9(8): e103973.

Figure 1 Relationship between percentage of stem length injured by *Melanagromyza sojae* and number of grains/plant in lower (F = 55.7; df = 30; P<0.0001), middle (F = 45.38; df = 30; P<0.0001) and upper (F = 213.2; df = 30; P<0.0001) canopy segments of soybean plants. Data points in the chart represent mean values from experiment A.

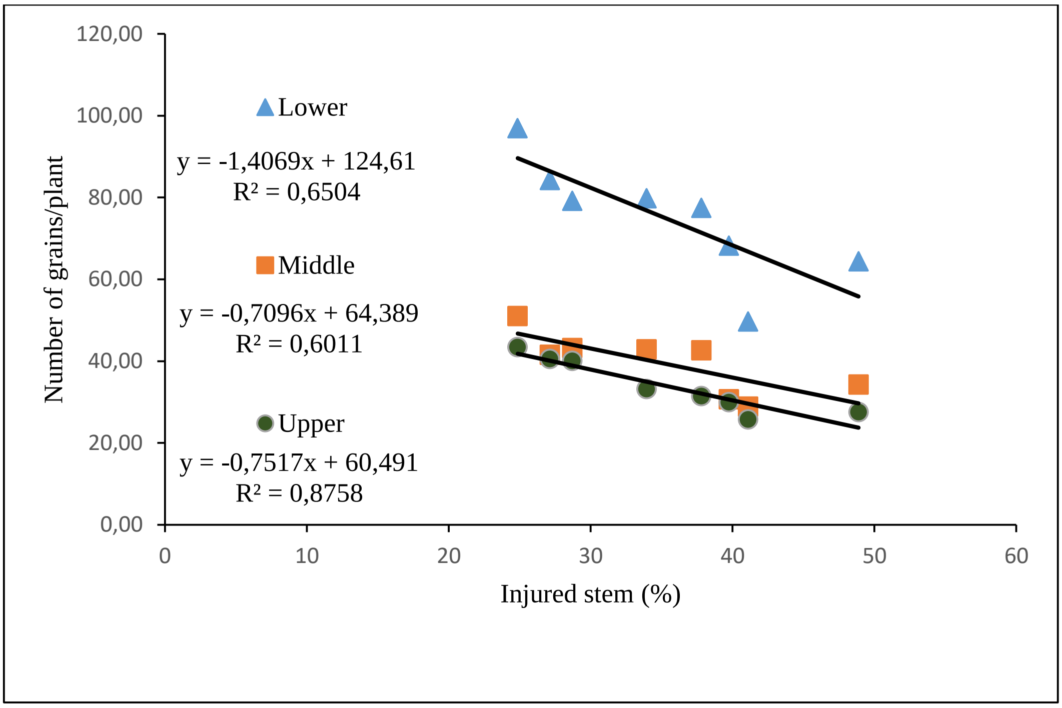


Figure 2 Relationship between percentage of stem length injured by *Melanagromyza sojae* and 1000-grain weight in lower (F = 173.5; df = 30; P<0.0001), middle (F = 99.22; df = 30; P<0.0001) and upper (F = 113.4; df = 30; P<0.0001) canopy segments of soybean plants. Data points in the chart represent mean values from experiment A.

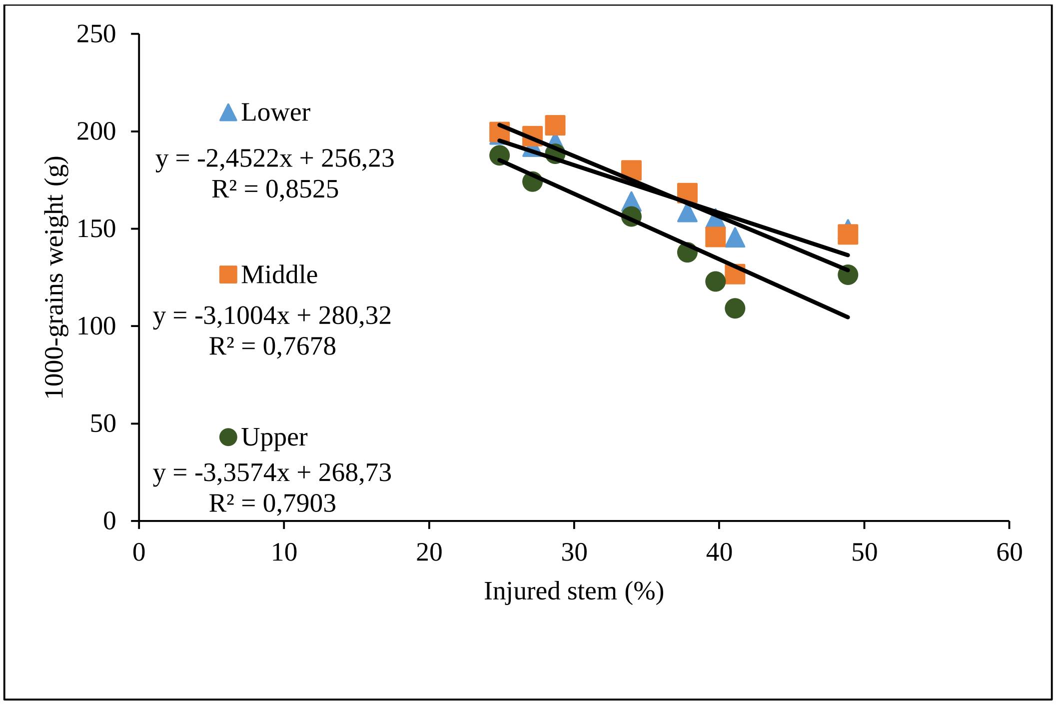


Figure 3 Relationship between percentage of stem length injured by *Melanagromyza sojae* and grain yield/plant in lower (F = 123.6; df = 30; P<0.0001), middle (F = 90.54; df = 30; P<0.0001) and upper (F = 163.6; df = 30; P<0.0001) canopy segments of soybean plants. Data points in the chart represent mean values from experiment A.

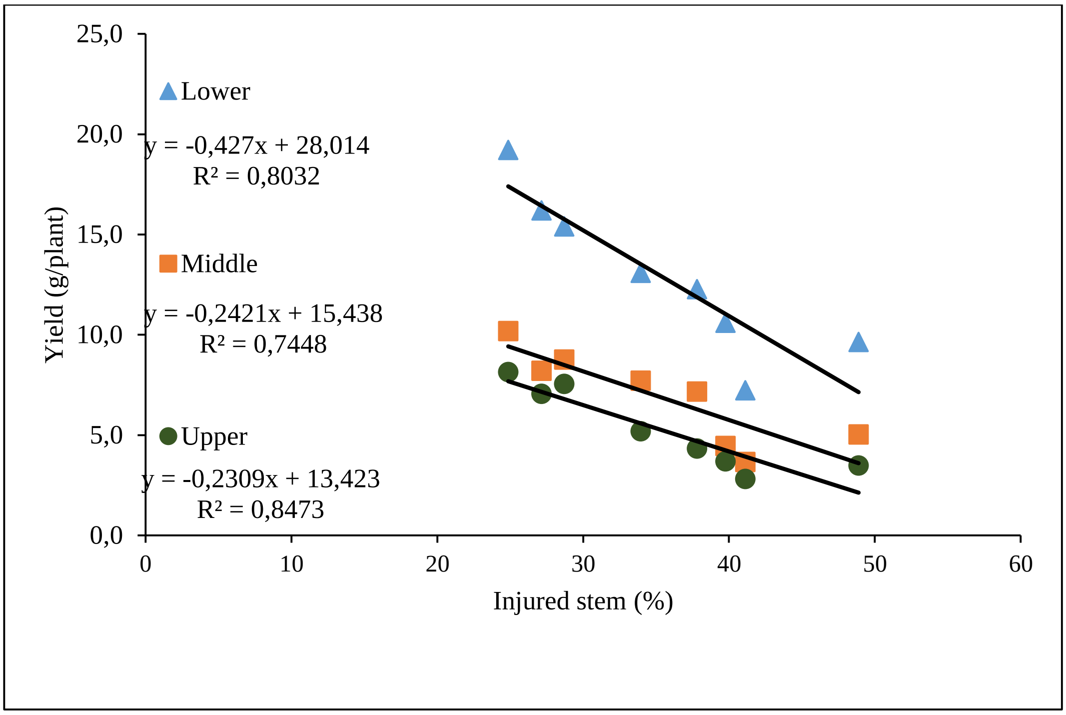


Table 1 Treatments, number of sprays and growth stages of the soybean plants at each spray. Experiment A, season 2019/2020, Santa Maria, RS, Brazil.

Treatment	Number of sprays	Growth stage at each spray
1	7	V3 – V6 – V8 – R2 – R3 – R4 – R5.2
2	6	V6 – V8 – R2 – R3 – R4 – R5.2
3	5	V8 – R2 – R3 – R4 – R5.2
4	4	R2 – R3 – R4 – R5.2
5	3	R3 – R4 – R5.2
6	2	R4 – R5.2
7	1	R5.2
8	0	-

Table 2 Treatments, number of sprays and growth stages of the soybean plants at each spray. Experiment B, season 2020/2021, RS, Brazil.

Treatment	Number of sprays	Growth stage at each spray
1	6	V5-V7-R1-R2-R3-R4-R5.3
2	5	V7-R1-R2-R3-R4-R5.3
3	4	R1-R2-R3-R4-R5.3
4	3	R2-R3-R4-R5.3
5	2	R3-R4-R5.3
6	1	R4-R5.3
7	0	-

4.8 Supplementary Material

Supplementary Table 1 Percentage of soybean stem length injured by *Melanagromyza sojae* in relation to insecticide sprays starting at different growth stages of the crop. Experiment A, season 2019/2020, Santa Maria, RS, Brazil.

Treatment	Growth stage at first spray	Growth stage at evaluation moment							
		V3	V6	V8	R1	R2	R3	R4	R5.2
1	V3	8.1[ns4]	14.0[ns]	43.9[ns]	47.1[ns]	29.7 a[3]	29.5 a	20.7 a	21.8 a
2	V6	0.9[ns]	30.3[ns]	59.3[ns]	64.0[ns]	36.2 a	27.0 a	27.3 a	23.0 a
3	V8	8.3[ns]	38.4[ns]	58.2[ns]	62.1[ns]	28.7 a	25.7 a	23.4 a	29.5 a
4	R2	5.0[ns]	15.7[ns]	56.1[ns]	54.9[ns]	46.0 b	36.7 b	30.8 a	31.2 a
5	R3	0.8[ns]	33.0[ns]	67.0[ns]	61.2[ns]	52.9 b	40.2 b	37.2 b	34.4 a
6	R4	1.7[ns]	35.8[ns]	59.4[ns]	49.6[ns]	44.2 b	37.1 b	44.8 b	48.9 b
7	R5.2	1.7[ns]	18.2[ns]	58.8[ns]	41.3[ns]	47.5 b	48.3 b	44.2 b	69.4 c
8	-	9.8[ns]	27.5[ns]	61.1[ns]	62.6[ns]	39.3 a	42.4 b	39.6 b	51.8 b
CV (%)[1]		284.61	104.92	39.56	39.46	31.89	36.50	34.95	44.51
SE[2]		4.00	8.83	7.25	6.90	4.09	4.13	3.70	5.45
F		0.927	1.147	0.808	1.515	4.463	3.666	6.297	9.160
P-value		0.4921	0.3459	0.5839	0.1785	0.0004	0.0022	<0.0001	<0.0001

[1]Coefficient of variation; [2]Standard error; [3]Means followed by the same letter do not differ among themselves by the Scott Knott test ($p \leq 0.05$); [4]Non-significant.

Supplementary Table 2 Percentage of soybean stem length injured by *Melanagromyza sojae* in relation to insecticide sprays starting at different growth stages of the crop. Experiment B, season 2020/2021, Santa Maria, RS, Brazil.

Treatment	Growth stage at first spray	Growth stage at evaluation moment						
		V5	V7	R1	R2	R3	R4	R5.3
1	V5	25.1 ns[4]	50.5 ns	73.4 ns	59.0 ns	54.0 ns	39.0 a[3]	45.9 a
2	V7	37.2 ns	29.1 ns	66.4 ns	50.0 ns	68.4 ns	35.6 a	44.9 a
3	R1	46.2 ns	58.4 ns	49.4 ns	48.6 ns	51.5 ns	32.5 a	33.6 a
4	R2	19.8 ns	67.5 ns	55.5 ns	46.3 ns	55.8 ns	52.6 b	56.3 b
5	R3	49.1 ns	53.4 ns	53.2 ns	51.2 ns	53.6 ns	46.4 b	58.0 b
6	R4	35.4 ns	51.6 ns	64.1 ns	55.9 ns	51.6 ns	47.4 b	59.9 b
7	-	33.1 ns	53.6 ns	67.2 ns	58.7 ns	55.3 ns	48.8 b	65.5 b
CV (%)[1]		98.18	54.89	39.90	43.56	35.49	34.07	37.62
SE[2]		10.90	9.02	7.74	7.27	6.25	4.65	6.18
F		0.927	1.664	1.272	0.484	0.868	2.582	3.173
P-value		0.4833	0.1478	0.2856	0.8174	0.5247	0.0285	0.0097

[1]Coefficient of variation; [2]Standard error; [3]Means followed by the same letter do not differ among themselves by the Scott Knott test ($p \leq 0.05$); [4]Non-significant.

Supplementary Table 3 Yield components following *Melanagromyza sojae* injury in relation to insecticide sprays starting at different growth stages of the crop, in lower, middle and upper canopy segments of the soybean plants. Experiment A, season 2019/2020, Santa Maria, RS, Brazil.

Treatment	Growth stage at first spray	Number of pods			Number of grains			1000-grain weight		
		Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper
1	V3	39.3[ns4]	20.3[ns]	16.7 a[3]	96.9[ns]	51.0 a	43.4 a	198.2 a	199.7 a	187.7 a
2	V6	33.3[ns]	16.9[ns]	15.1 a	79.2[ns]	43.2 b	40.0 a	194.5 a	203.0 a	188.4 a
3	V8	34.7[ns]	16.6[ns]	15.1 a	84.3[ns]	41.5 b	40.5 a	192.1 a	197.5 a	174.1 a
4	R2	34.5[ns]	18.3[ns]	13.7 a	79.8[ns]	42.9 b	33.2 b	164.0 b	179.9 b	156.2 b
5	R3	35.3[ns]	18.8[ns]	13.3 a	77.4[ns]	42.5 b	31.4 b	158.6 b	168.3 b	137.9 c
6	R4	36.9[ns]	20.4[ns]	14.4 a	68.2[ns]	30.6 c	30.0 b	155.3 b	145.6 c	122.9 c
7	R5.2	37.0[ns]	20.5[ns]	13.8 a	64.4[ns]	34.2 c	27.6 c	149.7 b	147.0 c	126.3 c
8	–	29.4[ns]	19.3[ns]	13.9 a	49.6[ns]	28.9 c	25.7 c	145.5 b	126.7 d	109.0 d
CV (%)[1]		25.52	12.24	9.32	28.28	12.62	9.53	5.36	5.89	7.55
SE[2]		4.49	1.16	0.675	10.60	2.48	1.62	4.54	5.03	5.67
F		0.429	1.756	2.667	1.801	9.010	16.313	22.663	32.811	29.398
P-value		0.8732	0.1500	0.0385	0.1401	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[1]Coefficient of variation; [2]Standard error; [3]Means followed by the same letter do not differ among themselves by the Scott Knott test ($p \leq 0.05$); [4]Non-significant.

Supplementary Table 4 Yield components following *Melanagromyza sojae* injury in relation to insecticide sprays starting at different growth stages of the crop, in lower, middle and upper canopy segments of the soybean plants. Experiment B, season 2020/2021, Santa Maria, RS, Brazil.

Treatment	Growth stage at first spray	Number of pods			Number of grains			1000-grain weight		
		Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper
1	V5	10.4 a[3]	11.4[ns4]	10.1b	19.3 a	21.3[ns]	21.5[ns]	210.2 a	208.2 a	179.3 a
2	V7	6.5 b	12.8[ns]	11.1 a	11.4 b	23.8[ns]	21.8[ns]	195.8 b	197.9 b	181.4 a
3	R1	11.1 a	10.0[ns]	9.2b	20.0 a	19.4[ns]	20.1[ns]	183.4 c	183.4 c	167.0 b
4	R2	9.9 a	11.6[ns]	10.7a	18.7 a	20.3[ns]	20.3[ns]	206.1 a	206.1 a	175.2 b
5	R3	12.3 a	10.2[ns]	9.7b	21.7 a	21.7[ns]	21.0[ns]	204,0 a	197.9 b	181.4 a
6	R4	5.1 b	13.1[ns]	11.4 a	8.9 b	23.9[ns]	24.3[ns]	173.2 d	179.3 c	171.1 b
7	-	9.6 b	9.5[ns]	9.5a	16.3 b	16.0[ns]	17.5[ns]	193.7 b	195.8 b	183.4 a
CV (%)[1]		21.88	20.20	9.82	25.59	21.12	12.37	2.29	2.63	2.60
SE[2]		1.00	1.12	0.50	2.09	2.19	1.29	2.23	2.57	2.29
F		6.264	1.560	2.808	4.796	1.494	2.419	34.871	17.440	7.066
P-value		0.0011	0.2158	0.0415	0.0044	0.2362	0.0683	<0.0001	<0.0001	0.0005

[1]Coefficient of variation; [2]Standard error; [3]Means followed by the same letter do not differ among themselves by the Scott Knott test (p≤0.05); [4]Non-significant.

Supplementary Table 5 Grain yield following *Melanagromyza sojae* injury in relation to insecticide sprays starting at different growth stages of the crop, in lower, middle and upper canopy segments and in whole soybean plants. Experiment A, season 2019/2020, Santa Maria, Brazil.

Treatment	Growth stage at first spray	Yield			Grain yield/plant
		Lower	Middle	Upper	
1	V3	19.2 a[3]	10.2 a	8.1 a	37.5 a
2	V6	15.5 a	8.8 b	7.5 a	31.8 a
3	V8	16.2 a	8.1 b	7.0 a	31.4 a
4	R2	13.2 b	7.7 b	5.2 b	26.1 a
5	R3	12.3 b	7.1 b	4.3 b	23.8 b
6	R4	10.6 b	4.5 c	3.7 c	18.7 b
7	R5.2	9.6 b	5.0 c	3.5 c	18.1 b
8	-	7.1 b	3.6 c	2.8 c	13.6 b
CV (%)[1]		31.61	15.52	11.11	20.06
SE[2]		2.05	0.53	0.29	2.52
F		3.665	18.294	48.234	10.396
P-value		0.0097	<0.0001	<0.0001	<0.0001

[1]Coefficient of variation; [2]Standard error; [3]Means followed by the same letter do not differ among themselves by the Scott Knott test ($p \leq 0.05$);

Supplementary Table 6 Grain yield following *Melanagromyza sojae* injury in relation to insecticide sprays starting at different growth stages of the crop, in lower, middle and upper canopy segments and in whole soybean plants. Experiment B, season 2020/2021, Santa Maria, RS, Brazil.

Treatment	Growth stage at first spray	Yield			Grain yield/plant
		Lower	Middle	Upper	
1	V5	4.0 a[3]	4.4[ns4]	3.8[ns]	12.3[ns]
2	V7	2.2 b	4.6[ns]	3.9[ns]	10.8[ns]
3	R1	3.6 a	3.4[ns]	3.3[ns]	10.3[ns]
4	R2	3.8 a	4.4[ns]	3.8[ns]	12.0[ns]
5	R3	4.3 a	4.2[ns]	3.7[ns]	12.3[ns]
6	R4	1.5 b	4.2[ns]	4.1[ns]	9.8[ns]
7	-	3.2 a	3.1[ns]	3.2[ns]	9.5[ns]
CV (%)[1]		25.34	20.05	13.37	16.41
SE[2]		0.41	0.41	0.25	0.90
F		6.151	1.913	1.894	1.734
P-value		0.0012	0.1337	0.1372	0.1704

[1]Coefficient of variation; [2]Standard error; [3]Means followed by the same letter do not differ among themselves by the Scott Knott test ($p \leq 0.05$); [4]Non-significant.

5 CONCLUSÕES

Neste trabalho, os resultados revelam a capacidade de *Melanogromyza* sojae causar danos à produtividade da cultura da soja nas condições de cultivo brasileiras. Desta forma, estas são as primeiras informações a respeito da redução de produtividade da soja geradas no continente americano. Os danos ocasionados pelo processo de alimentação da larva desta espécie afetam, principalmente, o número de grãos e a massa de mil grãos, de modo que estes decrescem linearmente à medida que a severidade dos danos aumenta. O resultado do decréscimo nestes dois componentes reflete-se em redução de produtividade de grãos, sendo de 0,9 g planta^{-1} para cada ponto percentual da haste principal da planta de soja com a presença de danos. Observa-se que a aplicação de inseticidas durante a fase vegetativa de desenvolvimento contribui para minimizar os danos de *M. sojae* e, consequentemente, reduzir as perdas de produtividade causadas por este inseto.

Estudos adicionais são necessários para determinar o impacto na produtividade causado por *M. sojae* em outras épocas de cultivo e regiões da América do Sul. Torna-se fundamental o estudo da suscetibilidade de cultivares ao ataque deste inseto-praga, bem como o entendimento da relação entre o ciclo da soja, a flutuação populacional e a dinâmica de danos do inseto sob cenários distintos. O desenvolvimento de métodos de monitoramento dos insetos adultos com o intuito de detectar o momento do início da infestação bem como a sua flutuação populacional ao longo do ano são fundamentais para o manejo deste inseto. Também se fazem necessárias pesquisas de eficácia de controle de inseticidas químicos e biológicos, pois atualmente no Brasil ainda não existem inseticidas com registro para o controle deste inseto.

REFERÊNCIAS

ADAK, T. et al. Residue and bio-efficacy evaluation of controlled release formulations of imidacloprid against pests in soybean (*Glycine max*)**. Journal of Environmental Science and Health**, v. 47, p. 226-231, 2012.

AGROFIT. **Agrofit: Sistema de agrotóxicos fitossanitários**. 2021. Disponível em: <http://extranet.agricultura.gov.br/agrofit_cons/principal_agrofit_cons>. Acesso em: 03 Out. 2021.

ARNEMANN, J. A. et al. Soybean Stem Fly, *Melanagromyza sojae* (Diptera: Agromyzidae), in the New World: detection of high genetic diversity from soybean fields in Brazil. **Genetics and Molecular Research**, v. 15, gmr.15028610, 2016.

BECHE, M. et al. Occurrence of *Syntomopus parisii* (Hymenoptera: Pteromalidae) parasitizing *Melanagromyza sojae* (Diptera: Agromyzidae) in Brazil and Paraguay. **Genetics and Molecular Research**, v. 17, gmr18074, 2018.

Bernardi, O. **Avaliação do risco de resistência de lepidópteros-praga (Lepidoptera: Noctuidae) à proteína Cry1Ac expressa em soja MON 87701 × MON89788 no Brasil.** Tese - Escola Superior de Agricultura Luiz de Queiroz, Piracicaba, 2012.

Ben-Dov, E. *Bacillus thurigiensis* subsp. *israelensis* and its dipteran-specific toxins. **Toxins**, v. 6, p. 1222-1243, 2014.

BHATTACHARJEE, N. S. Incidence of the stemfly, *Ophiomia phaseoli* (Tryon) on soybean. **Indian Journal of Entomology**, v. 42, p. 280-282, 1980.

BRIER, H.; CHARLESTON, K. **Soybean stem fly outbreak in soybean crops.** The Beatsheet, 2013. Disponível em: <http://thebeatsheet.com.au/crops/pulses/soybeans/soybean-stem-fly-outbreak-in-soybean-crops/>. Acesso em: 15 Jul. 2017.

CABI. **Species page: soybean stem miner *Melanagromyza sojae*.** 2020. Disponível em: <https://www.plantwise.org/knowledgebank/datasheet/33003>. Acesso em: 03 Out. 2021.

CÂMARA, G. M. S. Preparo do solo e plantio. In: SEDIYAMA, T.; SILVA, F.; BORÉM, A. (Org.). **Soja do plantio à colheita**. Viçosa: UFV, p. 66-109, 2015.

CHIANG, H. S.; NORRIS, D. M. Morphological and physiological parameters of soybean resistance to agromyzid beanflies. **Enviromental Entomology**, v. 12, p. 260-265, 1983.

CONAB. **Acompanhamento da Safra Brasileira: Grãos, safra 2020/21**. 2021. Disponível em: <https://www.conab.gov.br/info-agro/safras/graos/boletim-da-safra-de-graos>. Acesso em: 03 Ago. 2021.

CURIOLETTI, L. E. et al. First insights of soybean stem fly (SSF) *Melanagromyza sojae* control in South America. **Australian Journal of Crop Science**, v. 12, p. 841-848, 2018.

CZEPAK, C. et al. First record of the soybean stem fly *Melanagromyza sojae* (Diptera: Agromyzidae) in the Brazilian Savannah. **Pesquisa Agropecuária Tropical**, v. 48, p. 200-203, 2018.

DU, J. R.; Hong, L. M. An initial report of an experiment on controlling the agromyzid fly *(Melanagromyza sojae)* of soybean with carbofuran and the loss of yield caused by the pest. **Journal of Nanjing Agricultural University**, v. 5, p. 56-61, 1982.

FERREIRA, D. R. et al. The soybean stem fly found on Persian clover as an alternative wintering host in the soybean belt of South America. **Genetics and Molecular Research**, v. 19, gmr18627, 2020.

FOLLMANN, D. N. et al. Relações lineares entre caracteres de soja safrinha. **Sociedade de Ciências Agrárias de Portugal**, v. 40, p. 213-221, 2017.

GANGADRE, G. A.; KOGAN, M. Sampling stem flies in soybean. In: KOGAN, M.; HERZOG, D. C. (ed.) **Sampling methods in soybean entomology**. Heidelberg: Springer Science & Business Media, p. 394-403, 1980.

GASSEN, D. K.; SCHNEIDER, S. Ocorrência de *Melanagromyza* sp. (Dip. Agromizidae) danificando soja no sul do Brasil. In: **Reunião De Pesquisa da Soja da Região Sul**, 13, 1985, Porto Alegre. Soja: resultados de pesquisa 1984-85. Passo Fundo: EMBRAPA-CNPT, 108-109.

GUEDES, J. V. C. et al. Mosca-da-haste. **Cultivar Grandes Culturas**, v. 197, p. 28-31, 2015.

GUEDES, J. V. C. et al. First record of soybean stem fly *Melanagromyza sojae* (Diptera: Agromyzidae) in Paraguay confirmed by molecular evidence. **Genetics and Molecular Research**, v. 16, gmr16039707, 2017.

JADHAV, S. **Bio-ecology and management of stem fly, *Melanagromyza sojae* (Zehntner) (Agromyzidae: Diptera) in soybean ecosystem**. Tese -University of Agricultural Sciences, Dharwad, 2011.

JADHAV, S. N. et al. Assessment of crop loss estimation due to stem fly *Melanagromyza sojae* (Zehntner) in soybean ecosystem. **Journal of Experimental Zoology**, v. 16, p. 221-228, 2013.

LEE, S. Y. The mode of action of Endrin on the Bean Stem Miner, *Melanagromyza sojae* with special reference to its translocation in soybean plants. **Journal of Economic Entomology**, v. 55, p. 956-954, 1962.

POZEBON, H. et al. Arthropod invasions versus soybean production in Brazil: a review. **Journal of Economic Entomology**, v. 113, n. 4, p. 1591–1608, 2020.

POZEBON, H. et al. Highly diverse and rapidly spreading: *Melangromyza sojae* threatens the soybean belt of South America. **Biological Invasions**, v. 23, n. 5, p. 1591-1608, 2021(a).

POZEBON, H. **Pragas invasivas da soja no Brasil e o caso da mosca-da-haste,** *Melanagromyza sojae*: **Diversidade genética na América do Sul.** Dissertação – Universidade Federal de Santa Maria, Santa Maria, 2021(b).

SAVAJJI, K. **Biology and management of soybean stem fly *Melanagromyza sojae* (Zehntner) (Diptera: Agromyzidae).** Dissertação - University of Agricultural Sciences, Bengaluru, 2006.

SINGH, O. P.; SINGH, K. J. Seasonal incidence and damage of *Melanagromyza sojae* (Zehnt.) on soybean. **Indian Journal of Plant Protection**, v. 18, p. 271-275, 1990.

SPENCER, K.A. Agromyzidae (Diptera) of economic importance. **Economic Importance**, v. 9, 1-405, 1973.

STRAKHOVA, I. S. et al. The parasitoid complex (Hymenoptera, Eulophidae) of leafminer flies (Diptera, Agromyzidae) in the middle Volga Basin. **Entomology Review,** v. 93, p. 865-873, 2013.

STREETER, J. G.; JEFFERS, D. L. Distribution of total non-structural carbohydrates in soybean plants having increased reproductive load. **Crop Science**, v. 19, p. 729-734, 1979.

TALEKAR, N. S. Search for beanfly resistance in soybean, mungbean and snapbean. **Asian Vegetable Resistance & Development,** 1980. Disponível em: < https://worldveg.tind.io/record/8277/?ln=en>. Acesso em: 03 Out. 2021.

___________. Characteristics of *Melanagromyza sojae* (Diptera: Agromyzidae) damage in soybean. **Journal of Economic Entomology**, v. 82, p. 584-588, 1989.

___________. **Agromyzid flies of food legumes in the tropics**. New Delhi: Wiley Eastern, v. 297, s/p, 1990.

TALEKAR, N. S.; CHEN, B. S. Seasonality of insect pests of soybean and mungbean in Taiwan. **Journal of Economic Entomology**, v. 75, p. 34-37, 1983.

_______. Soybean in tropical and subtropical cropping systems. Tainan: Asian Vegetable Research and Development Center, p. 257-271, 1985.

TAY, W. T. et al. Mitochondrial DNA and trade data support multiple origins of *Helicoverpa armigera* (Lepidoptera, Noctuidae) in Brazil. Scientific Reports, v. 7, 45302, 2017.

THAPA, R. B. Redescription of *Melanagromyza sojae* (Zehntner) from India and Nepal. **Nepalese Journal of Biosciences**, v. 2, p. 64-70, 2012.

TROSSERO, M.; ZUIL, S.; FRANA, J. **Mosca del tallo de la soja, Melanagromyza sojae (Diptera: Agromyzidae), en el centro de Santa Fe.** Instituto Nacional de Tecnología Agropecuaria, 2020. Disponível em: <https://inta.gob.ar/sites/default/files/inta_rafaela_mosca_del_tallo_soja_web.pdf>. Acesso em: 31 mai 2020.

VAN DEN BERG, H. et al. Soybean stem fly, *Melanagromyza sojae* (Diptera: Agromyzidae), on Sumatra: seasonal incidence and the role of parasitism. **International Journal of Pest Management**, v. 41, p. 127-133, 1995.

VAN DEN BERG, H.; SHEPARD, B.; NASIKIN, B. M. Response of soybean to attack by stemfly *Melanagromyza sojae* in farmer's fields in Indonesia. **Journal of Applied Ecology**, v. 35, n. 4, p. 514-522, 1998.

VAN DER GOOT, P. De Agromyza-vliegjes der inlandse katjang gewassen op Java. Mededeelingen van het Instituut voor Plantenziekten, 78. English translation: **Tropical Vegetable Information Service** (1984). Taiwan: Asian Vegetable Research and Development Center, 1930.

VENKATESAN, T; KUNDU, G. G. Yield-infestation relationship and determination of economic injury level of stem fly, *Melanagromyza sojae* (Zehnt,) infesting soybean. **Journal of Entomological Research**, v. 18, n. 3, p. 265-170, 1994.

VITORIO, L. et al. First record of the soybean stem fly *Melanagromyza sojae* (Diptera: Agromyzidae) in Bolivia. **Genetics and Molecular Research**, v. 18, gmr18222, 2019.

WANG, C. L. Occurence and life history of *Melanagromyza sojae* on soybean. **Journal of Agricultural Research**, v. 28, p. 217-223, 1979.

WANG, J.; GAI, J. Mixed inheritance model for resistance to agromyzid bean fly (*Melanagromyza sojae,* Zehntner) in soybean. **Euphytica,** v. 122, p. 9-18, 2001.

YADAV, P. et al. Effect of weather factors on seasonal incidence of insect-pests of soybean. **Technofame: A Journal of Multidisciplinary Advance Research,** v. 4, p. 46-51, 2015.

Yu, H. et al. Acquisition of Cry1Ac protein by non-target arthropods in Bt soybean fields. **PLos One**, 9(8): e103973, 2014