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**PRAGAS INVASIVAS DA SOJA NO BRASIL E O CASO DA MOSCA-  
DA-HASTE, *Melanagromyza sojæ*: DIVERSIDADE GENÉTICA NA  
AMÉRICA DO SUL**

Santa Maria, RS  
2021



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*Melanagromyza sojae*: DIVERSIDADE GENÉTICA NA AMÉRICA DO SUL**

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

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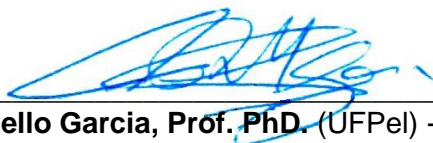
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Santa Maria, RS  
2021



*Dedico essa dissertação aos meus pais, Hermes e Erenita, e à minha irmã Simone.*





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*"Não se conforme com o padrão deste mundo, mas seja transformado pela renovação da sua mente"*  
*(Romanos 12:2)*



## RESUMO

### PRAGAS INVASIVAS DA SOJA NO BRASIL E O CASO DA MOSCA-DA-HASTE, *Melanagromyza sojae*: DIVERSIDADE GENÉTICA NA AMÉRICA DO SUL

AUTOR: Henrique Pozebon

ORIENTADOR: Prof. PhD. Jonas Andre Arnemann

A produção de soja no Brasil tem sido historicamente afetada por pragas exóticas, cuja introdução acarreta em graves prejuízos econômicos e ambientais. Entretanto, estimativas precisas dos impactos econômicos resultantes de tais invasões são praticamente inexistentes. A mosca-da-haste *Melanagromyza sojae* (Zehntner, 1900) (Diptera: Agromyzidae), recentemente introduzida em vários países da América do Sul, representa uma importante ameaça à produção de soja no continente devido ao seu alto potencial de dano e difícil controle, permanecendo pouco explorada em termos de caracterização genética. O presente trabalho apresenta dois artigos com o objetivo de avaliar o impacto das pragas invasivas na produção de soja brasileira, estimando o risco de invasões futuras; e elucidar o cenário atual da invasão por *M. sojae* na América do Sul, caracterizando geneticamente as populações presentes no continente. No primeiro artigo, através de uma revisão bibliográfica e comparação de séries históricas provenientes do banco de dados da FAO, constatou-se que as introduções de *Bemisia tabaci* MEAM1, *Tetranychus urticae* e *Helicoverpa armigera* no Brasil contribuíram para que o uso de inseticidas na soja triplicasse de 1990 a 2016. Por meio da avaliação de quatro critérios moduladores do potencial invasivo de uma praga (isto é, probabilidade de entrada, probabilidade de estabelecimento, características biológicas e disponibilidade de medidas de manejo), as espécies ausentes *Aphis glycines* e *Spodoptera litura* foram identificadas como de alto risco para o Brasil, enquanto *M. sojae* já se encontra estabelecida e apresenta potencial para crescer em ocorrência. No segundo artigo, a caracterização do gene mtCOI em 79 indivíduos de *M. sojae*, provenientes de diferentes localidades do Rio Grande do Sul, Santa Catarina, Goiás, Paraguai, Bolívia e Argentina, revelou a presença de 22 haplótipos diferentes de mosca-da-haste no continente. Espécimes coletados na safra 2018/19 foram identificados, submetidos à extração de DNA, amplificação via PCR e sequenciamento do gene mtCOI. As sequências obtidas foram analisadas em softwares específicos, juntamente com sequências de *M. sojae* previamente publicadas e disponíveis no GenBank. O haplótipo Msoj-COI-02 apresentou a maior frequência na América do Sul, seguido pelo haplótipo Msoj-COI-01, e é o possível ancestral a partir do qual se originaram as demais linhagens. Ainda, os haplótipos Msoj-COI-21, Msoj-COI-22 e Msoj-COI-23 foram identificados pela primeira vez no Rio Grande do Sul. A alta diversidade genética encontrada é uma evidência de que múltiplas introduções de *M. sojae* ocorreram na América do Sul, reiterando a necessidade de se reforçar as barreiras de biossegurança nacionais e desenvolver programas de manejo economicamente eficientes e ambientalmente sustentáveis para essa praga altamente invasiva.

**Palavras-chave:** Agromyzidae. Diversidade genética. Espécies invasivas. *Glycine max.* *Melanagromyza sojae*.



## ABSTRACT

### INVASIVE SOYBEAN PESTS IN BRAZIL AND THE STEM FLY *Melanagromyza sojae* CASE: GENETIC DIVERSITY IN SOUTH AMERICA

AUTHOR: Henrique Pozebon  
ADVISOR: Prof. PhD. Jonas Andre Arnemann

Soybean production in Brazil has been historically affected by exotic arthropod pests, whose introduction leads to severe economic and ecological damage. However, precise estimates of economic impacts resulted from such invasions are lacking. The stem fly *Melanagromyza sojae* (Zehntner, 1900) (Diptera: Agromyzidae), recently introduced and established in the soybean belt of South America, poses a considerable threat to soybean production in the continent due to its high damaging potential, remaining unexplored regarding genetic traits. This work comprises two chapters aimed at assessing the impact of invasive pests on Brazilian soybean production, identifying potential future invaders; and unravelling the current scenario of *M. sojae* invasion in South America, genetically characterizing populations of this pest from Brazil, Bolivia, Paraguay and Argentina. The first chapter revealed that *Bemisia tabaci* MEAM1, *Tetranychus urticae* and *Helicoverpa armigera* introductions in Brazil led to a threefold increase in insecticide usage from 1990 to 2016, based on a review of literature and analysis of historical data from the FAO statistical database. By evaluating four criteria related to potential invasiveness of arthropod pests (i.e. likelihood of entry, likelihood of establishment, biological traits and availability of management measures), the yet absent species *Aphis glycines* and *Spodoptera litura* were identified as high-risk potential invaders to Brazil, whereas the already established *M. sojae* is likely to increase its occurrence. The second chapter included the characterization of mtCOI gene in 79 *M. sojae* individuals from Rio Grande do Sul, Santa Catarina, Goiás, Paraguay, Bolivia and Argentina, revealing that 22 different *M. sojae* haplotypes are present in the continent. Specimens sampled during the 2018/19 cropping season underwent morphological identification, DNA extraction, amplification by PCR and sequencing of the mtCOI gene. The resulting sequences were analysed in specific softwares, together with *M. sojae* sequences previously published and available on GenBank. Haplotype Msoj-COI-02 was the most frequent in South America, followed by Msoj-COI-01, and is probably the ancestral haplotype from which the other strains have originated. Furthermore, novel haplotypes Msoj-COI-21, Msoj-COI-22 and Msoj-COI-23 were identified for the first time in Rio Grande do Sul. The high genetic diversity found suggests that multiple introductions of *M. sojae* female founders took place in South America, highlighting the need to strengthen biosecurity barriers and develop economically efficient and environmentally sustainable management programs for this highly invasive pest.

**Key-words:** Agromyzidae. Genetic diversity. *Glycine max.* Invasive species. *Melanagromyza sojae*.





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## LISTA DE ABREVIATURAS E SIGLAS

AMOVA	Analysis of Molecular Variance
ARG-CO	Argentina - Córdoba
AUS-NSW	Australia - New South Wales
BOL-SC	Bolívia - Santa Cruz
BRA-GO	Brasil - Goiás
BRA-RS	Brasil - Rio Grande do Sul
BRA-SC	Brasil - Santa Catarina
CABI	Centre for Agriculture and Bioscience International
CESB	Comitê Estratégico Soja Brasil
COI	Citocromo Oxidase Sub-Unidade I
COII	Citocromo Oxidase Sub-Unidade II
CONAB	Companhia Nacional de Abastecimento
COSAVE	Comite de Sanidad Vegetal
CytB	Citocromo B
DAE	Dias Após a Emergência
EFSA PLH	European Food Security Authority in Plant Health
EMBRAPA	Empresa Brasileira de Pesquisa Agropecuária
EPPO	European and Mediterranean Plant Protection Organization
FAO	Food and Agriculture Organization of the United Nations
FAOSTAT	FAO Statistical Database
IBGE	Instituto Brasileiro de Geografia e Estatística
IPPC	International Plant Protection Convention
MAPA	Ministério da Agricultura, Pecuária e Abastecimento
MEAM1	Middle-East Asia Minor 1
MED	Mediterranean
MIP	Manejo Integrado de Pragas
mtDNA	DNA Mitocondrial
NW1	New World 1
NW2	New World 2
PAR-AP	Paraguai - Alto Paraná

PAR-CA	Paraguai - Canindeyú
PAR-IT	Paraguai - Itapúa
PCR	Polymerase Chain Reaction
PRA	Pest Risk Analysis
SINDIVEG	Sindicato Nacional da Indústria de Produtos para Defesa Vegetal
SNP	Single Nucleotide Polymorphism
UFSM	Universidade Federal de Santa Maria
USDA	United States Department of Agriculture
VIGIAGRO	Vigilância Agropecuária Internacional
WTO	World Trade Organization



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## 1 INTRODUÇÃO

A agricultura apresenta uma importância estratégica para a economia do Brasil e outros países sul-americanos. Certas populações de insetos e ácaros podem atingir status de praga e causar perdas significativas à produção de grãos, fibras, frutas e hortaliças, demandando para o seu controle custos elevados e altos impactos ambientais. Além das pragas já estabelecidas no continente, o clima favorável e a localização geográfica facilitam a introdução de espécies exóticas, ampliando os problemas fitossanitários e prejudicando as exportações de *commodities* (SUGAYAMA et al., 2015; VILELA; ZUCHI, 2015). A introdução de pragas invasivas em ambientes agrícolas representa um grave problema de biossegurança, trazendo riscos econômicos e ambientais tanto para o país afetado quanto para as suas regiões fronteiriças. A falta de protocolos unificados de fiscalização entre diferentes países, aliada ao intenso comércio de bens agrícolas e tráfego humano, tem facilitado a dispersão de espécies invasivas para além de seus centros de origem (HURLEY et al., 2016; TAY et al., 2017; SEEBENS et al., 2017; TAY; GORDON, 2019).

A introdução de pragas exóticas resulta em perdas na produção agrícola e elevação nos custos de controle, conforme demonstrado por casos históricos de invasão no Brasil. A entrada do bicudo-do-algodoeiro, *Anthonomus grandis* (Boheman, 1843) (Coleoptera: Curculionidae) em 1980, praticamente inviabilizou a cultura nos estados de São Paulo e Paraná. Já em 1982, a introdução da traça-do-tomateiro, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) elevou o custo de produção do tomate em 25% (MICHEREFF FILHO; VILELA, 2001). Por fim, a invasão de *Helicoverpa armigera* (Hübner, 1809) (Lepidoptera: Noctuidae) em 2013 resultou em perdas econômicas estimadas entre 0,8 e 2 bilhões de dólares na safra 2012/13 (BUENO; SOSA-GÓMEZ, 2014; LOPES-DA-SILVA et al., 2014).

A soja [*Glycine max* (L.) Merrill], principal *commodity* de exportação cultivada na América do Sul, é atacada por diversas espécies de artrópodes capazes de ocasionar injúrias nas plantas e reduzir a produtividade de grãos. Muitas dessas pragas são exóticas, apresentando alta capacidade adaptativa e reprodutiva que facilitam seu estabelecimento no novo habitat. Não obstante, o impacto econômico dessas invasões na produção de soja brasileira permanece largamente

desconhecido. Nesse grupo de espécies invasivas inclui-se a mosca-da-haste *Melanagromyza sojae* (Zehntner, 1900) (Diptera: Agromyzidae), cujas larvas formam galerias no interior das hastes das plantas de soja e afetam seu crescimento e desenvolvimento, reduzindo a produtividade de grãos (TALEKAR, 1989). Nativa da Ásia, sua ocorrência foi recentemente confirmada no Brasil (ARNEMANN et al., 2016a), Paraguai (GUEDES et al., 2017), Bolívia (VITORIO et al., 2019) e Argentina (TROSSERO et al., 2020), indicando uma dispersão crescente na América do Sul.

As larvas de *M. sojae* formam galerias ao longo de toda a extensão da planta de soja, prejudicando a translocação de seiva nos tecidos do xilema (CHIANG; NORRIS, 1983). Como a injúria ocorre no interior das plantas, os sintomas expressos externamente são praticamente imperceptíveis. A espécie apresenta ciclo curto (média de 21 dias de ovo a adulto) e alta taxa de oviposição (média de 170 ovos fêmea<sup>-1</sup>), possibilitando a ocorrência de três a cinco gerações por ciclo da cultura (SPENCER, 1973; TALEKAR; CHEN, 1985). A ocorrência crescente de *M. sojae* em lavouras de soja “safrinha”, semeadas a partir de 31 de dezembro no sul do Brasil, aliada à escassez de informações sobre o potencial de injúria e alternativas de manejo eficazes, demonstram a necessidade imediata de aprofundar-se o entendimento acerca da bioecologia e dinâmica populacional dessa espécie no Brasil e América do Sul (FOLLMANN et al., 2017; POZEBON et al., 2020).

A identificação correta da espécie é um dos pressupostos do Manejo Integrado de Pragas - MIP (KOGAN, 1998), podendo ser aperfeiçoado com o advento de técnicas moleculares, como o uso de marcadores. A diferenciação de espécies da família Agromyzidae por meio de caracteres morfológicos é demorada e dispendiosa, devido ao tamanho diminuto dos insetos e semelhanças taxonômicas. Nesse sentido, o uso de ferramentas genéticas permite uma identificação rápida e efetiva. Além disso, são obtidas informações acerca da diversidade genética e estrutura populacional da espécie, contribuindo para que perdas econômicas sejam evitadas e auxiliando no desenvolvimento de estratégias de manejo.

Assim, o presente trabalho compõe-se de dois artigos com o objetivo de avaliar o impacto econômico das pragas invasivas na produção de soja brasileira, estimando o risco de invasões futuras; e elucidar o cenário atual da invasão por *M. sojae* na América do Sul, caracterizando geneticamente as populações presentes no Brasil, Bolívia, Paraguai e Argentina, países já colonizados pela praga. Os artigos são precedidos por um referencial teórico e sucedidos por uma discussão geral.

## 2 REFERENCIAL TEÓRICO

### 2.1 PRAGAS INVASIVAS NA AMÉRICA DO SUL

O crescimento acelerado da população humana durante os últimos dois séculos trouxe desafios significativos para a produção agrícola. Estudos projetam um aumento de 70% na demanda por alimentos até 2050 (FAOSTAT, 2020; FEDOROFF, 2009). As exigências em produtividade agrícola demandam inovações constantes no controle de organismos que atacam o sistema produtivo, tais como artrópodes capazes de desenvolver mecanismos adaptativos (p. ex. resistência a inseticidas). Uma parcela dessas espécies possui a capacidade de se disseminar para além das suas regiões endêmicas e se tornar uma espécie invasiva, ocasionando perdas agrícolas, restrições no mercado internacional e elevação dos custos de produção (ZISKA et al., 2011). Invasões de artrópodes exóticos constituem, portanto, uma ameaça grave à segurança alimentar no mundo todo, demandando a adoção de estratégias de biossegurança para proteger a economia e conservar a biodiversidade dos países-alvo (VENETTE, 2015).

De acordo com a Convenção Internacional de Diversidade Biológica (CBD, 1992), uma espécie é considerada exótica quando localizada fora de sua região endêmica. Se a espécie introduzida é capaz de produzir uma prole fértil e sobreviver no novo habitat, ela é considerada estabelecida. Por fim, se a espécie estabelecida expandir sua distribuição, ameaçando a biodiversidade nativa, ela passa a ser considerada uma espécie invasiva (MACK et al., 2000). A introdução de espécies invasivas pode estar associada às atividades humanas, como turismo, tráfego aéreo (p. ex. *A. grandis* no Brasil; RAMALHO; SANTOS, 1994), comércio internacional (p. ex. *T. absoluta* no Brasil; MICHEREFF FILHO; VILELA, 2001), introdução intencional para fins recreativos (p. ex. coelhos e raposas na Austrália; SHORT; SMITH, 1994) ou de controle biológico (p. ex. sapos da espécie *Bufo marinus* introduzidos na Austrália para o controle de coleópteros na cana-de-açúcar; VAN BEURDEN, 1981). Incursões podem ocorrer também de forma natural, como na dispersão de *Schistocerca gregaria* da África para a América do Sul e Caribe em 1988, provavelmente por causas climáticas (CAPINERA, 2008).

Certas características biológicas facilitam a dispersão de artrópodes invasivos, como tamanho reduzido, boa capacidade de vôo, alta taxa reprodutiva, polifagia e capacidade de suportar longos períodos de transporte e armazenamento de produtos agrícolas (CAPINERA, 2008). Complicações adicionais incluem ausência de inimigos naturais, condições edafoclimáticas favoráveis e disponibilidade de fontes alimentares. O comércio internacional de *commodities* potencialmente infestadas por pragas invasivas pode ser prejudicado pela falta de protocolos padronizados de quarentena, que disponham dos meios para uma rápida identificação e erradicação dos organismos suspeitos. Prevenir a entrada de uma espécie invasiva é mais economicamente viável do que manejar os impactos de seu estabelecimento (MAYNARD; NOWELL, 2009). À medida que a espécie se dispersa no espaço (área colonizada) e tempo (anos após a introdução), seu manejo torna-se gradualmente mais oneroso e menos eficaz (HARVEY; MAZZOTTI, 2014).

O Brasil apresenta um alto risco para invasões de pragas devido às suas dimensões continentais, fronteiras extensas (15,7 mil km com dez países diferentes), variedade de condições climáticas e fluxo intenso de importações e exportações (IBGE, 2015; OLIVEIRA et al. 2013). Desde o início do século XX, 65 espécies de importância econômica foram introduzidas no país (LOPES-DA-SILVA et al., 2014), iniciando com a mosca-das-frutas *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae) em 1901. A Figura 1 ilustra o aumento na frequência de introdução de pragas no Brasil: enquanto menos de uma introdução era registrada por ano antes de 1960, dois casos passaram a ser reportados anualmente na década de 1990, e mais de três por ano a partir dos anos 2000 (SUGAYAMA et al., 2015).

Três casos notórios ilustram os riscos que essas espécies exóticas trazem para a produção agrícola. O bicudo-do-algodoeiro (*A. grandis*), introduzido em 1980, praticamente inviabilizou o cultivo de algodão nos Estados de São Paulo e Paraná. A traça-do-tomateiro (*T. absoluta*), introduzida em 1982, aumentou os custos de produção da cultura em 25 % (MICHEREFF FILHO; VILELA, 2001). Por fim, a introdução de *H. armigera* em 2013 ocasionou perdas econômicas estimadas entre 0,8 e 2 bilhões de dólares na safra 2012/13 (LOPES-DA-SILVA et al., 2014; BUENO; SOSA-GÓMEZ, 2014). Outras pragas invasivas de alto impacto introduzidas no Brasil nas últimas décadas incluem o ácaro-rajado *Tetranychus urticae* (Koch, 1836) (Acari: Tetranychidae) e a mosca-branca *Bemisia tabaci* (Gennadius, 1889) MEAM1 (Middle-East Asia Minor 1) (Hemiptera: Aleyrodidae), ambas espécies altamente

polífagas. Os casos de invasão por *T. urticae*, *B. tabaci* e *H. armigera* no Brasil e seu impacto no uso de inseticidas são detalhadamente abordados no Artigo 1 dessa dissertação (páginas 49 a 66).

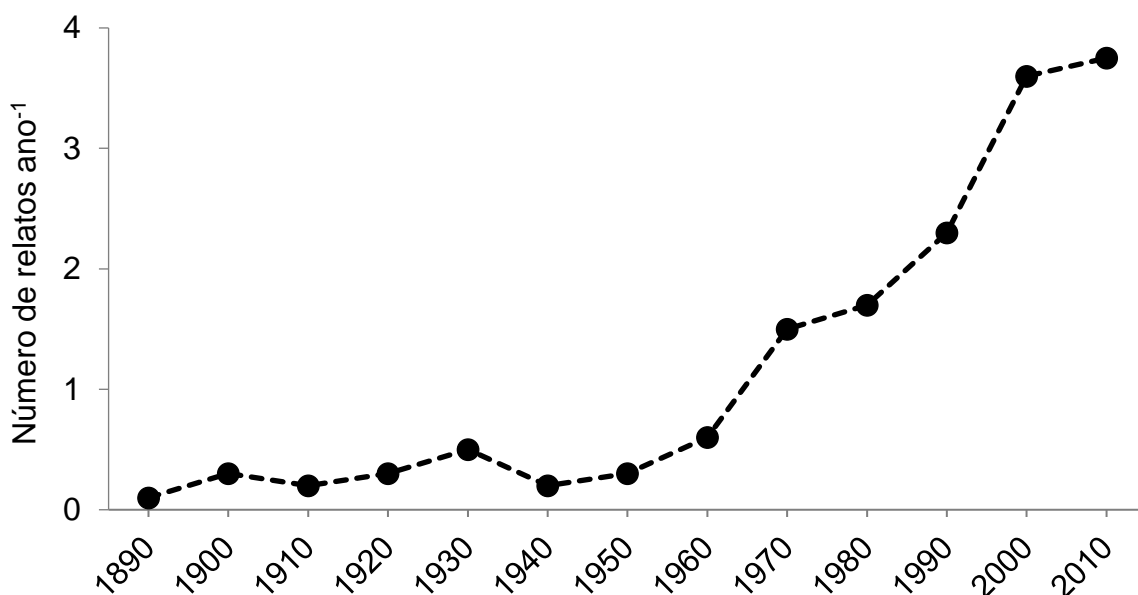


Figura 1 – Números de relatos científicos por ano de introdução de espécies-praga de interesse agrícola no Brasil

Fonte: Baseado em Sugayama et al. (2015)

A proeminência do Brasil no cenário agrícola moderno deve-se à sua extensão de áreas cultiváveis, clima favorável a diversas culturas, uso intensivo de insumos e suporte da pesquisa científica (BARROS; SPOLADOR; BACCHI, 2009; OLIVEIRA et al., 2013). Muitos desses fatores podem ser estendidos a países vizinhos sul-americanos, denotando a importância de prevenir-se a disseminação de pragas e manejar eficientemente a sua ocorrência nos locais já invadidos (LIEBHOLD; TOBIN, 2008). Argentina, Brasil, Chile, Paraguai e Uruguai são países-membros do *Comite de Sanidad Vegetal* (COSAVE), uma organização intergovernamental que visa facilitar a cooperação entre nações sul-americanas na solução de questões envolvendo pragas invasivas, como análises de risco, quarentena de plantas e certificação fitossanitária.

A soja é a oleaginosa de maior relevância na agricultura moderna (MACDONALD et al., 2015). Nativa do nordeste asiático, é atualmente cultivada em todos os continentes, com exceção da Antártida (CHUNG; SINGH, 2008). O Brasil e a América do Sul devem a essa cultura o grande impulso de mecanização das lavouras comerciais verificado nas últimas décadas, bem como a expansão das fronteiras agrícolas e modernização acelerada de outras culturas, como o milho. Com uma produção de 124 milhões de toneladas na safra 2019/20, o Brasil ultrapassou os EUA no ranking internacional e é agora o maior produtor mundial de soja (USDA, 2020). A área cultivada com a cultura alcança 37 milhões de hectares no país (32% destes no estado do Rio Grande do Sul; CONAB, 2020) e ultrapassa 58 milhões de hectares em todo o continente sul-americano (FAOSTAT, 2020). Brasil, Argentina, Paraguai e Bolívia concentram praticamente toda a produção do continente, correspondendo a 187 milhões de toneladas na safra 2019/20 (50% da produção mundial; USDA, 2020). Devido à grande extensão das áreas de cultivo de soja e à variedade de pragas associadas à cultura, cerca de 50 % dos agrotóxicos comercializados no Brasil são empregados na produção de soja (Figura 2).

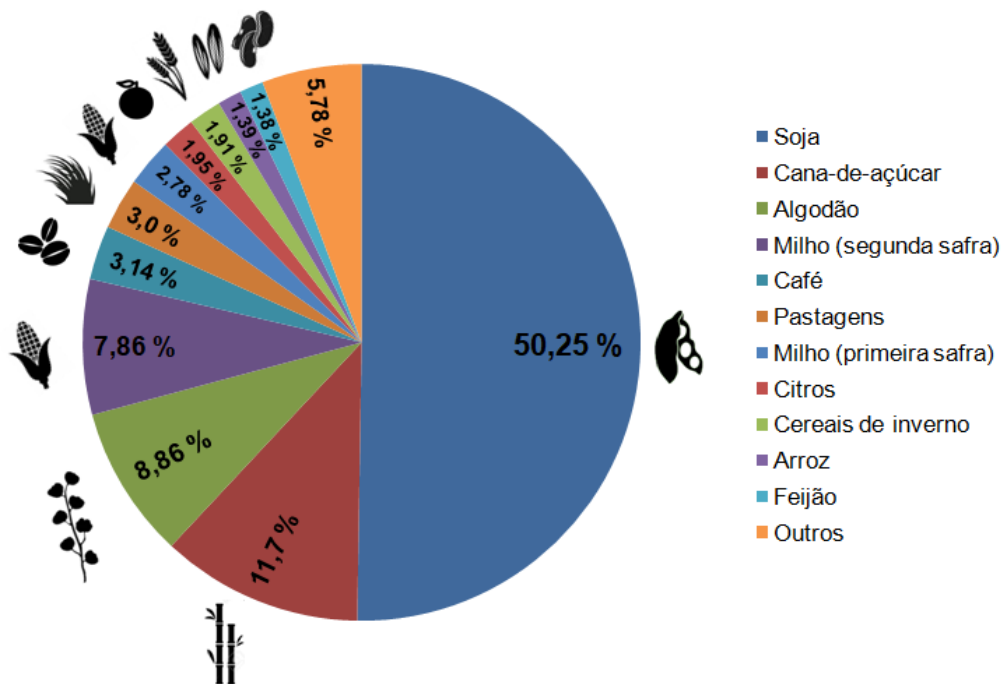


Figura 2 – Comercialização de agrotóxicos por cultura agrícola no Brasil em 2018

Fonte: Dados fornecidos por Patrícia Fédor (SINDIVEG)



Muitas dessas pragas são invasivas no Brasil, ocasionando perdas de produtividade e elevação dos custos de controle após sua introdução. Entretanto, a relação existente entre pragas invasivas da soja e aumento no uso de inseticidas nas últimas décadas permanece desconhecida. Além disso, os processos ecológicos que facilitam a entrada e estabelecimento de espécies invasivas no Brasil têm sido pouco explorados. A prevenção de entrada de pragas exóticas deve pautar-se em critérios de avaliação de risco, capazes de identificar invasões em potencial. Essas lacunas são abordadas no Artigo 1 dessa dissertação (páginas 49 a 66).

Certos fatores biológicos e ecológicos, como ciclo de vida curto, alta capacidade reprodutiva, hábito alimentar polífago e adaptabilidade a diferentes condições climáticas, podem conferir elevada capacidade invasiva a determinadas espécies de artrópodes. A lagarta *Spodoptera litura* (Fabricius, 1775) (Lepidoptera: Noctuidae) e o pulgão-da-soja *Aphis glycines* (Matsumura, 1917) (Hemiptera: Sternorrhynca) são exemplos de pragas da soja, relacionadas a esses fatores, que apresentam alta probabilidade de invadir o país no decorrer dos próximos anos. Já a mosca-da-haste *M. sojae*, também incluída nesse grupo, encontra-se firmemente estabelecida no Brasil e representa uma das principais ameaças à produção de soja na América do Sul em termos de pragas invasivas, devido ao seu alto potencial de dano, dificuldade de controle e crescente disseminação geográfica.

## 2.2 A MOSCA-DA-HASTE *Melanagromyza sojae* (ZEHNTNER)

### 2.2.1 Ocorrência na América do Sul

Nativa do leste asiático, a mosca-da-haste *M. sojae* destaca-se como uma das principais pragas da soja na Indonésia (VAN DEN BERG et al., 1998), China (WANG; GAI, 2001), Índia (THAPA, 2012) e Austrália (BRIER; CHARLESTON, 2013). As larvas dessa espécie abrem galerias no interior das hastes das plantas de soja e afetam a sua produtividade (SPENCER, 1990; TALEKAR; CHEN, 1985). Na América do Sul, o primeiro registro de moscas do gênero *Melanagromyza* infestando lavouras de soja ocorreu no Brasil (Rio Grande do Sul) em 1983, nos municípios de

Santa Maria e Passo Fundo (GASSEN; SCHNEIDER, 1985); por ocasião, a espécie não foi identificada. A ocorrência do gênero *Melanagromyza* foi reportada também no sul do Paraguai, durante a safra 2014/15 (BENÍTEZ-DÍAZ, 2015).

Posteriormente, a presença da espécie *M. sojae* foi confirmada por meio de caracterização morfológica e molecular no sul do Brasil (ARNEMANN et al., 2016a), Paraguai (GUEDES et al., 2017), centro-oeste do Brasil (CZEPAK et al., 2018), Bolívia (VITORIO et al., 2019) e Argentina (TROSSERO et al., 2020). Há também relatos de ocorrência nos estados de Minas Gerais e Bahia (CESB, 2018). Portanto, praticamente toda a região do Cone Sul da América do Sul (isto é, sul do Brasil, Argentina, Paraguai e Bolívia) encontra-se colonizada pela mosca-da-haste, com a introdução da praga no Uruguai permanecendo uma questão de tempo. Juntos, esses quatro países produziram 187 milhões de toneladas de soja em 58 milhões de hectares na safra 2019/20, correspondendo a cerca de 50% da produção mundial (FAOSTAT, 2020; USDA, 2020) e evidenciando a necessidade imediata de aprofundamento dos estudos acerca dessa praga altamente invasiva.

### **2.2.2 Morfologia e bioecologia de *Melanagromyza sojae***

Os adultos de *M. sojae* 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, com 2 mm de comprimento e coloração amarelada, passando a marrom ao longo do desenvolvimento. As larvas são amarelo-claro, translúcidas e quando completamente desenvolvidas medem cerca de 4 mm. Apresentam espiráculos anteriores pequenos em forma de botão, com oito poros minúsculos. Os espiráculos posteriores são separados e apresentam seis poros elevados em torno de uma estrutura em forma de chifre. Os ovos medem cerca de 0,35 mm (SPENCER, 1973; TALEKAR, 1990; THAPA, 2012). De caráter oligófago, a mosca-da-haste ataca apenas plantas da família Fabaceae (p. ex., *Phaseolus vulgaris*, *Pisum sativum*, *Vigna angularis*; DEMPEWOLF, 2004), tendo como hospedeiro preferencial a soja (VAN DER GOOT, 1930). A Figura 3 ilustra os principais caracteres morfológicos de *M. sojae*.

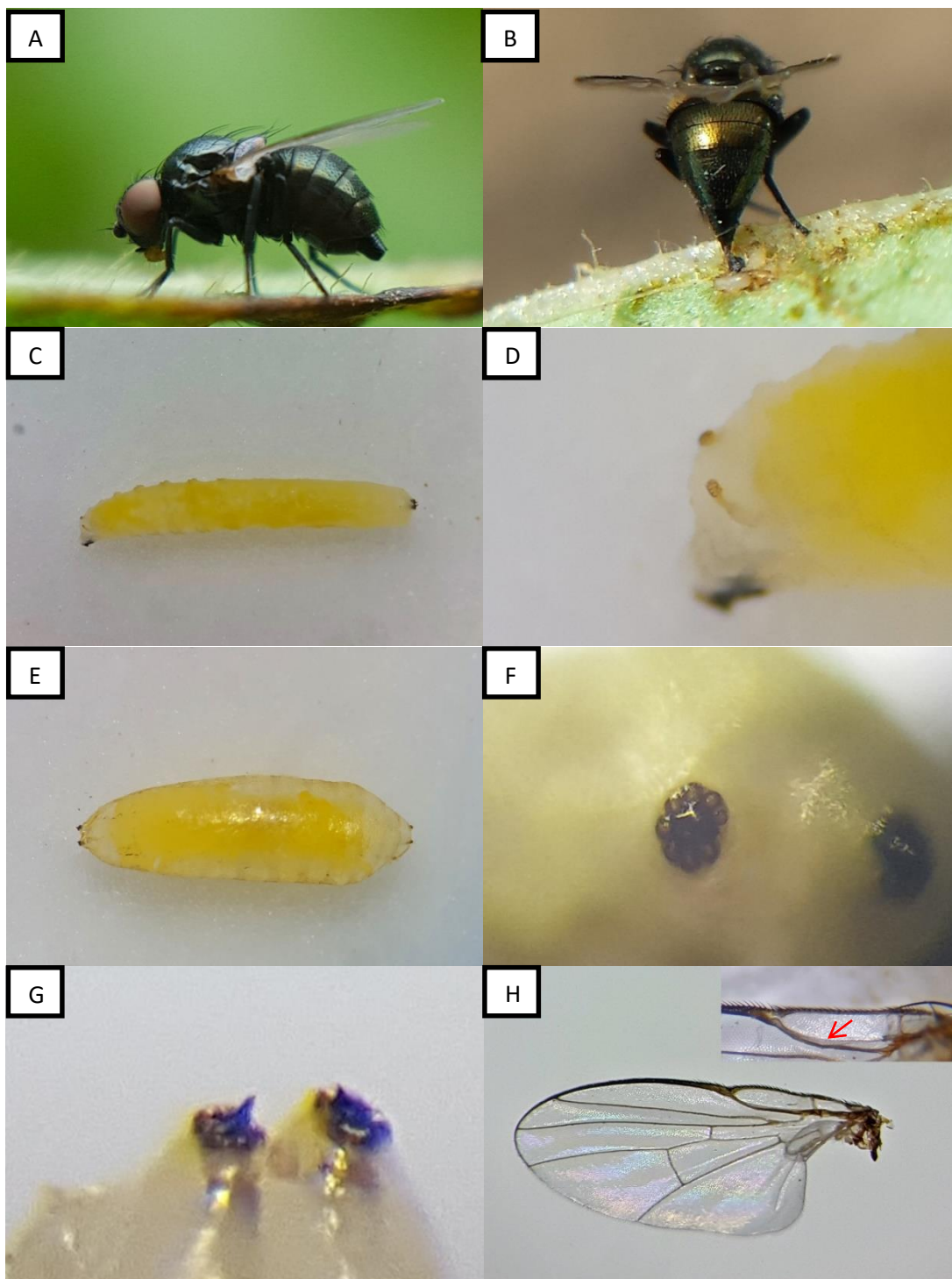


Figura 3 – Características morfológicas de *Melanagromyza sojae*: fêmea adulta (A), fêmea ovipositando (B), larva (C), espiráculos anteriores da larva (D), pupa (E), espiráculos posteriores da pupa (F, G), asa e detalhe da inserção da nervura subcostal (H)

Fonte: Vitorio et al. (2019)

O ciclo de vida de *M. sojae* compreende os estágios de ovo (dois a sete dias), larva (sete a doze dias), pupa (seis a onze dias) e adulto (10 a 46 dias), característicos de espécies com ciclo completo (WANG, 1979). O intervalo de ovo até adulto fértil varia de 16 a 26 dias, possibilitando a ocorrência de três a cinco gerações por ciclo da cultura (GANGADRE; KOGAN, 1980; SPENCER, 1973; VAN DER GOOT, 1930). As moscas perfuram a face adaxial dos folíolos de soja para alimentar-se do conteúdo celular extravasado, mas essas injúrias não afetam a produtividade de grãos (TALEKAR; CHEN, 1985). A oviposição ocorre no mesófilo de folhas jovens (trifolioladas ou unifolioladas), com um ou dois ovos depositados por furo, próximo à nervura central (LEE, 1962; THAPA, 2012; VAN DEN BERG et al., 1995). Cada fêmea deposita em média 170 ovos, variando de 41 a 270 (JADHAV, 2011; WANG, 1979), durante todo o ciclo da cultura da soja.

Após a eclosão, as larvas de *M. sojae* perfuram o mesófilo foliar em direção à nervura central, buscando atingir a haste principal da planta através do pecíolo. Cada larva produz uma galeria na taxa de 1,4 mm por hora (LEE, 1962), levando cerca de dois dias para adentrar a haste principal. Após esse período, as possibilidades de controle reduzem drasticamente, já que o inseto se torna praticamente inatingível por pulverizações de inseticida. Como resultado da alimentação das larvas na medula, formam-se túneis de coloração avermelhada que ocupam até 70% do comprimento da haste (SINGH; SINGH, 1990a). Em plantas recém-emergidas, quando a oviposição ocorre nas folhas unifolioladas, as larvas fazem galerias descendentes em direção ao solo, podendo ultrapassar a região do hipocótilo e chegar até a raiz principal. Se em seu caminho descendente a larva deparar-se com um segmento já broqueado por outra larva, ela irá inverter o sentido de alimentação e mover-se de forma ascendente na planta, podendo atingir o ponteiro e, conseqüentemente, ocasionar sua murcha (TALEKAR; CHEN, 1985).

Após completar quatro instares, a larva de *M. sojae* se alimenta das paredes da haste (ou seja, atravessa o xilema e floema) e produz um orifício de saída para a posterior liberação do adulto, o qual fica protegido com detritos durante a fase de pupa (VAN DER GOOT, 1930). Por fim, os adultos emergem dentro das galerias e abandonam a planta pelo orifício de saída feito pela larva (VAN DER GOOT, 1930; WANG, 1979). As fêmeas copulam cinco dias após a emergência e vivem de 15 a 36 dias, enquanto os machos vivem de 10 a 46 dias (SPENCER, 1973). A Figura 4 ilustra a dinâmica da injúria de *M. sojae* em plantas de soja.

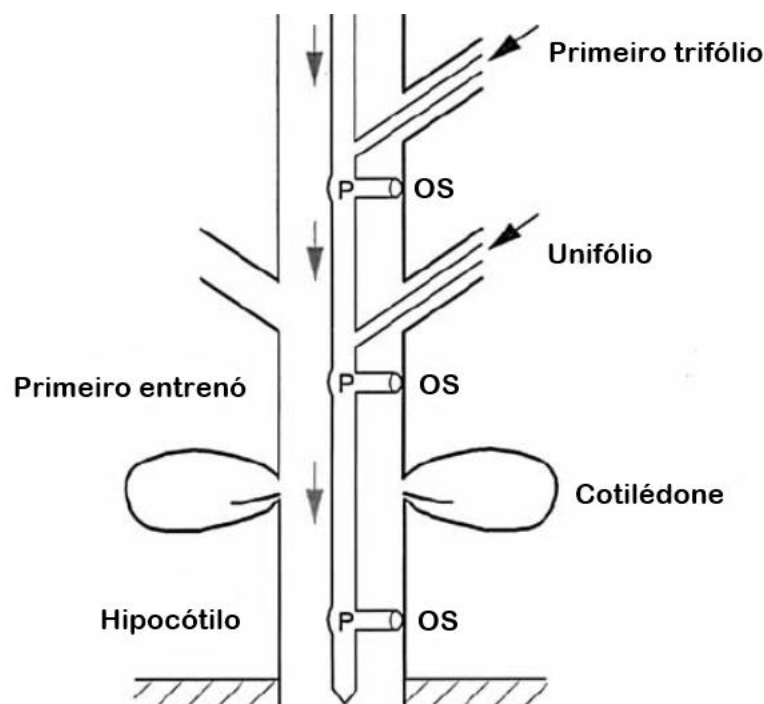


Figura 4 – Representação do terço inferior de uma planta de soja atacada por *Melanagromyza sojae*, detalhando as galerias de alimentação na medula central, os locais de formação da pupa (P) e orifícios de saída (OS)

Fonte: Van Den Berg, Shepard e Nasikin (1998)

No sul do Brasil, os maiores níveis de infestação por *M. sojae* ocorrem na segunda safra de verão ou soja “safrinha”, correspondendo às semeaduras realizadas após 31 de dezembro (CÂMARA, 2015; FOLLMANN et al., 2017). Segundo dados não oficiais, a área cultivada com soja safrinha no Rio Grande do Sul alcança cerca de 250 mil hectares ao ano, concentrados nas regiões norte e noroeste do estado, sucedendo o cultivo de milho precoce. O ciclo de vida curto de *M. sojae* e sua alta taxa reprodutiva permitem uma rápida colonização das lavouras de soja, sendo comum a ocorrência simultânea de várias larvas e pupas em uma mesma planta, tanto na haste principal quanto nas ramificações secundárias (GUEDES et al., 2015). Não obstante, é necessária a abertura da haste para verificar a presença dos indivíduos de *M. sojae* ou dos túneis de alimentação, já que as plantas injuriadas praticamente não apresentam sintomas externos (GANGRADE; KOGAN, 1980). Assim, a mosca-da-haste pode ser considerada uma “praga silenciosa” da soja, cuja ocorrência muitas vezes não é percebida pelos produtores.

### 2.2.3 Danos e manejo de *Melanagromyza sojae* em soja

Os danos ocasionados por *M. sojae* ocorrem durante sua fase larval, cuja injúria reduz a capacidade de translocação de água e nutrientes do xilema (CHIANG; NORRIS, 1983). Além do acúmulo de massa seca na planta ser restringido pela formação das galerias no interior da haste (TALEKAR, 1980; TALEKAR, 1989), a produção de grãos é afetada diretamente, já que a haste da planta de soja é o principal órgão de armazenamento de fotoassimilados que são translocados às sementes durante a fase de enchimento de grãos (STREETER; JEFFERS, 1979).

Altura de planta, área foliar total, número de flores, número de vagens, número de grãos e nodulação radicular por *Rhizobium* são alguns dos componentes de produtividade da soja afetados negativamente pelo ataque da mosca-da-haste (TALEKAR, 1989; VAN DEN BERG; SHEPARD; NASIKIN, 1998). A área foliar pode ser reduzida em mais da metade (Tabela 1). Sob altas infestações, ocorre ainda murcha e aceleração da maturação, podendo ser confundido com sintomas de doenças e nematoides (CZEPAK, 2018). Encurtamento dos entrenós, ramificação excessiva e engrossamento na base do caule são alguma das respostas fisiológicas frequentemente (embora não sempre) associadas ao ataque de *M. sojae*, podendo auxiliar na sua detecção. A Figura 5 ilustra as injúrias causadas por *M. sojae* e os principais sintomas do ataque expressos pela planta de soja.

Tabela 1 – Componentes de produtividade em plantas de soja sem e com injúria causada por *Melanagromyza sojae*. Cultivar utilizada: GC30067-0-8.

Componentes da soja	Sem injúria <sup>1</sup>	Com injúria
Nº de ramificações/planta	2,6 <sup>2</sup>	1,2
Altura de planta (cm)	51,9	41,8
Nº de trifólios/planta	12,3	9,0
Área foliar (cm <sup>2</sup> /planta)	1093,9	423,8
Área foliar (cm <sup>2</sup> /trifólio)	89,3	47,1

Fonte: Talekar e Chen (1985)

<sup>1</sup> Plantas protegidas do ataque de *M. sojae* com aplicações de ometoato 50 EC. Incidência de *M. sojae* na área "sem injúria": 6%; incidência na área "com injúria": 99%

<sup>2</sup> Diferença significativa pelo teste *t de student* a 1% de significância

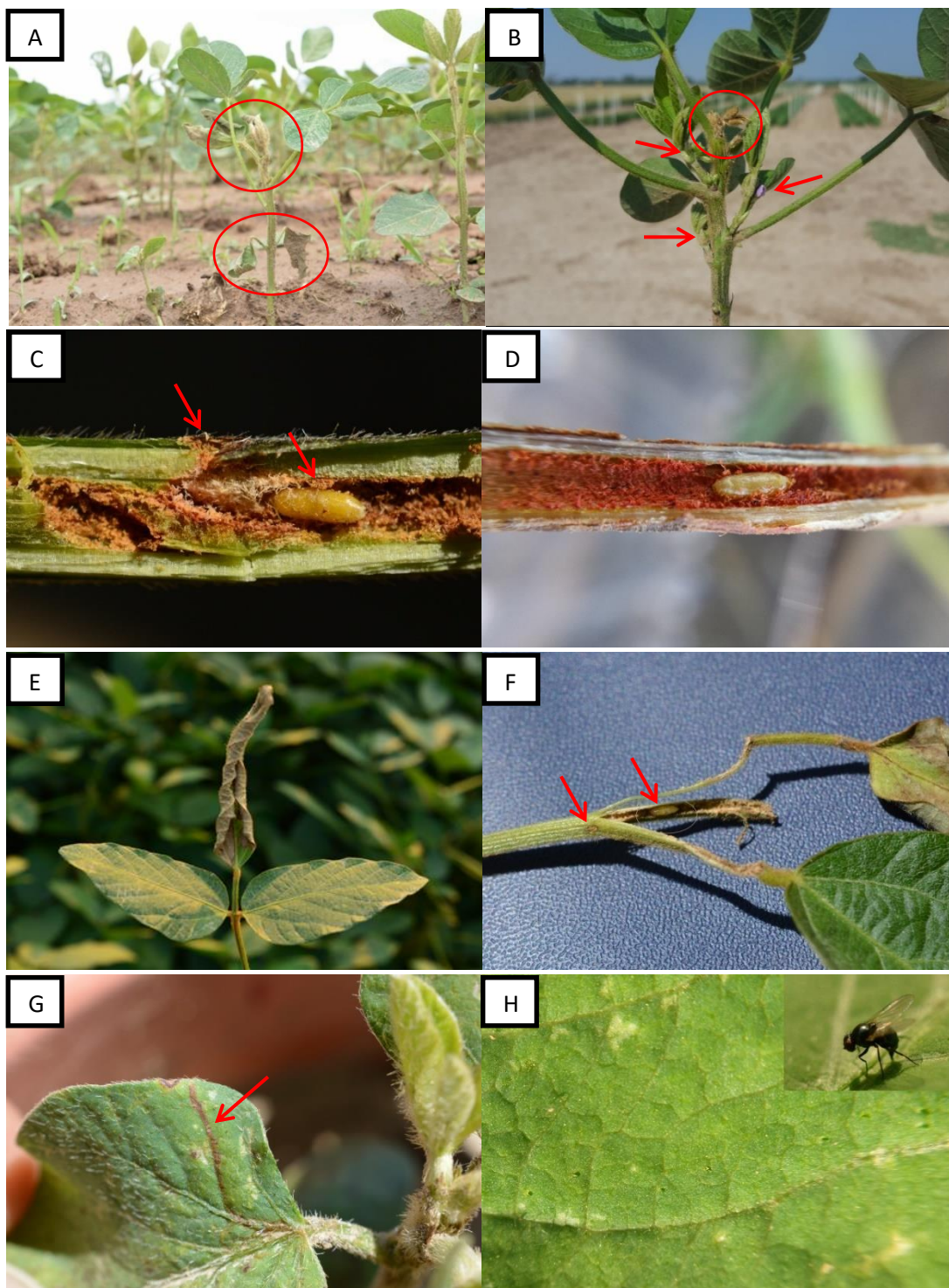


Figura 4 – Danos causados por *Melanagromyza sojae*: folhas unifolioladas e meristema apical necrosados (A), ápice e ramificações laterais mumificadas (B), pupa e orifício de saída (C), galeria com coloração avermelhada (D), folíolo necrosado (E), pecíolo exibindo pupa e furo de saída (F), folha unifoliolada exibindo nervura minada (G), puncturas de alimentação da fêmea adulta (H)  
Fonte: Vitorio et al. (2019)

A localização do orifício de saída pode ser um indicativo do momento da infestação da praga. Em infestações precoces, o orifício de saída é encontrado abaixo da cicatriz de inserção dos cotilédones. Em infestações tardias, o orifício de saída é encontrada nos entrenós acima da região do hipocótilo, pois o desenvolvimento das fibras do xilema nos entrenós próximos ao nível do solo dificulta o broqueamento pelas larvas (VAN DEN BERG et al., 1995; VAN DEN BERG; SHEPARD; NASIKIN, 1998).

Estimativas de redução de produtividade devido ao ataque de *M. sojae* variam de acordo com o local de cultivo, cultivar utilizada, data de semeadura e, principalmente, o estágio fenológico da planta de soja no momento da infestação (JADHAV et al., 2013a; SAVAJJI, 2006; TALEKAR; CHEN, 1985; VAN DEN BERG; SHEPARD; NASIKIN, 1998). Embora o inseto possa infestar plantas de soja ao longo de todo o ciclo de desenvolvimento da cultura, apenas ataques nos estádios iniciais de desenvolvimento resultam em perdas significativas na produtividade de grãos (TALEKAR, 1989). O período crítico para infestação por *M. sojae* situa-se entre quatro a cinco semanas após a emergência das plântulas; quanto mais tarde ocorrer o ataque, menor será o impacto na produtividade, pois em plantas já estabelecidas as galerias não atingem os tecidos condutores do xilema (CABI, 2020; SPENCER, 1973; TALEKAR; CHEN, 1985).

Perdas de produtividade foram estimadas em 2 % na Indonésia (VAN DEN BERG; SHEPARD; NASIKIN, 1998), 21 % em Taiwan (TALEKAR, 1989), 30 % na China (DU; HONG, 1982) e 41 % na Índia (JADHAV, 2011). Guedes et al. (2015) citam 36 % de redução sob as condições de cultivo do Brasil, mas Marques et al. (2021) observaram perdas de produtividade de até 61 % quando as plantas de soja não receberam aplicações de inseticidas antes do estágio fenológico R2 (florescimento pleno). Existe uma relação direta entre a percentagem de haste injuriada pela larva de *M. sojae* e a redução em estatura da planta e produção de grãos. Nos países asiáticos, dados da década de 80 indicavam que essa relação era de 0,18 cm de altura e 0,11 g de grãos reduzidos por planta de soja para cada ponto percentual de haste injuriada (BHATTACHARJEE, 1980; veja Figura 6). Dados posteriores indicaram reduções de 1,65 e 2,74 g de grãos por planta para cada 10 % de haste injuriada, de acordo com modelos de regressão linear e quadrática, respectivamente (VENKATESAN; KUNDU, 1994).



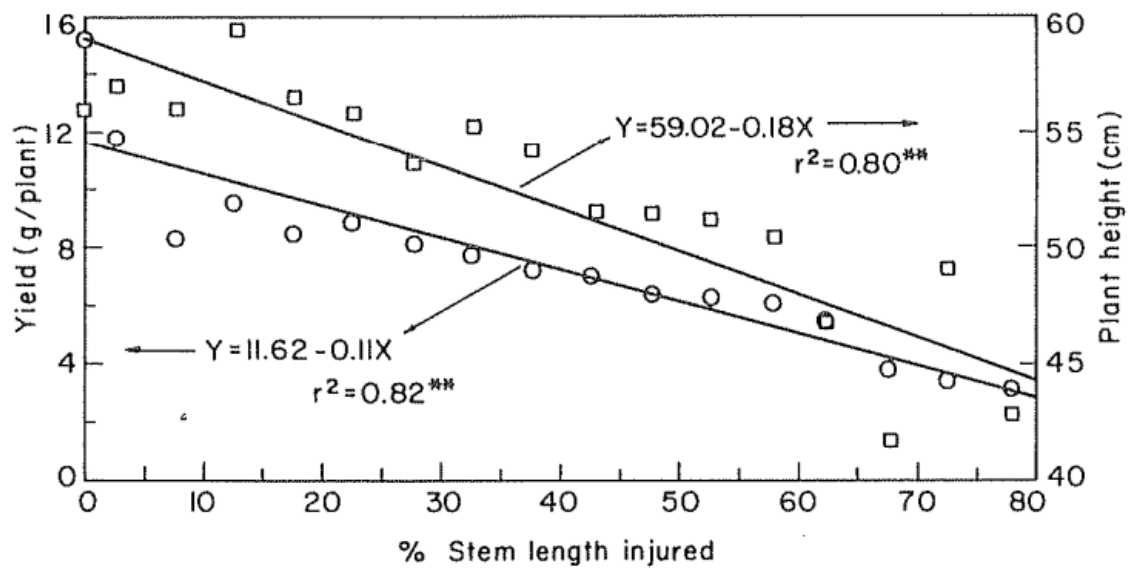


Figura 5 – Relação linear entre a porcentagem de haste da soja injuriada por *Melanagromyza sojae*, altura de planta (cm) e produtividade de grãos (g/planta)  
 Fonte: Bhattacharjee (1980)

Com base nesses valores, os autores supracitados propuseram um nível de dano econômico (NDE) para *M. sojae* de 6,4 % de haste injuriada. Em comparação, Kundu e Mehra (1989) sugeriram o valor de 26 % de haste injuriada como nível de controle. No Brasil, Marques et al. (2021) verificaram que cada ponto percentual de haste injuriada resultou numa redução de 1,02 g planta<sup>-1</sup> na produção de grãos. Considerando que as cultivares modernas apresentam menor IAF e menor estatura que as cultivares utilizadas há 40 anos, é compreensível que cada unidade de injúria causada por *M. sojae* gere uma redução maior na produtividade. Somado a isso, plantas de soja pulverizadas com inseticida antes do estágio fenológico R1 apresentaram menos danos por *M. sojae* e menor perda de produtividade, em comparação àquelas pulverizadas somente após o início do florescimento.

Embora os adultos de *M. sojae* não causem dano econômico à cultura da soja, são facilmente controlados por pulverizações de inseticidas ao sobrevoar a lavoura, ao passo que larvas e pupas permanecem enclausuradas e protegidas no interior das hastes. Outras possibilidades de controle incluem o tratamento de sementes, protegendo a cultura durante seu estabelecimento; e inseticidas com ação ovicida e efeito translaminar na folha, visando atingir os ovos depositados no mesófilo. Independente da estratégia escolhida, a janela de controle restringe-se ao

período anterior à entrada da larva na haste principal, e ao período posterior à emergência do adulto: no intervalo compreendido entre esses dois momentos críticos do ciclo de vida da praga, as chances de controle são praticamente nulas. Entretanto, diferentes estágios de vida do inseto costumam ocorrer simultaneamente na lavoura e, inclusive, nas mesmas plantas de soja, dificultando a tomada de decisão baseada apenas na biologia da praga (CURIOLETTI et al., 2018).

Nas regiões do mundo onde a espécie *M. sojae* é endêmica (isto é, no leste da Ásia), as formas de controle incluem o uso de cultivares de soja resistentes (TALEKAR, 1980), semeadura antecipada (TALEKAR; CHEN, 1983), controle biológico com parasitoides (TALEKAR, 1990; VAN DER BERG et al., 1995), eliminação de plantas voluntárias na entressafra (CZEPAK et al., 2018), uso de inseticidas aplicados via tratamento de sementes ou em parte aérea (ADAK, 2012; JADHAV et al., 2013b) e rotação de culturas com gramíneas, já que as espécies de Fabaceae são os únicos hospedeiros conhecidos de *M. sojae* (FERREIRA et al., 2020). Considerando as estratégias utilizadas em outros países, ao menos duas delas não se aplicam ao Brasil, que não dispõe de cultivares totalmente resistentes à praga e tampouco alteraria a época de semeadura, dada a estreita janela de encaixe de cultivos soja e milho. Os diferentes métodos de controle disponíveis para essa praga são detalhados abaixo.

Nos países asiáticos, parasitoides nativos (p. ex. *Gronotoma* sp., *Eurytoma* sp. e *Bracon* sp.) crescem em ocorrência concomitantemente ao crescimento populacional de *M. sojae*, atingindo até 70 % de parasitismo (TALEKAR, 1990). O parasitismo ocorre na fase larval e a emergência do parasitoide na fase de pupa da mosca-da-haste, o que não previne a injúria, mas reduz a intensidade das infestações subsequentes (VAN DEN BERG et al., 1995). Beche et al. (2018) reportaram parasitismo de *M. sojae* por *Syntomopus parisii* (De Santis, 1976) (Hymenoptera: Pteromalidae) no Brasil, com possibilidade de controle biológico por meio de liberação massal. A tolerância genética também é amplamente utilizada para o manejo da praga na Ásia, sendo que cultivares com medula estreita, folíolos menores, tricomas maiores e menor conteúdo de umidade na haste apresentam menor suscetibilidade ao ataque de *M. sojae* (CHIANG; NORRIS, 1983; TALEKAR; CHEN, 1985; VAN DEN BERG; SHEPARD; NASIKIN, 1998).

Outra tática de manejo de mosca-da-haste consiste em adequar a data da semeadura em função do fotoperíodo, de modo que o ciclo da soja não seja

encurtado. O ciclo mais longo permite maior acúmulo de matéria seca e, conseqüentemente, proporciona às plantas maior capacidade de superar as injúrias decorrentes do ataque de *M. sojae* (TALEKAR, 1989). Portanto, o desenvolvimento de programas de manejo deve priorizar a seleção de cultivares com tolerância intrínseca ao ataque de mosca-da-haste, seja por meio de ciclo longo (TALEKAR, 1989), medula central estreita (CHIANG; NORRIS, 1983) ou resistência por expressão de toxinas Bt com efeito de controle sobre dípteros (p. ex. Cry4Aa, Cry4Ba, Cry11Aa, Cyt1Aa, Cry10Aa and Cyt2Ba; BEN-DOV, 2014).

Na América do Sul, estratégias para controle de *M. sojae* permanecem escassas e pouco testadas. Embora haja 397 inseticidas registrados para utilização em soja no Brasil, nenhum deles está autorizado para o manejo de mosca-da-haste (AGROFIT, 2021). Além disso, alguns inseticidas citados na literatura para o controle de *M. sojae* sequer são comercializados no Brasil (p. ex. quinalfós, endrin, ometoato e monocotrofós) (LEE, 1962; TALEKAR; CHEN, 1985). Por outro lado, os inseticidas tiametoxam, imidacloprido, clorantraniliprole, espinosade, clorpirifós, cipermetrina e carbosulfano são comercializados no país e apresentam boa eficiência no controle dessa praga (ABDULLAH, 2001; DEBJANI et al., 2008; JADHAV et al., 2013b; KHANDHWE; NADAF; SHARMA, 2011; KUMAR; NGUYEN; GKVK, 2009a; KUMAR; NGUYEN; GKVK, 2009b). A alta capacidade de translocação dos neonicotinoides (p. ex. tiametoxam; GAZZONI, 2008) e das diamidas (p. ex. clorantraniliprole; PES et al., 2020) proporciona maiores chances de controle das larvas, ao passo que piretroides e outros inseticidas de choque são mais eficientes no controle de adultos de *M. sojae*.

Para as condições de cultivo da América do Sul, os ingredientes ativos clorantraniliprole, fipronil, imidacloprido e tiodicarbe apresentam boa eficiência de controle via tratamento de sementes, bem como imidacloprido, bifentrina e tiametoxam via aplicação foliar (CURIOLETTI et al., 2018). Os autores supracitados recomendam a aplicação de clorantraniliprole via tratamento de sementes, seguido por uma aplicação foliar de clorpirifós em até 10 dias após a emergência, repetida uma vez dentro de um intervalo inferior a 10 dias. Dessa forma, as plantas de soja são protegidas durante a fase de desenvolvimento mais sensível ao ataque de *M. sojae*. A rotação de cultura com gramíneas, como o milho, também é recomendada, uma vez que *M. sojae* desenvolve-se apenas em espécies leguminosas.

### 2.2.4 Identificação de *Melanagromyza sojae*

O Manejo Integrado de Pragas (MIP) envolve o uso harmonioso de diferentes estratégias de controle de pragas com base em parâmetros econômicos, ecológicos e sociais, com o objetivo de manter as populações das pragas abaixo do nível de dano econômico e, conseqüentemente, reduzir perdas na produção agrícola (KOGAN, 1998). A identificação correta da espécie é um dos pressupostos do MIP antes da adoção do manejo (CAMPO, 2012). No caso de pragas invasivas e espécies com grande similaridade taxonômica ou tamanho diminuto, como é o caso de moscas da família Agromyzidae, o processo de identificação convencional pode ser demorado e impreciso, por utilizar caracteres morfológicos internos ou genitália. A Figura 7, por exemplo, ilustra os diferentes aspectos dos espiráculos da larva em quatro espécies de Agromyzidae, enquanto a Figura 8 representa os diferentes locais de oviposição e alimentação dessas espécies em plantas de soja.

	<i>Ophiomyia phaseoli</i>	<i>Ophiomyia centrosematis</i>	<i>Melanagromyza sojae</i>	<i>Melanagromyza dolichostigma</i>
<b>Larva</b>				
Last instar				
Anterior spiracles				
Posterior spiracles				
<b>Pupa</b>				
Anterior spiracles				
Posterior spiracles				

Figura 6 – Características morfológicas das larvas e pupas de quatro espécies da família Agromyzidae

Fonte: Talekar e Chen (1985)

Diferentemente de *M. sojae*, as larvas das espécies *Ophiomyia phaseoli* e *Ophiomyia centrosematis* não se alimentam no interior da medula central das plantas de soja, mas sim próximas à epiderme. Já as larvas de *Melanagromyza dolichostigma* infestam exclusivamente a região do ponteiro, sendo que as fêmeas ovipositam na inserção peciolar no trifólio apical, e não no limbo foliar (como ocorre com *M. sojae* e *O. phaseoli*). Ainda, as fêmeas de *O. phaseoli* são as únicas que ovipositam também nos cotilédones das plântulas de soja; já as fêmeas de *O. centrosematis* não ovipositam em folha alguma, tampouco nos cotilédones, mas sim diretamente na epiderme (TALEKAR; CHEN, 1985). Portanto, o local de alimentação da larva no interior da haste é um indicativo prático (embora não totalmente seguro) para confirmação da espécie em questão.

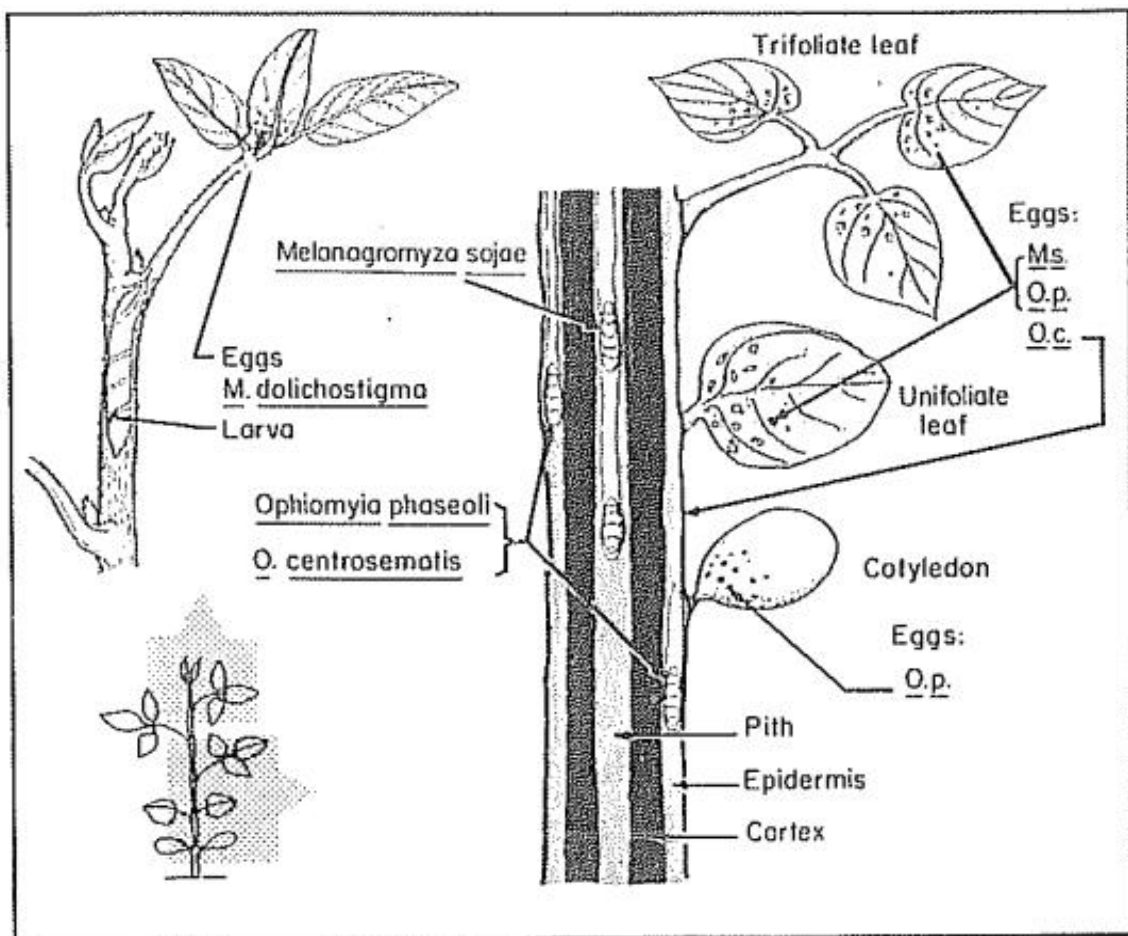


Figura 7 – Local de oviposição e hábito alimentar de quatro espécies da família Agromyzidae em plantas de soja

Fonte: Talekar e Chen (1985)

Além do aspecto dos espiráculos (Figura 7) e hábitos alimentares da larva (Figura 8), a inserção da nervura subcostal (Figura 3H) e o formato da genitália masculina do adulto também podem ser utilizados para identificação (DEMPEWOLF, 2004; THAPA, 2012). Entretanto, além de ser temporalmente dispendiosa, tal metodologia envolve um profundo conhecimento taxonômico e extensivo treinamento, acentuado pela ampla diversidade de espécies incluídas nesse grupo (mais de 2.000 espécies; THAPA, 2012). Nesse sentido, a utilização de marcadores moleculares apresenta-se como uma alternativa rápida e precisa de identificação, além de fornecer informações importantes acerca da diversidade genética e estruturação populacional da praga invasiva em questão.

### 2.3 FERRAMENTAS GENÉTICAS PARA IDENTIFICAÇÃO

O surgimento de novas técnicas de análise molecular nas últimas décadas proporcionou grandes avanços na pesquisa entomológica aplicada às pragas. A caracterização molecular é uma ferramenta que complementa a caracterização morfológica de insetos, proporcionando segurança e precisão na identificação da espécie. Além disso, as técnicas de biologia molecular podem ser utilizadas em estudos de diversidade genética e fluxo gênico entre populações, contribuindo para que potenciais perdas econômicas sejam evitadas e aperfeiçoando programas de monitoramento e manejo. Ferramentas genéticas também podem fornecer informações sobre a origem de uma espécie invasora e determinar se a introdução foi intencional ou não, o que por sua vez implica na identificação da rota de entrada e prevenção de invasões adicionais de mesma origem (SOSA-GÓMEZ et al., 2012).

Dentre essas ferramentas, o DNA mitocondrial (mtDNA) tem sido amplamente empregado como marcador molecular para estudos de genética de populações de pragas, devido à facilidade de manipulação, ausência de recombinação significativa e disponibilidade de marcadores moleculares universais (AVISE, 2000). Trata-se de uma molécula circular, compacta e com conteúdo gênico altamente conservado, diferenciando-se do DNA nuclear pelo menor número de genes (cerca de 1-2% do tamanho do genoma nuclear), estruturação em um duplo filamento circular (ao invés de uma dupla hélice), alta taxa de mutação devido à proximidade do mtDNA com a

cadeia respiratória (resultando em maior diversidade dentro e entre populações), e herança exclusivamente materna (haplóide), embora esse aspecto tenha sido recentemente contestado por alguns autores (NUNES; DOLEZAL; SCHLOTTERER, 2013; DOKIANAKIS; LADOUKAKIS, 2014). Os genes mitocondriais também estão presentes em maior número de cópias nas células, demandam um tamanho populacional menor que os genes nucleares para estudos genéticos, e podem ser facilmente isolados e replicados através de *primers* universais (ROLLINS et al., 2011; MOORE, 1995; PALUMBI et al. 2001). Alguns estudos apontam ainda que o uso de marcadores mitocondriais é mais adequado ao estudo de uma praga emergente nos estágios iniciais do processo invasivo (BOUBOU et al., 2011).

Tendo em vista esses fatores, é esperado que a identificação molecular de diferentes populações com base em genes mitocondriais seja mais rápida do que com genes nucleares, motivo pelo qual determinados segmentos do mtDNA tem sido utilizados internacionalmente como o “código de barras” de diferentes espécies (*DNA Barcoding*; ARNEMANN et al., 2016b). Dentro desse código genético, os segmentos mais comumente utilizados para tal fim são as regiões citocromo oxidase sub-unidade I (COI), citocromo oxidase sub-unidade II (COII), citocromo B (*Cytb*), 28S-D2 e ND4, devido ao alto nível de conservação desses genes (SIMON et al., 1994; GEBIOLA; BERNARDO; BURKS, 2010; ARNEMANN et al., 2016b). Os fragmentos de mtDNA são facilmente extraídos de artrópodes utilizando-se uma série de reagentes específicos, para em seguida serem replicados na ordem de milhões de cópias por meio de uma reação em cadeia da polimerase (PCR). Após a obtenção dos produtos amplificados de mtDNA, estes podem ser sequenciados e comparados com um banco de dados global (p. ex. GenBank), obtendo-se a identificação da espécie e permanecendo disponíveis para uso público.

O uso de marcadores moleculares com base no mtDNA tem sido de grande valia para a identificação de pragas em culturas agrícolas de alto valor agregado, como soja e algodão. Pragas da soja já caracterizadas por meio da região COI do mtDNA incluem as espécies *Heliothis virescens* (Fabricius, 1781) (ALBERNAZ et al., 2012), *Nezara viridula* (Linnaeus, 1758) (KAVAR et al., 2006) e *H. armigera* (ARNEMANN et al., 2019). No Brasil, Arnemann et al. (2016b) realizaram a anotação completa do genoma mitocondrial de *M. sojae* (Figura 9) e desenvolveram marcadores moleculares baseados no gene mtDNA COI da espécie, de forma a facilitar sua identificação.

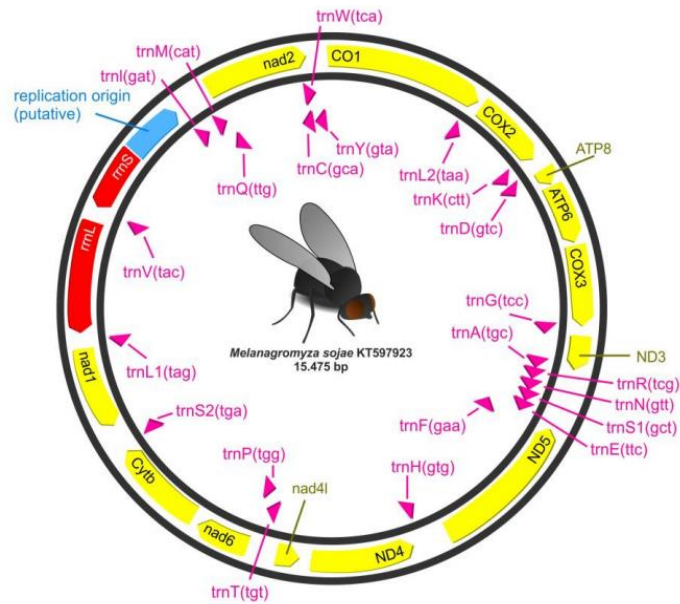


Figura 8 – Anotação do genoma mitocondrial completo de *Melanagromyza sojae*. Direções de tradução dos 27 genes indicadas por flechas  
 Fonte: Arnemann et al. (2016b)

Análises de variabilidade dentro da região COI do mtDNA podem auxiliar na compreensão dos padrões de dispersão da praga e fornecer indicadores de diversidade genética, como o número de haplótipos (ou seja, linhagens maternas) de *M. sojae* presentes na América do Sul (KANG et al., 2018; MADDEN et al., 2019; PIPER et al., 2019). A determinação da estrutura genética da população, bem como do fluxo gênico entre diferentes áreas geográficas, apresenta aplicação prática em programas de manejo, como a prevenção da dispersão de resistência ao ser rapidamente detectada em populações de campo (HENDRICHS et al., 2007; ANDOW, 2008). Estudos preliminares apontam para a presença de diferentes haplótipos em populações de *M. sojae* no sul do Brasil (ARNEMANN et al., 2016a), Paraguai (GUEDES et al., 2017) e Bolívia (VITORIO et al., 2019), sugerindo um cenário com múltiplas rotas de invasão da espécie na América do Sul. Entretanto, uma análise abrangente incluindo amostras de todos os países sul-americanos colonizados pela praga, e explorando as rotas de disseminação entre eles, permanece inexistente. Essas lacunas são abordadas no Artigo 2 dessa dissertação (páginas 67 a 85).



### 3 OBJETIVOS

#### 3.1 OBJETIVO GERAL

Avaliar o impacto econômico das pragas invasivas na produção de soja brasileira, estimando o risco de invasões futuras; e elucidar o cenário atual da invasão por *Melanagromyza sojae* na América do Sul, caracterizando geneticamente as populações presentes no Brasil, Bolívia, Paraguai e Argentina.

#### 3.2 OBJETIVOS ESPECÍFICOS

- Artigo 1 – Pragas invasivas *versus* produção de soja no Brasil: uma revisão<sup>1</sup>.
  1. Avaliar o impacto econômico causado por espécies invasivas de artrópodes na produção de soja brasileira nas três últimas décadas.
  2. Identificar as espécies ausentes de artrópodes-praga com maior probabilidade de serem introduzidas no Brasil nos próximos anos.
  3. Elucidar a situação atual da invasão por *M. sojae* no país e os prováveis cenários futuros para essa praga.
  
- Artigo 2 – Altamente invasiva e rapidamente disseminando-se: *Melanagromyza sojae* ameaça o cinturão da soja da América do Sul<sup>2</sup>.
  4. Identificar o(s) haplótipo(s) de *M. sojae* associado(s) ao cultivo da soja no Brasil, Bolívia, Paraguai e Argentina.
  5. Determinar a estrutura e diversidade genética das populações de *M. sojae* estabelecidas nos países supracitados.
  6. Sintetizar as principais estratégias de manejo disponíveis atualmente para o controle de *M. sojae* na cultura da soja.

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# Arthropod Invasions Versus Soybean Production in Brazil: A Review

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## Abstract

Soybean production in Brazil has been markedly affected by invasions of non-native arthropod species that feed on the crop, severely impacting biodiversity, food security, health, and economic development. Data on soybean production losses and increase in insecticide usage over the last two decades have not been explored in association with past invasion events, and the dynamics underlying the recent blitz of invasive species into Brazil remain largely unclear. We provide a review of arthropod invasions in the Brazilian soybean agroecosystem since 1990, indicating that the introductions of *Bemisia tabaci* (Gennadius) MEAM1 (Hemiptera: Aleyrodidae), *Tetranychus urticae* (Koch) (Acari: Tetranychidae), and *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) are likely correlated with periods of increase in insecticide usage for soybean production. Using these three cases as examples, we review factors that could lead to increased likelihood of future invasions by particular pests, outlining four possible criteria to evaluate potential invasiveness of non-native arthropods: likelihood of entry, likelihood of establishment, biological features of the species, and availability of control measures. *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) and *Aphis glycines* (Matsumura) (Hemiptera: Sternorrhynca) are examples of highly damaging soybean pests, related to one or more of these factors, that could be introduced into Brazil over the next years and which could lead to problematic scenarios. *Melanagromyza sojae* (Zehnter) (Diptera: Agromyzidae) also meets these criteria and has successfully invaded and colonized Brazilian soybean fields in recent years. Our review identifies current issues within soybean pest management in Brazil and highlights the need to adopt management measures to offset future costs and minimize lost revenue.

**Key words:** insect pest, insecticide usage, invasive species ecology, non-native species

Invasive species are plants, animals, or microorganisms not native to an ecosystem, whose intentional or unintentional introduction threatens biodiversity, food security, health, and economic development (Pimentel et al. 2001, Simberloff et al. 2013, Venette 2015). Accidental introductions of organisms into new environments have been facilitated by increase in world trade volumes and human travel activities (Hulme 2009; Lopes-da-Silva et al. 2014a, b; Tay et al. 2017), with the increase in global temperature potentially enabling these non-native species to better adapt to their new invasive range (Walther et al. 2009). Global agriculture is especially impacted by the invasion and establishment of non-native pests and pathogens, currently facing potential costs of over US\$540 billion per year if they continue to spread unchecked (Willis 2017). Along with weeds, arthropod pests are the main competitors with humans for agricultural resources, especially when high-input monocultures

are practiced over large areas (Oerke and Dehne 2004). Considering that food availability must double in the next 25 yr to keep pace with global population growth (McCouch et al. 2013), the advance of agriculture into new areas is widely predicted to favor the emergence of new pests (Venette 2015). Keeping agricultural exports ‘clean, green, and pest-free’ (Tay and Gordon 2019) is, therefore, of paramount importance to preserve food security worldwide.

Brazil stands out as a target for pest invasions due to its continental dimensions, extensive frontiers (>15,000 km by land and >7,000 km by sea, including 30 ports) and shared borders with multiple agricultural-intensive countries, wide range of climatic conditions, and intensive agricultural trade with other countries (Vilela and Zuchi 2015). Since the beginning of the 20th century and starting with *Ceratitidis capitata* (Wiedemann) (Diptera: Tephritidae) in 1901, 65 pest species of economic importance have been introduced into

the country (Lopes-da-Silva et al. 2014a, b). Invasion rate has also increased: while less than one introduction was reported per year before 1960, two cases were reported annually in the 1990s, and more than three after 2000 (Sugayama et al. 2015). The list includes key pests of major agricultural crops, such as *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in tomatoes and *Anthonomus grandis* (Boheman) (Coleoptera: Curculionidae) in cotton. The fact that pest invasions have not decreased after the establishment of sterner biosecurity protocols at the end of the 20th century (Lopes-da-Silva et al. 2014a, b) indicates that undetected invasions have risen in frequency, due to factors such as Brazil's broadening of commercial relations with other countries (Vilela and Zuchi 2015). Alternatively, the regulation programs adopted may have been ineffective in reducing invasion risks, signaling the need for protocol reevaluations and biosecurity strengthening at national borders.

The availability of data on agricultural losses is essential for the management of pest species, evaluation of plant protection practices and planning of food policies (Oerke and Dehne 2004). The annual production loss caused by all arthropod pests (native and non-native) in Brazilian agriculture has been estimated at 7.7%, on average, resulting in an annual impact of approximately US\$17.7 billion to the country's economy (Oliveira et al. 2014). According to the same author, 25 million tons of food, fiber, and biofuels are lost by the country every year due to the attack of arthropod pests, including 5.72 million tons of maize and 4.31 million tons of soybean, Brazil's two major export commodities. For soybean production alone, this represents an annual economic loss of US\$1.51 billion or US\$55 ha<sup>-1</sup>, exceeding the average amount spent annually on insecticides by soybean growers to control arthropod pests (around US\$30 ha<sup>-1</sup>; CONAB 2017). Oerke (2006) estimated potential and actual losses of soybean yield due to animal pests (i.e., arthropods, nematodes, slugs, and vertebrates) at 10.7 and 8.8%, respectively. Actual losses occur even with efforts to protect the crop (Onstad and Crain 2019).

Brazil ranks second in the world for soybean production (CONAB 2018), comprising most of the 55 million hectares of soybean grown in South America (FAOSTAT 2019) and providing abundant resources for arthropod pests that feed on it. Soybean-associated fauna in Brazil has shifted from 10 arthropod species in the 1960s to 37 in the new millennium (Panizzi and Corrêa-Ferreira 1997, Hoffmann-Campo et al. 2003). When non-native pests are introduced to this agricultural setting, immediate responses are often seen in the form of increased insecticide input per area. Annual expenditure for insecticides in Brazilian soybean production is over US\$1.3 billion, corresponding to nearly half of all insecticide sales in the country (around US\$3 billion in total sale value; Oliveira et al. 2014). Aside from direct costs related to on-field yield losses and increased insecticide usage, the introduction and spread of non-native species indirectly impacts international trade, ecosystem services, human and animal health.

Biosecurity schemes aim to prevent the introduction of organisms potentially invasive into the targeted country (Faulkner et al. 2014). Complete eradication of an invasive pest becomes less likely and more costly as the species spreads over space and time, meaning that prevention is the most cost-effective solution (Wittenberg and Cock 2005, Simberloff et al. 2013, Harvey and Mazzotti 2014). Failure in the early detection and removal of pest species will inevitably lead to long-term control efforts, as attested by the ongoing impacts of *Tetranychus urticae* (Koch) (Acari: Tetranychidae), *Bemisia tabaci* (Gennadius) MEAM1 (Middle-East Asia Minor 1) (Hemiptera: Aleyrodidae) and *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) on Brazilian soybean production during the last two decades (Grbić et al. 2011, Stacke et al. 2018, Arnemann et al. 2019a).

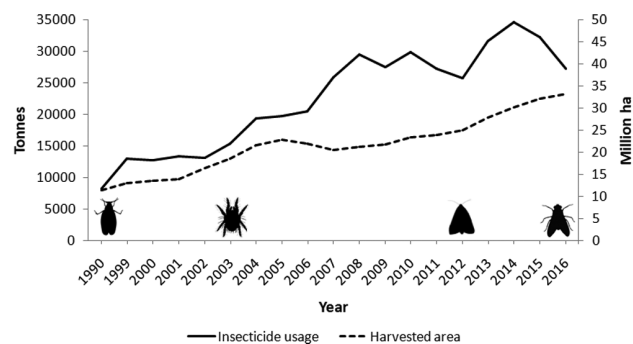
Historical data addressing pest-related impacts in Brazil over time are available (e.g., increase in insecticide usage per area), but have not yet been explored in association with past invasion events, nor have the mechanisms behind these successful invasions been clearly unraveled.

In this article, we review the impact of arthropod pest invasions on Brazilian soybean production, from 1990 to 2016, tracing a historical frame of insecticide usage and searching for correlations with major invasion events. Based on three current soybean pests, we also analyze the factors that could increase likelihood of future invasions by particular species, outlining possible criteria to evaluate potential invasiveness of non-native arthropods without resorting to risk assessment models. Two absent soybean pests and one recently established in Brazil are analyzed in-depth to illustrate this approach, emphasizing the likelihood of their introduction and potential impacts of an extensive population outbreak, respectively.

## Impact of Invasive Arthropods on Brazilian Soybean

Food production can be directly and/or indirectly impacted by arthropod pests. The second arise as a consequence of the first, and both result ultimately in increased production costs. Crop damages occurred in the field (pre-harvest) and/or during storage (post-harvest; Oerke 2006) lead to direct costs related to increased purchase and application of insecticides, medical expenses due to insecticide exposure and loss of working hours while recovering (50 unreported cases of poisoning estimated for each reported one; Dasgupta et al. 2001). Indirect impacts include damages related to environmental contamination (mainly on soil and groundwater used for drinking or irrigation; Pedlowski et al. 2012) and mortality of beneficial insects and other non-target organisms (Aktar et al. 2009). In the case of invasive pest species, restrictions of national and international trade and need for development of monitoring and management programs result in further indirect costs (Follet and Neven 2006, Vilela and Zuchi 2015).

Total insecticide usage by Brazilian soybean growers has more than tripled since 1990, with brief periods of curve stabilization or decrease (Fig. 1). While much of this growth is linked to cropland expansion, the increase in insecticide usage (3.3×) exceeds the growth



**Fig. 1.** Total insecticide and acaricide usage (tonnes) and harvested area (million hectares) with soybean in Brazil, from 1990 to 2016. Events of invasion and/or significant population increase are indicated, from left to right, for *Bemisia tabaci* MEAM1, *Tetranychus urticae*, and minor tetranychid mites (e.g., *Mononychellus planki*, *Tetranychus desertorum*, and *Tetranychus gigas*), *Helicoverpa armigera*, and *Melanagromyza sojae*. Data on insecticide usage and harvested area obtained from FAOSTAT database (2019). Insecticide usage on soybean production estimated as 45% of total insecticide usage on Brazilian agriculture each year (Oliveira et al. 2014, SINDIVIG 2018).

of harvested area (2.9×) with soybean in Brazil (FAOSTAT 2019). This increase can be linked both to higher number of sprayings per crop cycle and higher insecticide input per area, as a consequence of the increasingly diverse and hard-to-control fauna associated with soybean in the country (e.g., Hoffmann-Campo et al. 2003, Bueno et al. 2012).

Relationships between arthropod populations and soybean agroecosystems are dynamic and unstable, with secondary pests often shifting to primary status—and vice-versa. These changes in pest dynamics include native or established species rising in abundance, non-native pests being introduced in the landscape, and the possible combination of both. The population of defoliating caterpillars in Brazilian soybean fields, for instance, underwent drastic changes in its overall composition from 1975 (90% of *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Noctuidae), 10% of *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae)) to 1997 (80% of *C. includens*, 20% of *A. gemmatalis*), due to changes in cultural practices (e.g., cropping patterns, variety maturation date) and effects of pesticide applications (Guedes et al. 2015). Tetranychid mites, on the other hand, increased in occurrence after the introduction of soybean rust (*Phakopsora pachyrhizi* (Sydow & P. Sydow) [Pucciniales: Phakopsoraceae]) in Brazil in 2001, due to increased fungicide usage and consequent decrease in the population of natural entomopathogens (Roggia 2010).

Alterations in one or more of these aspects (cultural practices, efficiency of entomopathogens, and pesticide applications) are the main factors leading to changes in pest status, together with adjustments on economic injury levels and changes in host association (Kogan and Turnipseed 1987). Both native and non-native species can undergo changes in pest status, but the main increases in Brazilian insecticide usage since 1990 seem to have developed as a result of major invasion events recorded during this period: *B. tabaci* MEAM1 (introduced in the 1990s; Lima et al. 2002), *T. urticae* (introduced in the 1990s, ascended as a primary pest in 2002/2003; Roggia 2007), and *H. armigera* (likely introduced between 2006 and 2008, with maximum spread in 2012; Tay et al. 2013, 2017; Sosa-Gómez et al. 2016) (Fig. 1). The increase observed from 2006 to 2008 has been linked to a combined impact of recurrent soybean pests (caterpillars and stinkbugs) and weather-favored outbreaks of tetranychid mites and whiteflies (EMBRAPA 2007); nevertheless, the suspected arrival of *H. armigera* between 2006 and 2008 could have potentially contributed to the unexpected increase in insecticide usage per unit area during that time period. Similarly, insecticide applications in various countries have also increased since the arrival (ca. 2016) of the fall armyworm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) in the Old World (e.g., Ethiopia and India; Kassie et al. 2020, Togola et al. 2018). Arthropod invasions are, therefore, one of many factors leading to increase or decrease of insecticide usage, and often become the primary factor.

Insecticide usage per land area of soybean (Fig. 2) has steadily decreased since 2010, with a brief period of increase from 2012 to 2014. Genetically modified (GM) soybean cultivars expressing insecticidal Cry proteins from *Bacillus thuringiensis* (Berliner) (Bacillales: Bacillaceae) (Bt) were released for commercial use in Brazil in 2010, quickly becoming a key tool in the management of tropical lepidopteran pests. *Anticarsia gemmatalis* (velvetbean caterpillar), *C. includens* (soybean looper), and *Chloridea (Heliothis) virescens* (F.) (Lepidoptera: Noctuidae) (tobacco budworm) are major defoliators of soybeans in Brazil, and all are still highly susceptible to the Bt protein Cry1Ac expressed by soybean event MON 87701 × MON 89788 (Bernardi 2012). The commercial use of Bt soybeans explains the decrease in insecticide usage following the

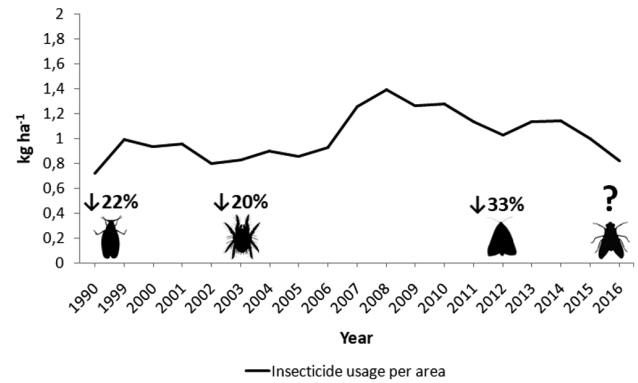


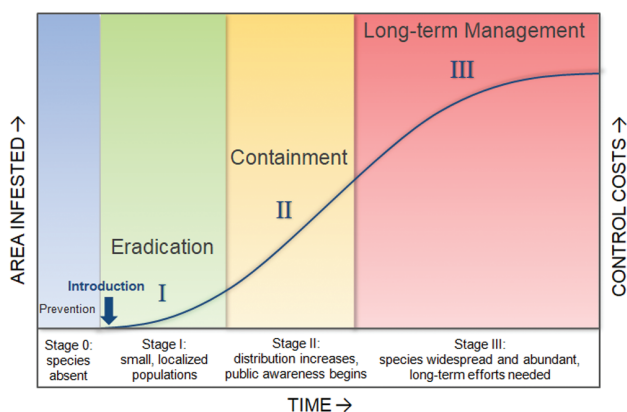
Fig. 2. Insecticide usage per land area ( $\text{kg ha}^{-1}$ ) grown with soybean in Brazil, from 1990 to 2016. Average soybean yield losses observed in plot trials under Brazilian growing conditions are indicated, from left to right, for *Bemisia tabaci* MEAM1 (Vieira et al. 2013), *Tetranychus urticae* and minor tetranychid mites (Arneemann et al. 2018), and *Helicoverpa armigera* (Osaki and Alves 2015). Data on insecticide usage and harvested area obtained from FAOSTAT database (2019). Insecticide usage on soybean production estimated as 45% of total insecticide usage on Brazilian agriculture each year (Oliveira et al. 2014, SINDIVEG 2018). '?' indicates no data available for *Melanagromyza sojae*.

release of this technology in 2010 (Figs. 1 and 2). It is also an example of how pest dynamics in an agricultural setting are rapidly modified by changes in host association. Before 2009, *S. frugiperda* was rarely spotted in soybean fields (Guedes et al. 2015); 2 yr later, it composed 20% of the caterpillar population (Stecca 2011), due to its low susceptibility to Cry1Ac (Bernardi et al. 2014).

Bt cultivars can, therefore, be described as a two-sided coin within soybean pest management: as an effective tool to control *C. includens*, *A. gemmatalis* and other minor defoliating caterpillars, it led to a considerable decrease on insecticide usage; and because of its low efficacies against *S. frugiperda* coupled with reduced spraying, this previously inconspicuous pest was able to rise unchecked in occurrence and importance within the Brazilian soybean agroecosystem. The adjustment of insecticide usage per land area (Fig. 2) reveals another noteworthy fact. It shows that Brazilian soybean growers have not willingly increased insecticide usage over time unless serious threats (such as major events of pest invasion) demanded so; and when efficient alternative technologies became widely available (such as Bt cultivars), farmers were very quick to adopt them and reduce insecticide usage.

*Helicoverpa armigera* has shown susceptibility to Cry1Ac (e.g., Yu et al. 2013, Dourado et al. 2016), but this is apparently restricted to early growth stages of the insect: sixth instar larvae are 80 times less susceptible to Cry1Ac than the first instar (Silva 2018), probably due to enhanced proteolysis inside the insect's midgut. Accordingly, following the population outbreaks of *H. armigera* throughout Brazil in 2011 and 2012 (Czepak et al. 2013), insecticide usage increased again until 2014 (Figs. 1 and 2). The novel soybean transgenic event DAS-81419-2 (Conkesta technology; Dow AgroSciences, LLC, Indianapolis, IN) expresses Cry1Ac, Cry1E, and PAT proteins (Fast et al. 2015) and is highly effective against *H. armigera* (Marques et al. 2017), representing a valuable tool for the management of this pest in the years to come; but its broad use at commercial scale has yet to occur.

Identifying where a non-native species is at in the invasion curve is the first step to planning management programs (Harvey and Mazzotti 2014), which may fall into one of four possible categories (Fig. 3). Preventing the establishment of an invasive



**Fig. 3.** The three stages of a typical pest invasion, following its introduction in a new environment, and the management measures pertaining to each stage. As the species spreads over space and time, control becomes both increasingly costly and ineffective, hence the importance of interrupting the progression of the invasion as early as possible. Curve model and respective actions obtained from [Agriculture Victoria \(2010\)](#) and [Harvey and Mazzotti \(2014\)](#), with our naming of each stage.

pest (e.g., restricting the trade of commodities that may contain non-native species at its points of origin and ports of entry) is the most cost-effective way to reduce its impacts ([Leung et al. 2002](#), [Simberloff 2006](#), [Tay and Gordon 2019](#)), followed by eradication, provided that the introduction is detected at an early stage. Eradication becomes increasingly unlikely as populations grow and become established, with efforts being redirected to the containment of the species' core population and prevention of its spread to new areas; this is the stage of the invasion at which public awareness typically begins. Finally, eradication of invading populations that become too widespread will no longer be possible, and will require ongoing future management actions aiming at population suppression and resource protection ([Agriculture Victoria 2010](#)).

The detection of *H. armigera* as a devastating component long after its introduction in Brazil, for instance, constrained management decisions to minimize the impact of the pest in high-value commercial crops ([Sosa-Gómez et al. 2016](#)). This suggests that stages 1 and 2 of the invasion ([Fig. 3](#)) were gone and its correspondent actions (eradication and containment), off the table. Complicating the situation has been the report by [Arneemann et al. \(2019b\)](#) that showed multiple entry points for the introductions of *H. armigera* into the South American continent, and thereby increasing the challenge for effective containment and eradication. As a rule, the spreading of an invasive species over space and time invariably increases its control costs and decreases its effectiveness, as evident from the recent spread of *S. frugiperda* across the African and Asian continents ([Goergen et al. 2016](#), [Otim et al. 2018](#), [Tay and Gordon 2019](#), [Jones et al. 2019](#)). Resources directed at prevention result in an estimated 1:100 return on investment; long-term management, on the other hand, provides a return 20-fold lower, at best (1:1–5) ([Agriculture Victoria 2010](#)).

### Three Case Studies on Current Soybean Pests

Gathering accurate data on geographic distribution and economic impact of invasive pests is the first step towards developing agricultural and environmental protection policies ([Lopes-da-Silva et al. 2014a, b](#)). Precise assessments of the economic impact caused by arthropod pests are, however, difficult to obtain due to the wide range of factors influencing their damage potential, such as environmental

conditions, plants species under cultivation, socioeconomic conditions of the farmers and level of technology used ([Oliveira et al. 2014](#)). Nonetheless, in-depth analyses of each particular pest can provide an overall picture of its impacts and, in the case of invasive species, give useful insights on how to evaluate the likelihood of future invasions.

We review three exemplary cases of arthropod invasion that occurred in Brazil since 1990: the invasive whitefly, *B. tabaci* MEAM1; the two-spotted spider mite, *T. urticae*; and the cotton bollworm, *H. armigera*. The three species are highly damaging to soybean production and have likely led to increased insecticide usage in the country ([Figs. 1 and 2](#)), among other consequences. Each pest will be analyzed according to the following aspects: status of worldwide distribution, host range, date of first detection in Brazil, current extent of distribution in Brazil, average yield losses observed in plot trials, stability of occurrence throughout cropping seasons and level of resistance to insecticides.

#### *Bemisia tabaci* MEAM1

The *Bemisia tabaci* whitefly species complex consists of at least 30 cryptic species that are morphologically indistinguishable (reviewed by [De Barro et al. 2011](#)). As a group, they rank second among the top arthropod pests in the world by scientific publication numbers, with 36 different plant genera serving as hosts, resistance reported for 56 different insecticides and capacity to transmit over 100 viruses ([Willis 2017](#)). Various species currently regarded as within the *B. tabaci* species complex, such as the sub-Saharan African 2 (SSA2; see [Boykin et al. 2018](#)), the Middle-East Asia Minor 1 (MEAM1, previously known as 'B-biotype'; [Brown et al. 1995](#)), and Gennadius' original *B. tabaci* species ([Tay et al. 2012](#); Mediterranean or MED, previously 'Q-biotype', see [De Barro et al. 2011](#)) have spread from their presumed native ranges to global locations, predominantly following international ornamental and agricultural trade pathways ([De Barro et al. 2011](#), [Hadjistylli et al. 2015](#), [Elfekih et al. 2018](#), [Tay and Gordon 2019](#)). To date, 165 countries have reported the presence of at least one *B. tabaci* whitefly species ([Fig. 4](#)), making it the highest global distribution of all insect pests ([EPP0 2019](#)).

Two whitefly species, NW1 (New World 1, formerly referred to as 'A-biotype') and NW2 (New World 2) occur indigenously in Brazil ([Marubayashi et al. 2013](#)), but soybean yield losses due to whitefly attack only became significant after the accidental introduction of the MEAM1 species during the 1990s ([Lourenço and Nagai 1994](#)). High infestations of *B. tabaci* MEAM1 have been reported in Brazil from that decade onwards ([Ramiro 2000](#)), moving from the status of occasional pest to major pest of soybean crops ([Lima et al. 2002](#)). More recently, multiple introductions of the invasive MED species were detected in the country ([da Fonseca Barnosa et al. 2015](#); [de Moraes et al. 2017](#)). The southern Brazilian introduction probably originated from Argentina and Uruguay, border countries that had also previously reported its occurrence ([Grille et al. 2011](#)), while a separate introduction detected in São Paulo and Paraná states was likely associated with ornamental trade pathways ([de Moraes et al. 2017](#)). While populations of the MED species tend to dominate crops grown under greenhouse conditions ([Kontsedalov 2012](#)), the MEAM1 species typically prevails in open field and is still the predominant species of *B. tabaci* in Brazil ([de Moraes et al. 2018](#)).

Total losses caused by *B. tabaci* MEAM1 on Brazilian agriculture since 1995 have been estimated at US\$3 billion ([Czepak 2010](#)), with 12 to 30% of soybean yield losses observed in plot trials ([Vieira et al. 2013](#)). Infestation levels by *B. tabaci* MEAM1 vary little between cropping seasons, with this species remaining an important soybean pest throughout the years. The pest is currently spread throughout

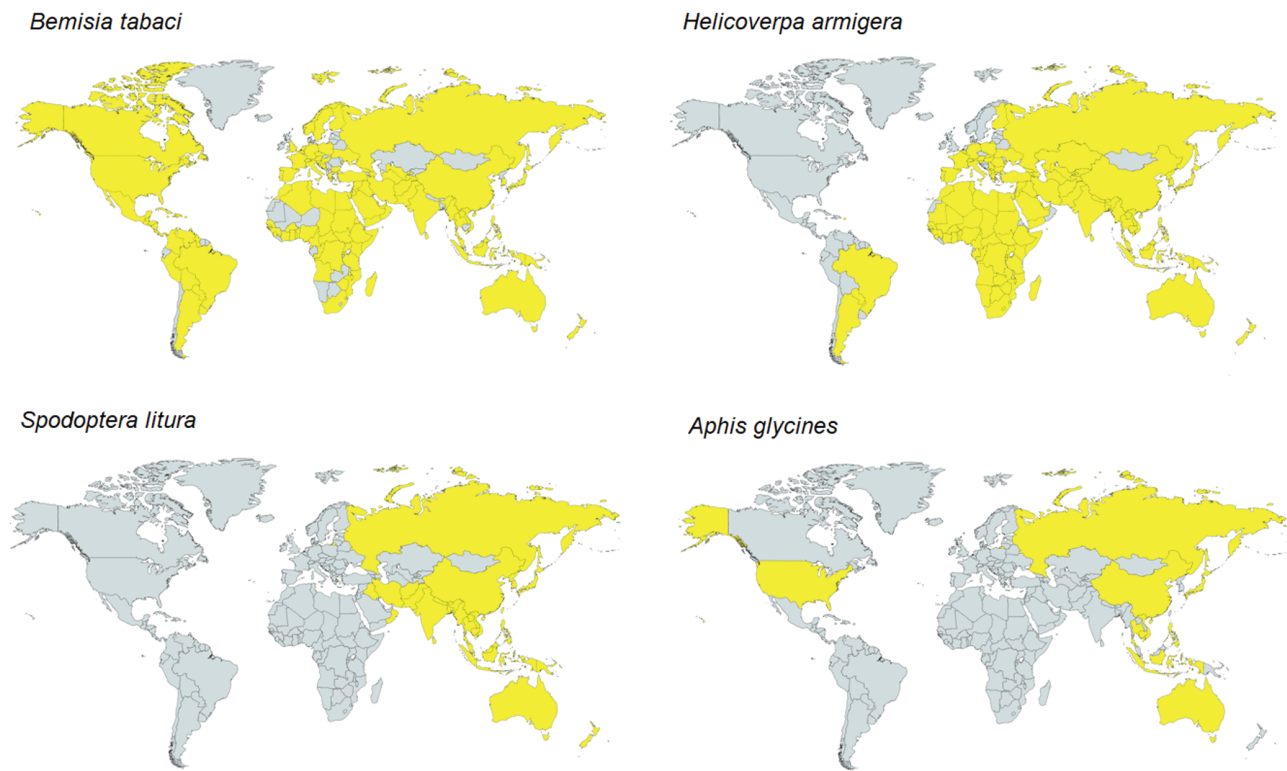


Fig. 4. Current worldwide distribution of *Bemisia tabaci*, *Helicoverpa armigera*, *Spodoptera litura*, and *Aphis glycines*. Distribution data obtained from EPPO Global Database (2019).

most Brazilian states (de Moraes et al. 2018), but occurs at higher infestation levels in the center-west region (Vieira et al. 2012). Insecticide resistance and control failures are also more frequent in this region (Dângelo et al. 2018), with control costs reaching US\$75 ha<sup>-1</sup> (EMBRAPA 2017) since *B. tabaci* MEAM1 outbreaks began. Short life cycle (16–25 d; Sottoriva 2010), high oviposition rate (160 eggs per female; Malumphy et al. 2017), uneven distribution in the plant (adults in the upper parts, nymphs in the lower; Pozebon et al. 2019) and increasing insecticide resistance (Dângelo et al. 2018) contribute to the pest survival on soybean fields, as attested by the low control efficacy provided by all insecticides currently employed in Brazil (Arnemann et al. 2019a).

High levels of resistance to azadirachtin, spiromesifen, lambda-cyhalothrin, cartap, and chlorantraniliprole have been detected in field populations of *B. tabaci* MEAM1 from Brazil (Dângelo et al. 2018), with the latter pointing to a potential development of cross-resistance to the related anthranilic diamide cyantraniliprole, as recently reported for *B. tabaci* MED in China (Wang et al. 2018). The MED species is considered to evolve stable resistance to neonicotinoid and other novel insecticides more rapidly than MEAM1 (Jones et al. 2008, McKenzie et al. 2012), including the widely used imidacloprid and bifenthrin (Karunker et al. 2008, Wang et al. 2020). Both whitefly species (MEAM1 and MED) have also shown resistance to pymetrozine, either in specific form or in cross-resistance with neonicotinoids (Gorman et al. 2010).

### *Tetranychus urticae*

Although reported as occurring in Brazilian soybean fields since the 1990s (Link et al. 1999), *T. urticae* became a major pest in the crop after unprecedented population outbreaks during the 2002/2003 and 2003/2004 summer cropping seasons (Roggia 2007), in association

with minor mite species (*Mononychellus planki* (McGregor) [Acari: Tetranychidae], *Tetranychus desertorum* (Banks) [Acari: Tetranychidae], and *Tetranychus gigas* (Pritchard & Baker) [Acari: Tetranychidae]; Guedes et al. 2007). Inappropriate use of pyrethroid insecticides (leading to mortality of natural enemies; Degrande 1998) and increased use of fungicides for the control of soybean rust *Phakopsora pachyrhizi* (affecting the population of entomopathogenic fungi that naturally controls mite populations; Roggia 2010) have been pointed out as causes for the increasing occurrence of *T. urticae* in soybean fields.

Currently dispersed throughout 111 different countries (Willis 2017), the two-spotted spider mite is a cosmopolitan pest with extensive host plant range and high level of insecticide resistance. Wind currents are thought to have spread the species across temperate landscapes of the Northern Hemisphere, while human-mediated plant transport carried it into other countries, such as Brazil (Navajas 1998, Mendonça 2009). It is one of the most polyphagous arthropod herbivores, feeding on 1,100 plant species from 140 different families (Grbić et al. 2011), and displaying resistance to 95 different insecticides and acaricides (the highest level among all arthropod species; Willis 2017). Short life cycle, abundant progeny and arrhenotoky have facilitated the rapid development of insecticide resistance in populations of *T. urticae* and other economically important Acari (van Leeuwen et al. 2010). Abamectin, acrinathrin, and melbemectin are among the acaricides commonly used for *T. urticae* control in Brazil and whose field efficacy has decreased worldwide (Sato et al. 2005, Vassiliou and Kitsis 2013, Çağatay et al. 2018), raising serious concerns about the future management of two-spotted spider mites in indoor and outdoor environments.

Soybean yield losses caused by tetranychid mites under Brazilian conditions range from 6 to 44% in plot trials (Arnemann et al. 2018). *Tetranychus urticae* is currently dispersed across all Brazilian

states, but field populations remain low when rainfall is abundant throughout the cropping season. Despite difficulties involved in its control and high potential for damage, *T. urticae* continues to shift annually between the status of primary and secondary soybean pest in Brazil, mainly due to its response to weather conditions (e.g., population increase during drought events; Wright et al. 2006) and plant factors (e.g., cultivar susceptibility; Arnemann et al. 2015). It has been suggested, nonetheless, that global warming will markedly increase the worldwide impacts of tetranychid mites in agriculture, due to accelerated development at higher temperatures (Migeon et al. 2009).

### *Helicoverpa armigera*

Considered one of the most important invasive pests in the world, the Old World cotton bollworm *H. armigera* is currently found in 143 different countries (Fig. 4), documenting 50 host genera and resistance to 49 insecticides (Willis 2017). Its annual costs to global agriculture have been estimated at over US\$5 billion (Sharma 2005), and a successful introduction in North America could put at risk US\$78 billion of agricultural output (Kriticos et al. 2015). *Helicoverpa armigera*'s presence was first confirmed in Brazil in 2013, based on morphological features (Czepak et al. 2013) and molecular characterization (Tay et al. 2013) after significant crop losses in the 2012/2013 cropping season; however, evidence suggests that it was already present in the country at least since October 2008, having reached its maximum spread as detected across northern and southern Brazil by August 2012 (Sosa-Gómez et al. 2016). Further studies on the mitochondrial DNA of South American populations revealed the complexity associated with global biosecurity network, with the detection of multiple incursion pathways for the pest into Brazil and its neighboring countries (Arnemann et al. 2019b) that was potentially associated with international trade routes (Arnemann et al. 2016a, Tay et al. 2017, Gilligan et al. 2019).

Economic losses caused by *H. armigera* in Brazilian soybean production during 2012/2013 summer cropping season were estimated at US\$0.8 billion (Buono and Sosa-Gómez 2014), and could reach as high as US\$2.5 billion year<sup>-1</sup> in a scenario of zero control (Osaki and Alves 2015). If left unmanaged, Brazilian farmers would have to increase soybean production by 30% to compensate for this yield loss. The pest is currently spread throughout all Brazilian states, but field populations have remained low compared to other defoliating caterpillars (e.g., *C. includens*, *S. frugiperda*; Guedes et al. 2015). Soybean yield losses caused by the cotton bollworm under Brazilian conditions range from 28 to 36% in plot trials (Osaki and Alves 2015). Despite its high potential for damage in soybeans, chemical insecticides employed for the control of *H. armigera* in Brazil have so far displayed satisfactory efficacy (e.g., Perini et al. 2016, Kuss et al. 2016), preventing the reoccurrence of such economic losses as when it was first detected in the country.

Several cases of insecticide resistance in *H. armigera* populations have, nonetheless, been reported worldwide, such as to carbamates (e.g., methomyl, thiodicarb) and most commercial pyrethroids (e.g., alpha-cypermethrin, beta-cypermethrin, deltamethrin, lambda-cyhalothrin, bifenthrin) in Australia (Umina et al. 2018). Widespread pyrethroid resistance has indeed been demonstrated by the presence of multiple cytochrome P450 CYP337B3 alleles in global populations of *H. armigera* including in Brazilian populations (Walsh et al. 2018), with the CYP337B3 gene originally absent in the related *H. zea* (Pearce et al. 2017) being also found to have been introgressed into *H. zea* (Anderson et al. 2016; Anderson et al. 2018) after the pest's arrival in the New World. Bt soybeans

currently grown in Brazil express the protein Cry1Ac, which is regarded as ineffective against the cotton bollworm (Yu et al. 2013, Dourado et al. 2016); however, the transgenic event DAS-81419-2 (expressing Cry1Ac, Cry1F, and PAT proteins) which is highly effective against *H. armigera* (Fast et al. 2015, Marques et al. 2017) is expected to be released for commercial use in Brazil soon, and will represent a valuable addition for the management of this pest in soybean fields.

### Overview

The three major arthropod pests that invaded Brazil since 1990, economically impacting its main grain crop, are among the top invasive pests worldwide: they are widespread, have a large host range, are highly damaging to soybean and have developed resistance to many insecticides. Accordingly, each of these events of invasion and/or significant population increase likely led to significant increases in the total amount of insecticide and acaricide used in the country (Fig. 1), as well as increased insecticide input per area (Fig. 2). The unfolding of such events reveals the key role played by invasive pests in the current agricultural scenario and the particular vulnerability of Brazil and other South American countries regarding this issue.

### Which are the Next Invasive Pests?

Preventing the introduction of non-native, potentially harming species is more cost-effective than dealing with the effects of its establishment—whether by eradication, containment, or suppression (Wittenberg and Cock 2005, Simberloff et al. 2013, Harvey and Mazzotti 2014). Unraveling the mechanisms behind the successful establishment of introduced species and outlining strategies to anticipate new invasions is a key step for mitigating their impacts (Gallien and Carboni 2017). Predicting future invasions is, however, a matter of great complexity, subject to formal processes established by international treaties. The International Plant Protection Convention (IPPC) determines that any phytosanitary measure against introduction and spread of non-native pests must be justified by a science-based pest risk analysis (PRA) (WTO 2009), and should not unduly restrict trade (Mumford 2002, Simberloff 2006).

When a non-native species is submitted to PRA, the probabilities of entry, establishment and spread in the new environment are evaluated under a specific suite of mathematical models, built on biological and bioclimatic parameters (Robinet et al. 2012). Potential economic impacts are also assessed, based on one of four quantitative techniques currently available (partial budgeting, partial equilibrium analysis, input output analysis, or computable general equilibrium analysis) (Soliman et al. 2010). The conclusions of the PRA are then used to decide whether and how non-native pests should be regulated and how much effort may be justifiably applied on prevention and management (FAO 2007). Although precise and reliable, invasive species risk assessments are time-consuming (Faulkner et al. 2014), data-intensive (Hayes and Barry 2008), not suited for screening numerous species (McClay et al. 2010) and devoid of methodologies for some taxa (Kumschick and Richardson 2013).

Another process often employed is the functional similarity approach, which determines patterns of similarity among coexisting species and their deviations from neutrality (Gallien and Carboni 2017). It relies on the assumption that invasion success increases with taxonomic diversity, indicating that invasive species benefit from resourceful sites as much as native ones—the rich get richer hypothesis, proposed by Stohlgren et al. (2003) and challenged by



Parker et al. (2010). The method itself includes the calculation of an index summarizing species similarity and a statistical test to estimate deviations from null expectations (Gallien and Carboni 2017). These analyses, however, demand large datasets (Gotelli 2000) and the use of several null models (Chalmandrier et al. 2013) to correctly infer the dominant processes driving community assembly and invasion.

Less exhaustive than full pre-border risk assessments is the creation of ‘watch lists’, used to identify threats that require monitoring (e.g., ‘warn list’ of Nehring and Klingenstein 2008, ‘black list-warning list’ of Essl et al. 2011). Faulkner et al. (2014) developed a method for creating watch lists using three predictors of invasion success (history of invasion, environmental suitability, and propagule pressure). The ‘alert list’ of the Belgian Harmonia system is similarly based on three criteria (the taxon is absent from Belgium, present in neighboring countries that are eco-climatically similar, and has potential for high environmental impact; Branquart 2007). Watch lists can be used to direct inspection efforts (Bacon et al. 2012) and prioritize post-border assessments (Parrott et al. 2009), by picking from a global pool of absent species those with high likelihood of being introduced and becoming invasive in the absence of control measures (Faulkner et al. 2014). According to Raymond et al. (2011), ‘qualitative modeling approaches can deliver consistent results and provide assistance in the development of strategic contingency plans (...) despite high levels of uncertainty regarding interactions within the underlying ecosystem’.

Quantitative analyses are, therefore, useful for overcoming challenges in invasive ecology, but present their own downsides; the same can be said of qualitative approaches. Whatever the method of choice, biosecurity screenings carried out by governmental entities represent an important source of data. The Brazilian Ministry for Agriculture, Livestock and Supply currently lists 316 arthropod species of 10 different families as absent quarantine pests (i.e., potentially harmful pests that are not yet present in the country; MAPA 2018), many of which are major soybean pests in other countries. The International Agricultural Vigilance System (VIGIAGRO), a governmental subdivision within MAPA, is responsible for screening of non-native arthropod pests at Brazil’s ports of entry. It encompasses 110 vigilance unities, distributed among seaports and riverports (30), airports (27), land frontiers (26), and inner customhouses (27) (MAPA 2014). The unities are not, however, evenly distributed across Brazil’s whole extent: most are located on south-eastern and southern states, the demographic and economic hubs of the country (Fig. 5).

In 2014, the Brazilian Institute for Agricultural Research (EMBRAPA) and the Brazilian Society for Agricultural Defense (SBDA) mapped the critical points for potential pest introduction in the country, based on geographical proximity with other countries, international entry routes and cultivation belts of major Brazilian crops (Spadotto et al. 2014). Although some critical points of entry (Fig. 6) overlap with vigilance unities (Fig. 5), many of them do not, indicating that eventual introductions along these pathways would go unnoticed. One hotspot for soybean pest invasion was also identified, at the western end of Bahia state (around the municipality of Barreiras), due to intensive cultivation of soybean, maize, and cotton coupled with the presence of one airport and absence of any vigilance unity, and could potentially explain why this region was one of the first to be affected by *H. argmigera* upon its arrival in the country (Spadotto et al. 2014).

Hirose and Moscardi (2012) listed 70 arthropod species as potential incoming threats to Brazilian soybean production, ranked along four levels of invasion risk according to their importance within native range and ecological similarities (or niche overlaps) with pests already established in Brazil (Kogan and Turnipseed

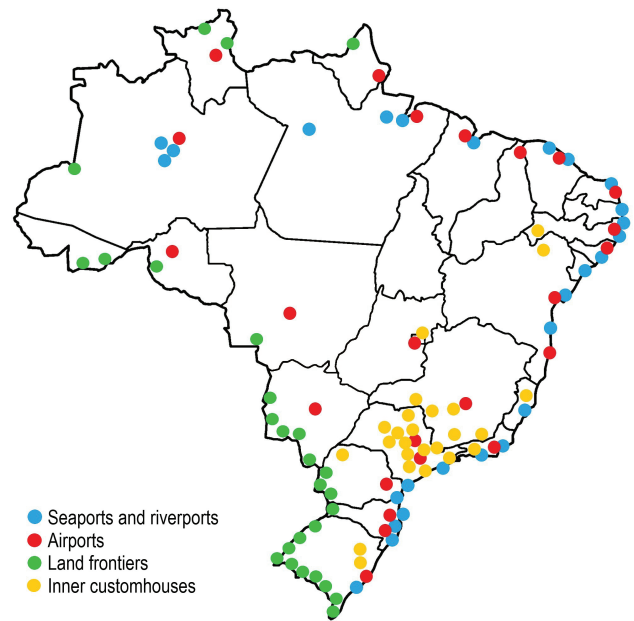


Fig. 5. The International Agricultural Vigilance System (VIGIAGRO) keeps 110 vigilance unities across Brazil, distributed among seaports and riverports (30), airports (27), land frontiers (26), and inner customhouses (27), to screen for exotic arthropod pests at ports of entry. Data on location of vigilance unities provided by MAPA (2014).

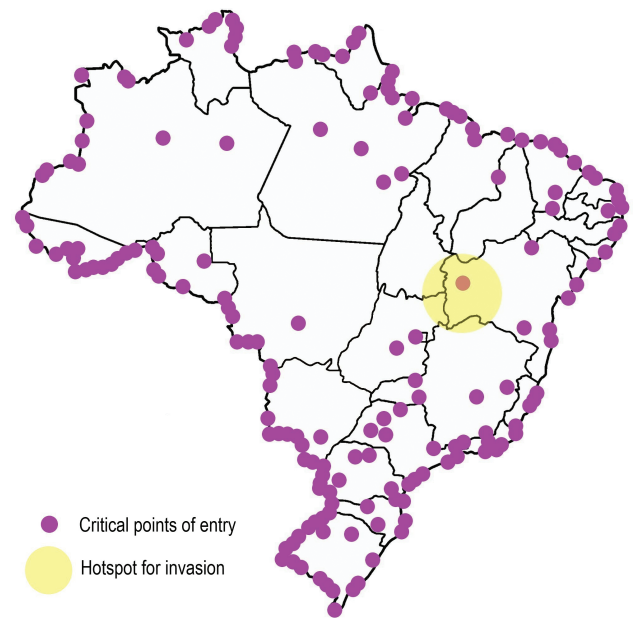


Fig. 6. Critical points of entry for agriculturally important pest species into Brazil, and one invasion hotspot identified for soybean pests at the western end of Bahia state. Data on location of entry points provided by Spadotto et al. (2014).

1987), as well as damage potential on soybean, presence of specific natural enemies, likelihood of insecticide resistance development and management hindrances due to biological traits. While a complete risk assessment would be needed to determine which of these species present the highest probability of invading Brazil in the future, certain qualitative elements can serve as criteria to evaluate the threat posed by specific pests and discuss likely scenarios of invasion.

Invasive biology has long struggled to identify which traits make some species more likely to become invasive than others. Certain demographic and competitive traits, like high fecundity, short juvenile periods, and fast growth rate, have been shown to promote invasion success (e.g., Van Kleunen et al. 2010, Dawson et al. 2011, Richardson and Pyšek 2012, Moodley et al. 2013). Tolerance of a wide range of environmental conditions (i.e., ecological competence) and ability to live off of a wide range of food types (i.e., polyphagy) have also been pointed out, since these biological features facilitate fast colonization of new environments (Capinera 2008). Another aspect associated with potential invasiveness is high dispersal ability, which includes adept flight capacity and the ability to endure long transport and/or storage of agricultural goods. All these characteristics are shared in some degree by the invasive pests addressed on the previous section (e.g., *B. tabaci* MEAM1, *T. urticae* and *H. armigera* in the Brazilian scenario, and also *S. frugiperda* in the global scenario), and it is reasonable to presume that future invaders will also likely present similar traits.

Regarding the usefulness of traits as invasiveness markers, no conclusive position has been reached to date. Kolar and Lodge (2001) found that 86% of the invasive species from a list could be identified from traits alone; however, many similar traits were also found in noninvasive species, requiring other explanations. Van Wilgen and Richardson (2011) argued that invasion success increases with phylogenetic distance between introduced and native species. Leffler et al. (2014) suggested that differences in trait values have limited power to predict future invasions, but their view has also been challenged (Dawson et al. 2015) and spurred further debate. A reasonable framework of comparison to triage future threats must, therefore, rely on more factors, such as the likelihood of a species' entry and establishment into the targeted country.

Countries intensely connected by trade and travel routes are more likely to exchange non-native species among them (Hulme 2009), as repeated patterns of human movement provide high propagule pressure and multiply the opportunities for establishment (Verling 2005). As a consequence, invasive soybean pests established in United States and China present high chances of reaching Brazil during the next years, since the three countries are the current major players on international soybean trade. The scenario where an invasive population established in one country becomes the source of invasion into another country is known as bridgehead invasion and is believed to have played a key role in shaping the distribution of many recent agricultural pests worldwide (Guillemaud et al. 2011).

In the case of polyphagous pests, introduction could also occur via other crops traded with minor trading partners. Transportation of plant material across borders stands out as the main introduction pathway for non-native pests into Brazil, with human activities being linked to 65% of the cases (Lopes-da-Silva et al. 2014a, b). Brazil is connected by land with 10 different South American countries (the third-longest land border in the world), and non-native pests could be first introduced into these other countries before appearing in Brazil, as occurred with *H. armigera* in Peru (Tay et al. 2017), Argentina, Paraguay, and Uruguay (Arnemann et al. 2019b), and with *B. tabaci* MED in Argentina and Uruguay (Grille et al. 2011, de Moraes et al. 2017). The biosecurity practices of neighboring countries are, therefore, as important to Brazil as its own.

Non-native species are continually introduced in many regions of the world, but not all thrive in the new environment. For an introduced species to establish and become invasive, it must survive a series of stages along the so-called introduction-naturalization-invasion continuum (Richardson and Pyšek 2012). As a rule, recently introduced species are primarily influenced by propagule pressure,

naturalized species are more influenced by climatic suitability and reproductive characteristics, and invasive species are highly influenced by their dispersal and competition traits (Dietz and Edwards 2006, Theoharides and Dukes 2007, Richardson and Pyšek 2012). Other interaction mechanisms, such as facilitative and multi-trophic interactions, should be further considered within invasive ecology (Gallien and Carboni 2017).

Three mechanisms or filters are thought to establish invasiveness in a newly introduced species: environmental, biotic, and dispersal filtering (Theoharides and Dukes 2007, Richardson and Pyšek 2012). First, local conditions (e.g., environment match) 'filter species based on their ecological niches and physiological adaptations'. Then, biotic competition (e.g., enemy release hypothesis) 'selects the alien species that can coexist within local communities through exploitation of unused resources or replacement of native species'. Finally, human intervention (e.g., management measures) 'determines which species spread into natural communities across the landscape'. The combination of these three assembly processes determines the success of introduced species outside their native range (Richardson and Pyšek 2012).

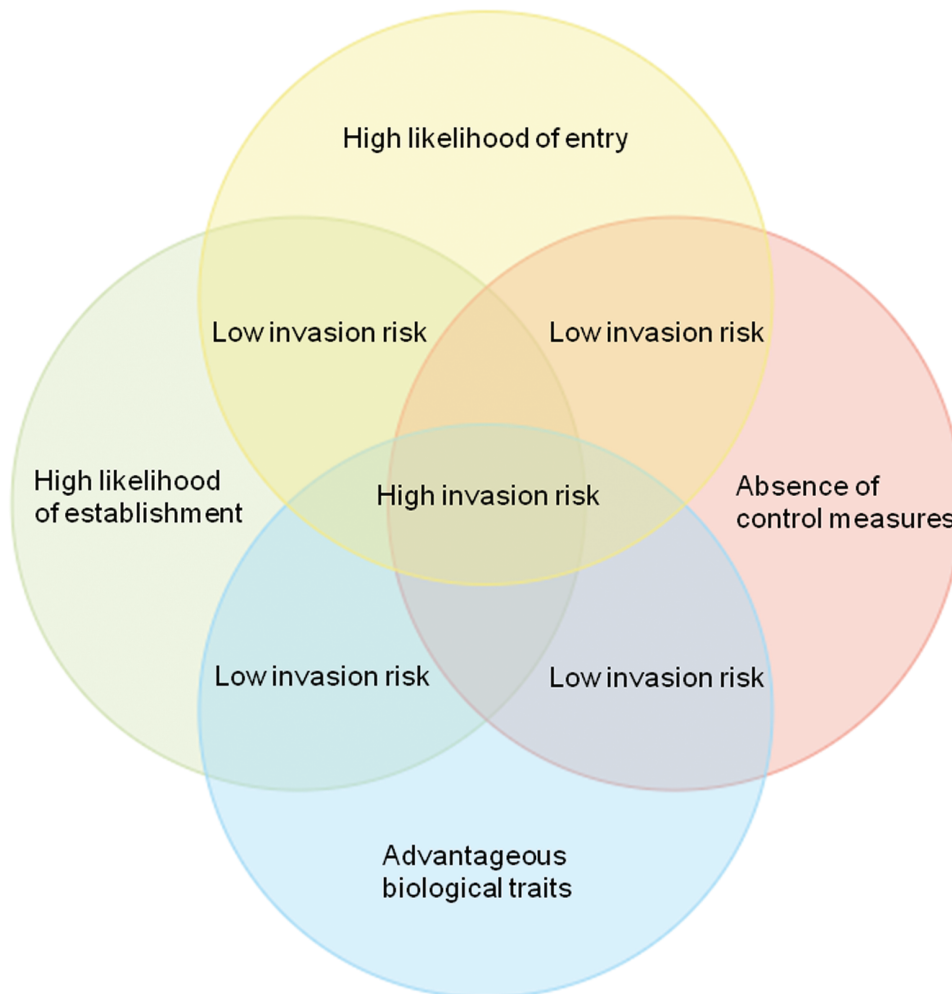
The main factors driving the invasiveness of current and future arthropod pests can, therefore, be summarized as 1) likelihood of entry (invasion history, propagule pressure, and strength of biosecurity protocols); 2) likelihood of establishment (environment match, presence of plant hosts, and absence of natural enemies in the new environment); 3) biological features of the species (feeding habit, dispersal ability, growth, and reproduction rate); and 4) availability of management measures. Invasion events can result from one or a combination of these factors, but are not necessarily limited to them. These criteria can be summarized in an 'invasive box', to illustrate how these factors might be shifted to the advantage of the country's biosecurity or the species' invasiveness (Fig. 7); or in an 'invasive diagram', to show different levels of invasion risk according to the intersection of these four criteria (Fig. 8). For instance, species known to be invasive elsewhere in the world and suitable to establish are potentially invasive but can be restricted by disadvantageous biological traits or presence of control measures.

The invasive box and diagram are meant to illustrate concepts, rather than serve as formal analytical models (as is the case with pest risk modeling); nonetheless, they are useful as tools to quickly evaluate the potential invasiveness of certain arthropod pests into a targeted country, paving the way for in-depth assessments of each species. They also relate to the four-stage actions allotted along a typical invasion curve (Fig. 3). Estimating a species' likelihood of entry into the country is the first step on preventing its introduction (first action). Eradication of an introduced species (second action) is directly dependant on its likelihood of establishment. The interplay of competitive biological traits, like high fecundity, short juvenile periods and fast growth rate, determines the species' ability to spread across the landscape and, thus, the possibility of its containment (third action). Finally, long-term management (fourth action) will not be possible unless efficient control measures are available.

We review three examples of non-native arthropod species that appear to be related to one or more of these factors, and present potential to invade Brazil and/or impact soybean production during the next years: the cluster caterpillar, *Spodoptera litura* (F.) (Lepidoptera: Noctuidae); the soybean aphid, *Aphis glycines* (Matsumura) (Hemiptera: Sternorrhynca); and the soybean stem fly, *Melanagromyza sojae* (Zehnter) (Diptera: Agromyzidae). The three species are currently widespread throughout major soybean-producing countries of Asia and Oceania, with dissemination increasing into other regions of the world. While *M. sojae*'s presence

Criterion	Biosecurity in advantage	Invasive pest in advantage
Likelihood of entry	Pest present in countries geographically and economically apart	Pest present in countries geographically and economically near
Likelihood of establishment	Unsuitable climate and plant hosts in the targeted country, natural enemies present	Suitable climate and plant hosts in the targeted country, natural enemies absent
Biological features	Monophagy, long lifecycle, slow reproduction, slow growth, limited flight capacity	Polyphagy, short lifecycle, rapid reproduction, rapid growth, adept flight capacity
Management measures	Readily available in the targeted country or under short-term development	Unavailable in the targeted country or under long-term development

**Fig. 7.** The invasive box providing qualitative summaries of factors within four possible criteria to evaluate potential invasiveness of certain arthropod pests into a targeted country, and to illustrate how they can shift to the advantage of the country's biosecurity or the species' invasiveness.



**Fig. 8.** Venn diagram illustrating the level of invasion risk of an arthropod pest into a targeted country, according to the interplay of four criteria. Intersection of four criteria determines high invasion risk; three criteria, intermediate invasion risk; and two criteria, low invasion risk. Membership of a species in one or no criterion of the diagram indicates very low and negligible invasion risk, respectively.

has already been confirmed in Brazil (Arnemann et al. 2016b, Czapak et al. 2018), *S. litura* and *A. glycines* remain absent and, if introduced, would inevitably increase insecticide usage, since alternative control strategies require time to be brought into commercial

use (e.g., incorporating aphid resistance into commercial soybean lines; Gardiner et al. 2009, Hanson et al. 2019) or have limited efficacy (e.g., poor control of the *Spodoptera* complex by certain Cry proteins; Yu et al. 2013, Bernardi et al. 2014).

### *Spodoptera litura*

Also known as cluster caterpillar and taro caterpillar, *S. litura* is currently present (whether as an invasive or native species) throughout most of Asia and Oceania (Fig. 4), including major soybean-producing countries (China, Australia; EPPO 2019). It is a polyphagous pest with high reproductive capacity (Qin and Ye 2007) and ability to migrate over long distances by flight (Armes et al. 1997), feeding on plants from at least 40 botanical families (EFSA PLH Panel 2019). This host range includes 112 economically important crops within Brassicaceae, Fabaceae, Poaceae, and Solanaceae, such as cotton, tobacco, mungbean, groundnut, eggplant, rice, cabbage, leafy vegetables, and soybean (Punithavalli et al. 2014).

*Spodoptera litura* larvae are leaf eaters although at high population densities almost all plant parts are eaten. Yield losses in soybean are directly linked to larval density (Lee et al. 2006), with infestation reaching its peak during the reproductive phase of the crop (Punithavalli et al. 2014), when soybean plants are most susceptible to defoliation (Bhattacharjee and Ghude 1985). The species is present in tropical and subtropical regions where there are no, or few, days of frost within a year, making Brazil an ideal spot for its establishment from north to south. While severe winter temperatures appear to limit the pest's development and prevent population outbreaks, the adults are able to extend their geographical range into cooler temperate regions during summer months (Sullivan 2007).

Regular interceptions of *S. litura* on ornamentals and leafy vegetables have been reported at E.U. and U.S. borders, making it a potential quarantine pest for Europe (EFSA PLH Panel 2019) and North America (Sullivan 2007). Outbreaks have occurred in E.U. glasshouses and been eradicated (EFSA PLH Panel 2019). The cluster caterpillar was also one of the first agricultural pests in India to develop resistance to insecticides, becoming sequentially resistant to benzene hexachloride (Srivastava and Joshi 1965), carbaryl (Mukherjee and Srivastava 1970), endosulfan (Verma et al. 1971), lindane and malathion (Ramakrishnan et al. 1984), cypermethrin, fenvalerate, and monocrotophos (Armes et al. 1997). Nonetheless, novel molecules such as chlorantraniliprole, spinosad, and emamectin benzoate have shown promising results against *S. litura* (Gadhiya et al. 2014, Patil et al. 2014).

*Spodoptera litura* meets, therefore, three of the four requirements for successful invasiveness: it is established in countries that trade intensively with Brazil, such as China (likelihood of entry); is well adapted to climatic conditions found in Brazil (and in its neighboring countries) and to plant species available as hosts in the country (likelihood of establishment); is polyphagous, endures long transport and storage and presents adept flight capacity (advantageous biological features). It may be satisfactorily controlled with insecticides already used in Brazil (availability of management measures), although resistance is rapidly increasing worldwide, and emergency approvals of potential insecticides for its control should be set in place as part of the pest management and preparedness strategy.

### *Aphis glycines*

Though less globally widespread (Fig. 4), *A. glycines* has expanded its range from China to north-central United States and southern Canada (EPPO 2019). With its establishment in North America has come a concurrent increase in insecticide usage (Ragsdale et al. 2011), costs to farmers (Song et al. 2008) and occurrence of resistance to insecticides (Hanson et al. 2017). Under optimal conditions, soybean aphids can double its population within 1.5 d (McCornack et al. 2004); under field conditions, this period rises to 7 d (Ragsdale et al. 2007). Facilitating the aphid's establishment is the previous

introduction of non-native plant species to North America that are necessary for the pest to shelter and feed on throughout its lifecycle.

The soybean aphid uses *Rhamnus* spp. as an overwintering host and soybean as a summer host. The introduction of both of these plants to North America pre-dated the subsequent arrival of *A. glycines*, producing conditions that made it easier for the aphid to spread throughout North America (Heimpel et al. 2004). This general phenomenon has been described as invasional meltdown, whereby positive interactions between non-native species facilitate one another's invasion by increasing likelihood of survival and ecological impact (Simberloff and Von Holle 1999). Had the management of *Rhamnus cathartica* (L.) (Rosales: Rhamnaceae) been more successful in North America, the risk of *A. glycines* outbreaks by an invasional meltdown could potentially have been avoided (Heimpel et al. 2010, Bahlai et al. 2014).

While not yet validated, this theory suggests that efforts to prevent future invasive pests of soybeans (or any crop) in Brazil require a comprehensive effort to limit the introduction and establishment of any non-native species. *Rhamnus sphaerosperma* (Sw.) (Rosales: Rhamnaceae) is the only species of the genus *Rhamnus* currently present in Brazil, occurring in seven states (all soybean producers; Lima 2015); but its viability as winter host to *A. glycines* has not yet been assessed. Likewise, summer temperatures in the soybean-growing regions of Brazil are suitable for *A. glycines* development (optimal at 27.8°C), but often surpass the maximum threshold (34.9°C) (McCornack et al. 2004).

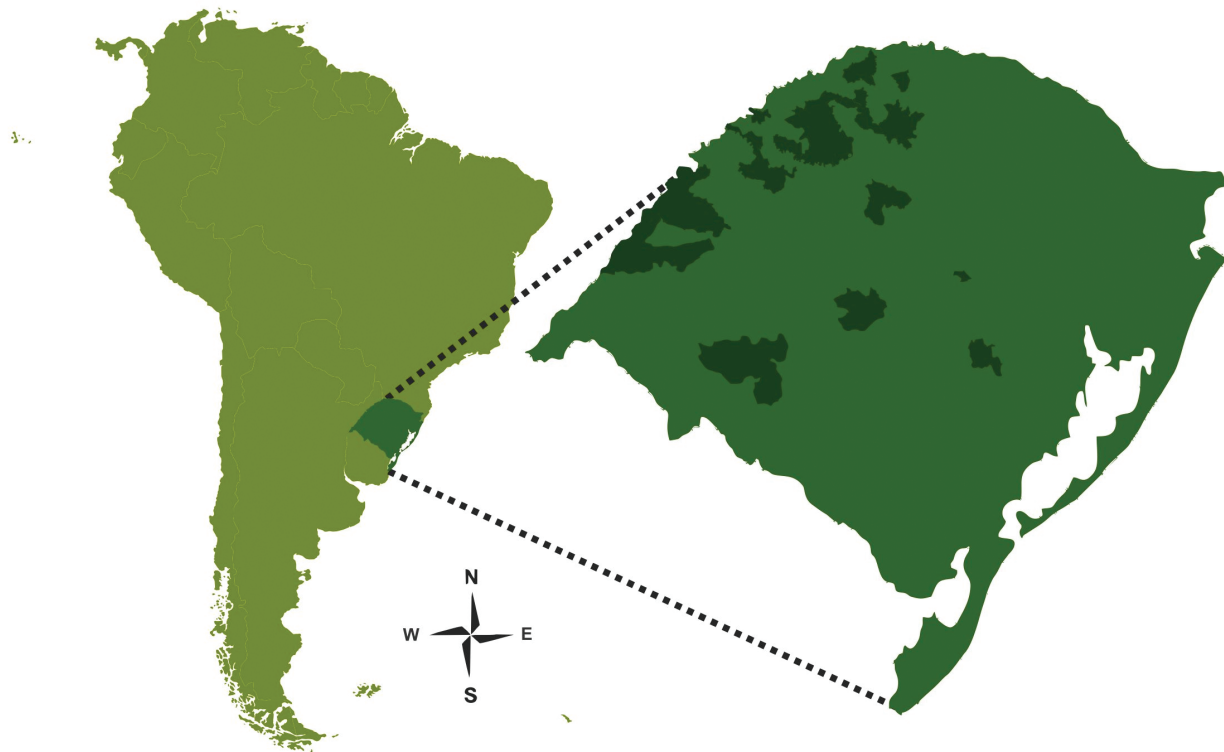
The consequences for the establishment of *A. glycines* have been profound for soybean production in the United States. Since its arrival, foliar-applied insecticide use increased fourfold (Yang and Suh 2015), even with the development of economic thresholds to help farmers determine their need (Ragsdale et al. 2011). The use of insecticides has resulted in sub-populations of soybean aphids developing resistance to pyrethroids (Hanson et al. 2017). Seed-applied neonicotinoid insecticides are available and commonly used to protect soybeans from early-season pests (North et al. 2018), but alone, they are insufficient to prevent yield loss during an aphid outbreak (Johnson et al. 2009, Krupke et al. 2017).

Resistance to *A. glycines* has been discovered in soybean germplasm and displayed the capacity to replace insecticides when used in a pyramid of two or more genes (McCarville et al. 2014), but it is currently available only through a limited number of commercial sources that primarily service organic soybean production (Hanson et al. 2019). Biocontrol agents are a significant source of mortality for soybean aphids, but may be limited due to insecticide use (Varenhorst and O'Neal 2012), the landscape in which soybeans are grown (Gardiner et al. 2009) and lack of specialist parasitoids that are considered responsible for keeping soybean aphids as a sub-economic pest in China (Liu et al. 2004).

*Aphis glycines* meets, therefore, three of the four requirements for successful invasiveness: it is established in countries that trade intensively with Brazil, such as United States and China (likelihood of entry); presents high reproductive capacity through parthenogenesis (advantageous biological features); and if introduced would lead to serious control issues due to insecticide resistance (absence of efficient management measures). On the other hand, it is not highly polyphagous and needs specific plant hosts to overwinter (which may or may not exist in Brazil), making its likelihood of establishment in the country uncertain.

### *Melanagromyza sojae*

Differently from *S. litura* and *A. glycines*, *M. sojae* is already present in southern Brazil (Arnemann et al. 2016b), center-west Brazil



#### Municipalities with *M. sojae*:

- |                       |                        |                  |                    |
|-----------------------|------------------------|------------------|--------------------|
| - Boa Vista do Buricá | - Herveiras            | - Roque Gonzales | - São Luiz Gonzaga |
| - Campo Novo          | - Independência        | - Rosário        | - São Martinho     |
| - Catuípe             | - Itaqui               | - Santa Maria    | - Seberi           |
| - Cerro Largo         | - Miraguaí             | - Santa Rosa     | - Tenente Portela  |
| - Cruz Alta           | - Palmeira das Missões | - Santo Ângelo   | - Três de Maio     |
| - Giruá               | - Pântano Grande       | - Santo Augusto  | - Três Passos      |
| - Guarani das Missões | - Porto Lucena         | - São Borja      | - Vista Gaúcha     |

**Fig. 9.** Municipalities in which *Melanagromyza sojae* has been detected in second-crop soybean (97–100% of plant infestation) during 2018/2019 summer cropping season, RS, Brazil. Dark shade indicates the municipalities, normal shade indicates the Rio Grande do Sul state, light shade indicates South America.

(Czepak et al. 2018), Paraguay (Guedes et al. 2017) and Bolivia (Vitorio et al. 2019), and preliminary analysis of mitochondrial DNA has revealed the possibility of multiple introduction pathways linking these countries (Vitorio et al. 2019). Although still absent or sparse in most Brazilian states, *M. sojae* has increased in incidence year by year in southern Brazil, being currently found in at least 28 municipalities of Rio Grande do Sul State (Fig. 9). This geographic range includes traditional soybean-growing regions (i.e., northern half of Rio Grande do Sul State) as well as new frontiers of the crop (i.e., southern half of Rio Grande do Sul State), attesting to the adaptability of the pest and its potential to hit production-threatening levels even in the absence of extensive land area grown with soybeans.

Soybean stem boring by *M. sojae* has long been a problem in many regions of the world (Dempewolf 2004), including Indonesia (van den Berg et al. 1998), China (Wang and Gai 2001), India (Thapa 2012), Australia (Brier and Charleston 2013) and parts of Russia (Strakhova et al. 2013). Quarantine inspections have often intercepted specimens at the frontiers of European Union, United States, and Japan (Roque and Auger-Rosenberg 2006, Kamiji and Iwazumi 2013). Soybean yield losses caused by *M. sojae* in plot

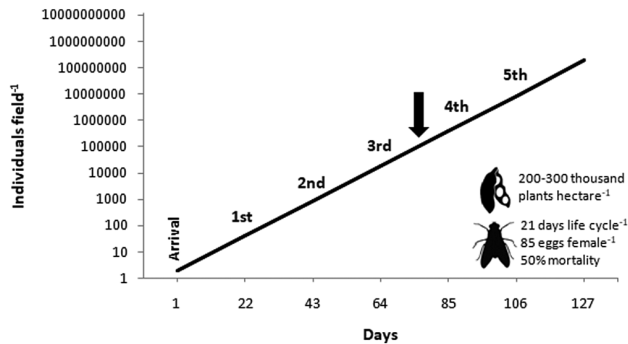
trials range from 33 to 41% in India (Jadhav 2011), but its damage potential under Brazilian conditions has not yet been determined. Detection is often compromised by the pest's small size (2–3 mm; Hirose and Moscardi 2012) and lack of external injury symptoms on the plant (Talekar and Chen 1985), while short lifecycle (16–26 d; Spencer 1990) and high oviposition rate (75–95 eggs female<sup>-1</sup>; Jadhav 2011) enable infesting populations to complete at least five generations per crop cycle.

An estimated population growth curve for *M. sojae* is provided on Fig. 10, considering 50% of natural mortality due to environmental resistance factors (e.g., death by age, unfavorable weather conditions, natural enemies, pathogens, and cannibalism). The curve shows that, in the absence of control measures, a founding population of one female and one male would need between three and four generations to colonize all soybean plants in one hectare (one individual per plant); and, at the end of the fifth generation, would have produced over 200 million descendants. This is a hypothetical rate of growth, and could be reduced to zero due to adverse conditions; field reports by soybean growers and technicians, however, point to the contrary. When *M. sojae* hits a soybean field during late summer season, almost all plants reach the end of its cycle, displaying some

degree of stem injury, regardless of region, cultivar and cultural practices.

Due to positive correlation with hot, dry weather conditions (Talekar and Chen 1983, Gopali et al. 2007), infestation outbreaks of *M. sojae* in Brazil have so far remained restricted to second-season soybean (i.e., soybean sowed after maize harvest, from December 31 onwards; Follmann et al. 2017), which is not usually grown in other Brazilian states besides Rio Grande do Sul. In soybean-growing regions of Asia, however, early-season infestations by *M. sojae* have long been reported, with subsequent peaks in larval activity occurring periodically until the end of the hot season (Kwon et al. 1981, Singh and Singh 1990a, Singh and Singh 1990b). *Melanagromyza sojae* typically overwinters as pupae inside dead soybean stems, but in the northern Brazilian states (hotter and drier than Rio Grande do Sul) active larvae have been found inside volunteer soybean plants as late as May (Czepak et al. 2018). This scenario is known as green bridge, whereby overlapping crops make food resources available for polyphagous insects (such as *M. sojae*) throughout the whole year (Oliveira et al. 2014).

The conjunction of these factors (suitable climatic conditions, natural adaptations in the pest's behavior, successful overwintering,



**Fig. 10.** Estimated growth curve for a *Melanagromyza sojae* population founded by one female and one male, considering a female/male ratio of 1:1, average lifecycle of 21 d, average oviposition rate of 85 eggs female<sup>-1</sup>, and 50% of natural mortality. The arrow indicates the point at which one hectare containing 200,000 to 300,000 soybean plants is fully colonized (at least one individual per plant). Generations are indicated along the curve, from the arrival of the founding pair to the fifth generation.

and presence of green bridges), coupled with increasing use of early-maturing soybean varieties (more susceptible to *M. sojae* attack; Talekar 1989), could soon expand the occurrence of *M. sojae* to Brazil's main-season soybeans. If such scenario develops, 55 million hectares of South American soybeans would face a major production threat, with few alternatives at hand to manage it. While some studies have explored insecticides for *M. sojae* control (Curioletti et al. 2018) and possible parasitoid species for use as a biocontrol agent (Beche et al. 2018), the concealed feeding habit of the larvae poses a considerable challenge for effective control. If infestation is virtually unavoidable due to winter survival, efforts should be directed to select and/or develop soybean cultivars displaying some degree of resistance to the pest, whether by late maturation (Talekar 1989), narrow pith cavity (Chiang and Norris 1983) or expression of Bt toxins effective against dipteran insects (e.g., Cry4Aa, Cry4Ba, Cry11Aa, Cyt1Aa, Cry10Aa, and Cyt2Ba; Ben-Dov 2014). Should the industry decide on Bt soybeans targeting *M. sojae*, Bt resistance management strategies for this pest would also be needed.

Determining the origin and genetic traits of the invasive population are the most effective ways to identify dissemination pathways available to the pest (Sugayama et al. 2015). The reconstruction of invasion routes with genetic-based methods 'can address fundamental questions in ecology and practical aspects of the management of biological invasions in agricultural settings' (Guillemaud et al. 2011), helping to mitigate economic impacts resulted from the invasion and prevent the occurrence of new ones (Estoup and Guillemaud 2010). Accordingly, efforts should now be focused on disentangling the introduction pathways of *M. sojae* into Brazil and other South American countries through whole-genome sequencing approach, elucidating the origin(s) of the invasive influx, the mean(s) by which it crossed the existing biosecurity barriers of these countries, and the strategies to be designed in order to prevent future introductions of other pests along the same paths.

**Overview**

The risk assessment of potential invasive pests allows the adoption of management measures at early stages of the invasion before control becomes exceedingly costly and with reduced effectiveness. Prediction of future invasions and adoption of phytosanitary measures must be justified by science-based pest risk analysis, but the potential invasiveness of non-native arthropods can also be reviewed

Criterion	Biosecurity in advantage	Invasive pest in advantage
Likelihood of entry		
Likelihood of establishment		
Biological features		
Management measures		

**Fig. 11.** The invasive box can be used to preliminarily evaluate potential invasiveness of future arthropod pests into Brazil. The silhouettes in each row represent, from left to right: *Spodoptera litura* (not yet introduced), *Aphis glycines* (not yet introduced), and *Melanagromyza sojae* (introduced and becoming highly invasive).

and evaluated according to four primary criteria: likelihood of entry, likelihood of establishment, biological traits of the species and availability of management measures. *Spodoptera litura* and *A. glycines* are examples of highly damaging soybean pests, related to one or more of these factors, that could potentially be introduced to Brazil in the near future (Fig. 11). *Melanagromyza sojae* also meets these criteria and has successfully invaded and colonized Brazilian soybean fields, demanding development and presenting challenges for the adoption of management measures to offset future costs and minimize lost revenue.

## Conclusions

Understanding historical cases of pest invasion in Brazil is useful to mitigate future potential economic losses and other detrimental effects, through enhancement of border surveillances and adoption of science-based government policies on agricultural trade and plant protection. The introductions of *B. tabaci* MEAM1, *T. urticae*, and *H. armigera* in the country are likely correlated with periods of increased insecticide usage in soybean production. Likelihood of entry, likelihood of establishment, biological attributes of the species and availability of management measures are among the main factors driving the invasiveness of arthropod pests. These factors can be quickly evaluated for each particular species, helping to predict the probability of future invasions. *Spodoptera litura* and *A. glycines* are among the non-native pests that could invade Brazil during the coming years and impact soybean production. *Melanagromyza sojae* is established in the country with potential to develop into a major soybean pest, and urgent actions to develop adequate management strategies for this pest under Brazilian soybean-growing conditions are needed.

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# Highly diverse and rapidly spreading: *Melanagromyza sojae* threatens the soybean belt of South America

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**Abstract** The soybean stem fly, *Melanagromyza sojae*, an Asian native insect, has successfully established in Brazil, Argentina, Paraguay and Bolivia. These countries are among the lead global soybean producing nations, being collectively known as the soybean belt of South America. Infestation levels of *M. sojae* grow by the year, facilitated by the lack of efficient management strategies. Previous studies have revealed a high number of maternal lineages in *M. sojae* populations from Southern Brazil and Paraguay,

but a comprehensive survey on genetic diversity combining samples from all countries within the South American soybean belt remains absent. We used the mitochondrial DNA cytochrome oxidase I partial gene (mtCOI) to characterize specimens of *M. sojae* collected in fourteen Brazilian sites and one Argentine site, and then combined our mtCOI data with previously published data from Australia, Bolivia, Paraguay, and other Brazilian sites, to investigate genetic diversity in this invasive agricultural pest species. Based on the molecular characterisation of the mtCOI gene, haplotypes Msoj-COI-01 and Msoj-COI-02 have the highest frequencies in the continent. The

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high genetic diversity found is evidence of introductions involving multiple female founders into the continent, and the high proportion of unique mtDNA haplotypes identified from Brazil, Paraguay and Bolivia ( $\sim 50\%$ ) suggests potential novel introductions have taken place. The findings from our study will contribute to a better understanding of *M. sojae* genetic diversity in South America, supporting the development of management strategies for this highly invasive pest and assisting with biosecurity preparedness of other emerging Agromyzidae flies of economic importance.

**Keywords** Agromyzidae · Genetic diversity · *Glycine max* · Molecular characterization · Soybean stem fly

## Introduction

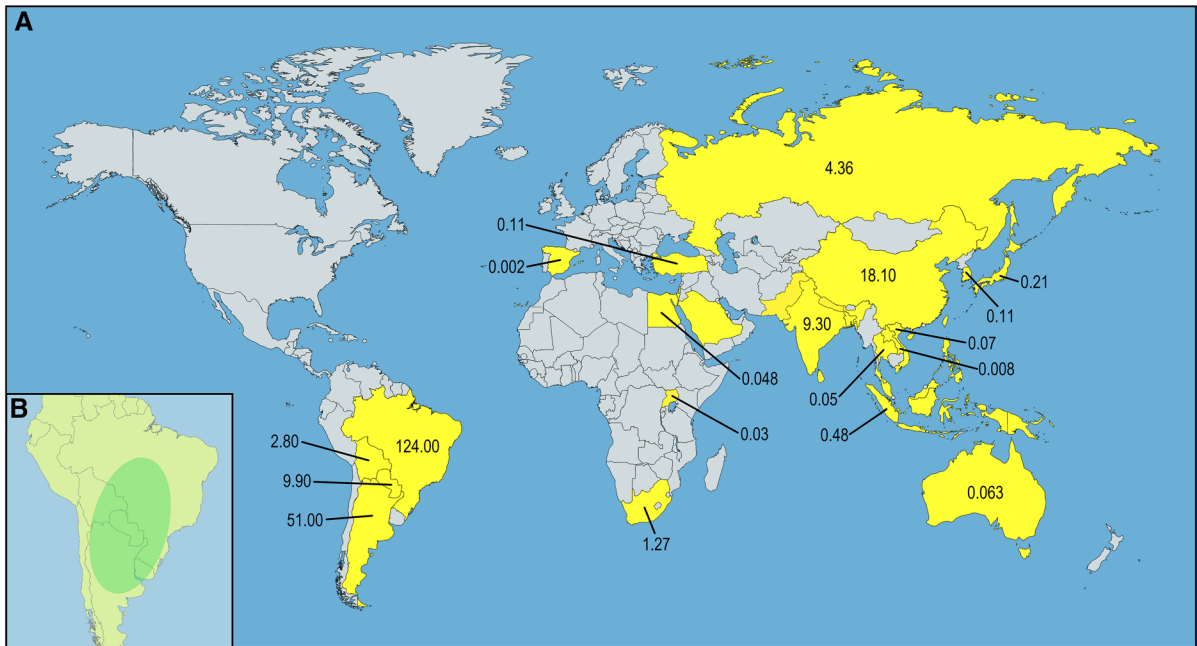
Soybean production in South America is concentrated on Brazil, Argentina, Paraguay and Bolivia, in that order. Together, these four countries grow 187 million metric tons of soybean on 58 million hectares annually (50% of the world production; FAOSTAT 2020, USDA 2020), being collectively known as the ‘soybean belt’ of the continent. South American farmers have historically struggled against many pests and pathogens, which thrive successfully in this tropical/subtropical region and severely impair soybean yield. Non-native arthropod species are among the most damaging and hard-to-control pests within soybean agroecosystems, frequently leading to increased insecticide usage following its introduction into the new environment (Pozebon et al. 2020). Among the long list of arthropod invasions in South America is included the soybean stem fly, *Melanogromyza sojae* (Zehnter) (Diptera: Agromyzidae). Native to East Asia (Spencer 1973), this species was confirmed as occurring in Southern Brazil in 2015 (Arnemann et al. 2016a) and Central-West Brazil in 2018 (Czepak et al. 2018), but is probably present in the country since 1983 (Gassen and Schneider 1985). Field reports also indicate that the pest has reached

Brazilian states of Minas Gerais (Southeast region) and Bahia (Northeast region) (CESB 2018). Molecular characterization subsequently confirmed its occurrence in Paraguay (Guedes et al. 2017), Bolivia (Vitorio et al. 2019) and Argentina (Trossero et al. 2020) (see Fig. 1 for the species’ current known worldwide distribution).

*M. sojae* larvae injure soybean plants by boring the main stem and feeding on the pith, leading to yield losses estimated to range between *ca.* 2% in Indonesia (van den Berg et al. 1998) to 40% in India (Jadhav et al. 2013), and reaching as high as 50% in untreated plots in Thailand (Pitaksa et al. 1996). High oviposition rate (170 eggs female<sup>-1</sup> on average; Wang 1979) and short lifecycle (16–26 days; Spencer 1990) allow the occurrence of at least five generations per crop cycle. Infestation outbreaks are correlated with sowing date and maturation rate, being more frequent on late season (Talekar and Chen 1983) and more damaging on early-maturing soybean cultivars (Talekar 1989). As such, *M. sojae* has become the main pest of second-season soybean (i.e. soybean sowed after maize harvest, from December 31<sup>th</sup> onwards; Follmann et al. 2017), typically grown on *ca.* 250 thousand hectares of Rio Grande do Sul and Santa Catarina states (Southern Brazil). Late soybean cultivation and survival of volunteer plants in the fields, coupled with presence of alternative overwintering plant hosts (e.g. Persian clover *Trifolium resupinatum*; Ferreira et al. 2020), have allowed the species’ populations to survive winter conditions and hit the summer cropping season at production-threatening levels. While some studies have proposed chemical (e.g. Curioletti et al. 2018) and biological (e.g. Beche et al. 2018) strategies for *M. sojae* management, control efficiency is often impaired by the concealed feeding habit of the larvae.

Increasing international trade and human travel activities have facilitated the dispersal of invasive arthropod species (Hurley et al. 2016; Tay et al. 2017; Seebens et al. 2017). The spreading of an invasive species over space and time increases control costs and decreases control effectiveness (Harvey and Mazzotti 2014; Pozebon et al. 2020), as observed in the recent spread of *Spodoptera frugiperda* across Africa and Asia (Goergen et al. 2016; Tay and Gordon 2019). Variability analysis within standard DNA barcode region has provided preliminary evolutionary genetic understanding of invasive pests and enabled a starting point to determine dispersal patterns, while also

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**Fig. 1** Worldwide distribution of *Melanagromyza sojae* (yellow colour) and soybean production (million metric tons) of each country in the 2019/20 cropping season (A). In detail (B) the ‘soybean belt’ of South America, where soybean

production in the continent is concentrated. *M. sojae* distribution data obtained from CABI (2020). Soybean production data obtained from FAOSTAT (2020) and USDA (2020)

assisting with molecular species diagnostics (Kang et al. 2018; Madden et al. 2019; Piper et al. 2019). Examples of such approach, as applied to non-native arthropod species in Brazil, include *Helicoverpa armigera* (Tay et al. 2013; Arnemann et al. 2019) and the eucalyptus pest *Thaumastocoris peregrinus* (Machado et al. 2020), while Arnemann et al. characterised the full mitochondrial genome of *M. sojae* (2016b) and developed the cytochrome oxidase I (mtDNA COI) gene as DNA marker (2016a) to facilitate further analyses of the pest genetic diversity in South America. Previous studies have revealed the presence of several mtCOI haplotypes in *M. sojae* populations from Southern Brazil (Arnemann et al. 2016a), Paraguay (Guedes et al. 2017) and Bolivia (Vitorio et al. 2019), hinting at a multiple invasion scenario involving either multiple female founders, and/or involving more than one incursion pathway into the continent. However, a comprehensive analysis combining samples from all countries within the South American soybean belt, and unravelling dispersal routes among them, is still lacking.

In this study, we sequenced part of the mtDNA COI gene of 18 *M. sojae* specimens collected in different

localities of Rio Grande do Sul state (Southern Brazil), Goiás state (Central-West Brazil) and Córdoba province in Argentina. Previously published sequences from Brazil, Paraguay and Bolivia were added to the dataset. Haplotype patterns, genetic diversity and the current status of management strategies available for this highly invasive pest, based on data from literature and collected in the present work, are analysed and discussed.

## Material and methods

### Sampling procedure

Fly larvae were collected from the stems of soybean plants that showed characteristic symptoms of injury by *M. sojae* (van den Berg et al. 1998), in 13 municipalities of Rio Grande do Sul state (Southern Brazil), one municipality of Goiás state (Central-West Brazil) and one municipality of Córdoba province (Argentina), from March 2019 to April 2019 (Table 1, Fig. 2). At each site, the larvae found within soybean stems were manually detached and placed into 1.5 mL

**Table 1** Sample list, countries of origin, sample size, host plants and collection dates of *Melanagromyza sojae* individuals used/sequenced by population. Mitochondrial DNA sequences from this study were combined with other sequences deposited in Genbank (Arnemann et al. 2016a; Guedes et al. 2017; Vitorio et al. 2019). Haplotype ID numbers refer to Msoj-COI-01 to 23. Newly identified haplotypes are indicated by '†'.

Population acronyms refer to Brazilian states of Rio Grande do Sul (RS), Santa Catarina (SC) and Goiás (GO); Paraguayan departments of Alto Paraná (AP), Canindeyú (CA) and Itapúa (IT); Bolivian department of Santa Cruz (SC); Argentine province of Córdoba (CO) and Australian state of New South Wales (NSW)

Country	Population (sample size)	Municipality	Source	Sampling date	Haplotypes	Reference	
Brazil	Rio Grande do Sul (31)	Boa Vista do Buricá	Soybean	Apr.2015	01, 08	Arnemann et al. (2016a, b, c)	
		Campo Novo	Soybean	Apr.2015	02	Arnemann et al. (2016a, b, c)	
		Catuípe	Soybean	Apr.2019	21 <sup>†</sup>	This study	
		Condor	Soybean	Apr.2019	09	This study	
		Cruz Alta	Soybean		Apr.2015	01, 02, 06	Arnemann et al. (2016a, b, c)
					Apr.2019 (1)		This study (1) (3)
		Dois Irmãos das Missões	Soybean	Apr.2015	13	This study	
		Giruá	Soybean	Apr.2019	22 <sup>†</sup>	This study	
		Itaqui	Soybean	Apr.2019	02	This study	
		Novo Machado	Soybean		Apr.2015	04	Arnemann et al. (2016a, b, c)
					Apr.2019		
		Porto Lucena	Soybean	Apr.2019	09	This study	
		Santa Maria	Persian clover	Aug.2019	02	Ferreira et al. (2020)	
		Santa Rosa	Soybean	Apr.2019	01	This study	
		Santo Augusto	Soybean	Apr.2019	13	This study	
		Santo Cristo	Soybean	Apr.2019	23 <sup>†</sup>	This study	
		São Borja	Soybean	Apr.2019	13	This study	
		Seberi	Soybean	Apr.2019	02	This study	
		Três de Maio	Soybean		Apr.2015	03, 04	Arnemann et al., (2016a, b, c)
					Apr.2019	02, 09	This study
		Tucunduva	Soybean	Apr.2015	01	Arnemann et al. (2016a, b, c)	
		Santa Catarina (12)	Descanso	Soybean	Apr.2015	02, 07	Arnemann et al., (2016a, b, c)
					Apr.2015	01, 02, 03	Arnemann et al., (2016a, b, c)
					Apr.2015	05, 09	Arnemann et al. (2016a, b, c)
					Apr.2015	03, 10	Arnemann et al. (2016a, b, c)
					Apr.2015	01, 04	Arnemann et al. (2016a, b, c)
		Goiás (3)	Silvânia	Soybean	Mar.2019	02, 13	This study



**Table 1** continued

Country	Population (sample size)	Municipality	Source	Sampling date	Haplotypes	Reference
Paraguay	Alto Paraná (10)	San Alberto	Soybean	Dec.2015	01, 03, 11, 12, 16	Guedes et al. (2017)
		Santa Rita	Soybean	Dec.2015	01, 09, 14, 15	Guedes et al. (2017)
	Canindeyú (5)	Corpus Christi	Soybean	Dec.2015	01, 07, 13	Guedes et al. (2017)
	Itapúa (8)	Pirapó	Soybean	Dec.2015	01, 02, 08, 09, 13, 17	Guedes et al. (2017)
Bolivia	Santa Cruz (7)	Cuatro Cañadas	Soybean	Jan.2017	13, 19	Vitorio et al. (2019)
		Fernández Alonso	Soybean	Sep.2016	02, 13	Vitorio et al. (2019)
		Santa Rosa del Sara	Soybean	Mar.2017	02	Vitorio et al. (2019)
		Santa Cruz de la Sierra	Soybean	Jan.2017	13	Vitorio et al. (2019)
		Yapacani	Soybean	Nov.2016	20	Vitorio et al. (2019)
Argentina	Córdoba (1)	Cañada de Luque	Chickpea	Nov.2019	11	This study
Australia	New South Wales (2)	Casino	Soybean	Mar.2013	02	Arnemann et al. (2016a, b, c)

vials containing 98% ethanol to preserve their DNA. Each vial was labelled with the municipality, date, and coordinates of the collection site. Vials from all sample collection sites were sent to the Crop Protection Department of Federal University of Santa Maria, Santa Maria, RS, Brazil, where they were stored at  $-20\text{ }^{\circ}\text{C}$  until genomic DNA extraction.

#### DNA extraction, PCR amplification, and COI-gene sequencing

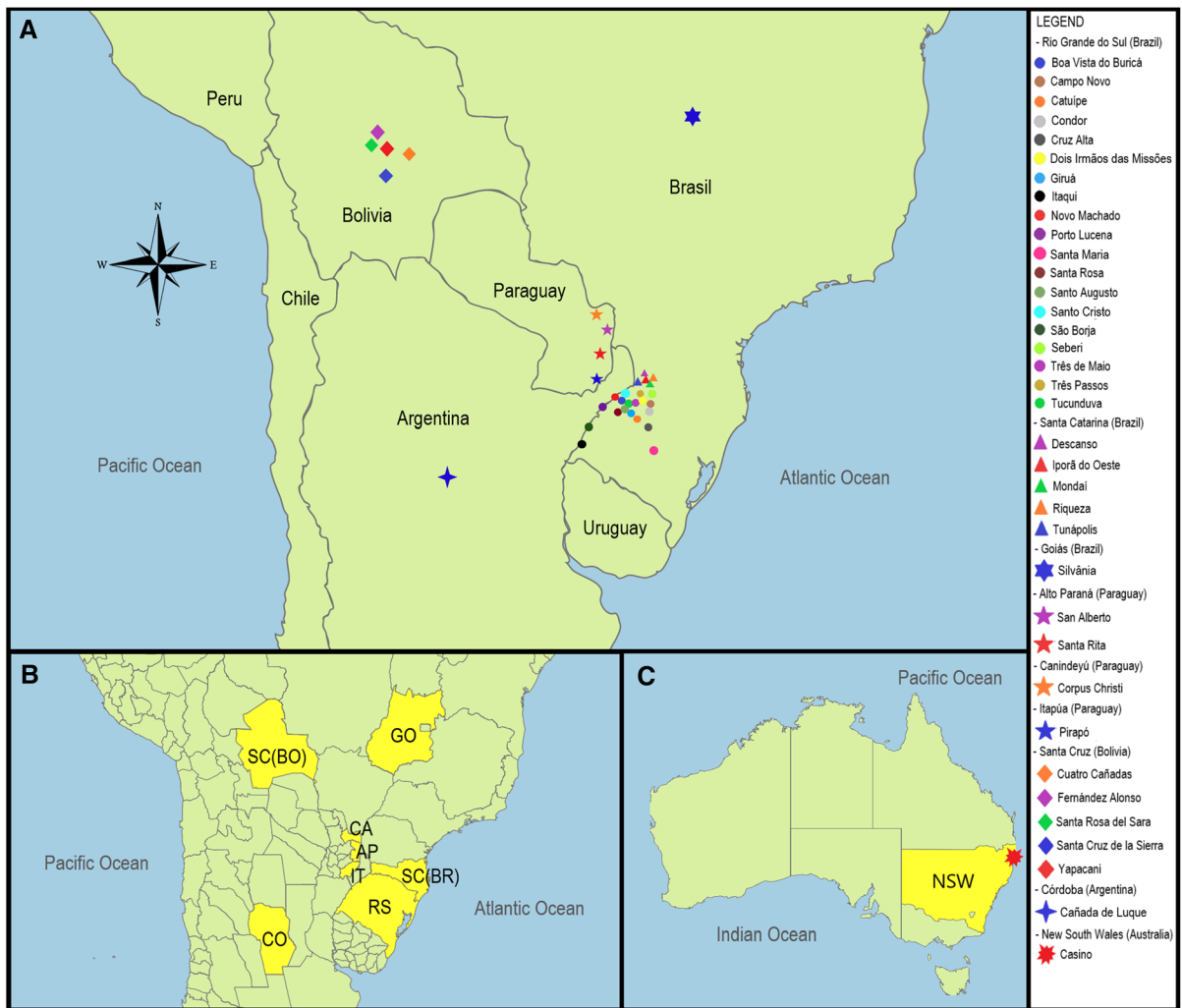
The identification of all *M. sojae* specimens was initially confirmed based on morphological traits and feeding behaviour within the plants (Talekar and Chen 1985) prior to being used in DNA extraction. DNA extraction was performed individually for each specimen using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Each ethanol-preserved larva was removed from the specimen vial and left to air dry on a paper towel. The entire body was individually macerated in a 1.5 mL tube containing 180  $\mu\text{L}$  of buffer ATL and 20  $\mu\text{L}$  of proteinase K and incubated at  $56\text{ }^{\circ}\text{C}$  for 24 h. Subsequently, genomic DNA was purified in a silica-based matrix and eluted in 35  $\mu\text{L}$  of buffer AE. The concentration of DNA was assessed in a spectrophotometer (NanoDrop<sup>TM</sup> 1000, Thermo Scientific, Wilmington, DE, USA).

A fragment of the mitochondrial COI gene was amplified from 18 individuals through polymerase

chain reaction (PCR) using the primers SSF-COI-F and SSF-COI-R and PCR conditions as described in Arnemann et al. (2016a). Briefly, each PCR reaction was performed with a final volume of 25  $\mu\text{L}$  composed by 2.5  $\mu\text{L}$  of JumpStart<sup>TM</sup> 10X reaction buffer; 1.25  $\mu\text{L}$  of dNTP mix (10 nM of each); 2.0  $\mu\text{L}$  of each primer (10 pM); 0.25  $\mu\text{L}$  of JumpStart<sup>TM</sup> DNA Polymerase (2.5 U/ $\mu\text{L}$ ) (Sigma-Aldrich, St. Louis, MO, USA); 2  $\mu\text{L}$  of template DNA (05–100 ng/ $\mu\text{L}$ ); and 15.0  $\mu\text{L}$  of ultra-pure water. PCR amplification consisted of an initial denaturation step at  $95\text{ }^{\circ}\text{C}$  for 5 min, followed by 34 cycles at  $95\text{ }^{\circ}\text{C}$  for 30 s,  $61\text{ }^{\circ}\text{C}$  for 30 s and  $72\text{ }^{\circ}\text{C}$  for 30 s, and a final extension at  $72\text{ }^{\circ}\text{C}$  for 5 min. Amplified products were resolved on 1.0% agarose electrophoresis gel, pre-stained with Nancy-520 DNA gel stain (Sigma-Aldrich) and visualized using a gel documentation system. Successfully amplified PCR products were sequenced by ACTGene Molecular Analyses (Alvorada, RS, BR), using the BigDye Terminator method on an ABI 3500 Genetic Analyser (Applied Biosystems, Foster City, CA, USA).

#### Data analysis

Quality assessment, trimming, editing, and analysis of each DNA sequence were performed using the softwares Pregap4 and Gap4 within the Staden package (Staden and Bonfield 2000). Geneious R9 (Biomatters



**Fig. 2** Municipalities (A) and states, departments or provinces (B) where the specimens of *Melanagromyza sojae* were collected in South America and Australia (C), in the present and previous studies. Acronyms refer to Brazilian states of Rio Grande do Sul (RS), Santa Catarina (SC) and Goiás (GO);

Paraguayan departments of Alto Paraná (AP), Canindeyú (CA) and Itapúa (IT); Bolivian department of Santa Cruz (SC); Argentine province of Córdoba (CO) and Australian state of New South Wales (NSW)

Ltd., New Zealand) was used to retrieve and align sequences with a length of 740 base pairs (bp). Presence of premature stop codons was checked through translation of the partial mtCOI contigs into protein sequences, by selecting the invertebrate genetic code 5 for amino acid translation in Geneious R9. The 18 COI sequences generated in this study were combined with 61 published sequences from different municipalities of Rio Grande do Sul state (Brazil; 17 sequences), Santa Catarina state (Brazil; 12 sequences), Alto Paraná department (Paraguay; 10 sequences), Canindeyú department (Paraguay; 5

sequences), Itapúa department (Paraguay; 8 sequences), Santa Cruz department (Bolivia; 7 sequences), and from New South Wales state (Australia; 2 sequences). The final dataset therefore consisted of 79 COI sequences from South America and Australia.

Estimates of haplotype diversity ( $h \pm SE$ ) and nucleotide diversity ( $\pi \pm SE$ ) were carried out using the molecular evolution software package DNA Sequence Polymorphism (DnaSP) version 5.10.01 (Librado and Rozas 2009). Tajima's D (Tajima 1989) was computed for all populations with at least

four samples, to evaluate deviations from the standard neutral model. The inference and visualization of genetic relationships among intraspecific sequences used to generate a haplotype network were conducted using TCS network (Clement et al. 2000) within the program PopART (Leigh and Bryant 2015). Analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was conducted on Arlequin 3.5 (Excoffier et al. 2005); the sequences from Argentina and Australia were excluded from AMOVA test due to small sample size. Separate analyses of unlinked loci were also performed, using the locus-by-locus option in Arlequin 3.5. Pairwise uncorrected (p) nucleotide distances (i.e. estimates of evolutionary divergence) between all *M. sojae* haplotypes and populations were calculated in MEGA X (Kumar et al. 2018) with 1000 bootstrap replications. The rate variation among sites was modelled with a gamma distribution (shape parameter = 1). All ambiguous positions were removed for each sequence pair (pairwise deletion option).

## Results

Fragments of mtDNA from 18 individuals of *M. sojae*, collected in 14 Brazilian sites (Rio Grande do Sul and Goiás states) and one Argentine site (Córdoba province), were amplified and sequenced. Combining our data with sequences previously deposited in GenBank (Arnemann et al. 2016a; Guedes et al. 2017; Vitorio et al. 2019), we obtained a total of 79 *M. sojae* mtDNA COI sequences. The trimmed 740 bp sequences (nucleotide positions 46 to 786 of the mitochondrial *M. sojae* genome, GenBank accession number KT597923) showed no ambiguity and no premature stop codons. Based on a consensus SNP (single nucleotide polymorphism) profile matching haplotype Msoj-COI-02 (Arnemann et al. 2016a), a total of 24 SNPs were identified from the 740 bp partial COI region (Fig. 3). This resulted in 22 mtDNA haplotypes, three of which (named Msoj-COI-21, Msoj-COI-22 and Msoj-COI-23) were novel haplotypes. Haplotype Msoj-COI-18, previously identified in Paraguay (Guedes et al. 2017), was found to be identical to Msoj-COI-01, and thus removed from our haplotype list; all sequences matching it were renamed as Msoj-COI-01. The new mtDNA haplotypes Msoj-COI-21, Msoj-COI-22 and Msoj-COI-23 generated from this study were submitted to GenBank (accession numbers: MW222178, MW222179 and MW222180,

respectively). All base substitutions identified involved transition (i.e. purine/purine; pyrimidine/pyrimidine) substitutions (sixteen T/C, eight G/A). Also, of the 24 variable (polymorphic) sites, 14 were singleton variable sites (two variants) and 10 were parsimony informative sites (two variants); there were no variable sites with three or four variants.

The observed nucleotide diversity between countries was low and ranged from 0.0037 to 0.0043 (Table 2). The highest nucleotide and haplotype diversities were found in Santa Catarina state (Brazil) and Alto Paraná department (Paraguay), probably due to larger sample size in these localities. For the Tajima's D estimates, all countries analysed (Brazil, Paraguay and Bolivia) showed negative D-values, indicating high level of low frequency polymorphisms (i.e. excess of rare alleles). This indicates that *M. sojae* populations within these countries are not constant in size or close to mutation-drift equilibrium, which is expected from an invasive species outside its native range. However, all D-values were associated with non-significant *P*-values (> 0.10), making further interpretations unreliable. Unique haplotypes within Brazil, Paraguay and Bolivia constituted ca. 50% of all haplotypes identified in each country (Table 2), indicating that separate invasion events may have introduced the species in each country from overseas.

The pairwise uncorrected (p) genetic distances between all *M. sojae* haplotypes were low (ranging from 0.00 to 0.014; see Fig. 4) as expected for the intra-species level comparison (e.g. Scheffer 2000; Arnemann et al. 2016a). Paraguay presented the highest evolutionary divergence from Argentina, Bolivia and Brazil (0.005; see Fig. 5). Analysis of Molecular Variance (AMOVA) detected no genetic structure at various hierarchical levels (Table 3), with almost all variation (98.82%) accounted for at the within population (i.e. within states) level; the variation observed among separate geographical groups (i.e. among countries) was small, and among populations within groups (i.e. among states), negligible. Locus-by-locus AMOVA presented similar results, with the majority of variation detected within populations for all 24 polymorphic loci (Supplementary Table 1). The associated *P*-values were non-significant for all loci except 192 and 603 (at the among groups level), further supporting the lack of genetic structure in the invaded range.

SNP position	54	72	81	99	126	144	237	258	264	273	276	288	291	334	354	360	388	414	549	552	601	648	675	771	n
Consensus	T	C	A	G	C	T	G	T	C	T	C	C	T	G	T	T	C	A	G	G	G	T	T	T	
Haplotype																									
Msoj-COI-1	*	*	*	*	*	*	A	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	16
Msoj-COI-2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	21
Msoj-COI-3	*	*	*	*	T	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	4
Msoj-COI-4	*	*	*	*	*	C	*	*	*	*	*	*	*	*	*	*	T	G	*	*	*	*	*	C	3
Msoj-COI-5	*	*	*	A	*	C	*	*	*	*	*	*	*	*	*	*	T	G	*	*	*	*	*	C	1
Msoj-COI-6	*	*	*	*	*	*	*	*	*	*	T	*	*	*	*	*	*	*	*	*	*	*	*	*	1
Msoj-COI-7	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	C	*	*	*	*	*	*	*	*	2
Msoj-COI-8	*	*	*	*	*	A	*	*	*	*	*	*	*	*	C	*	*	G	*	*	*	*	C	*	2
Msoj-COI-9	*	*	*	*	*	C	*	*	*	*	*	*	*	*	*	*	T	*	*	*	*	*	*	*	6
Msoj-COI-10	C	*	*	*	*	*	A	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	1
Msoj-COI-11	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	T	G	*	*	*	*	*	*	2
Msoj-COI-12	*	*	*	*	*	*	A	*	*	C	*	*	*	*	*	*	*	*	*	*	*	*	C	*	1
Msoj-COI-13	*	*	*	*	*	C	*	*	*	*	*	*	*	*	*	*	T	G	*	*	*	*	*	*	10
Msoj-COI-14	*	*	*	*	*	*	A	C	*	*	*	*	T	*	*	*	*	*	*	*	*	A	*	C	1
Msoj-COI-15	*	*	G	*	*	*	A	*	*	*	*	*	*	*	*	*	*	*	A	*	*	*	C	*	1
Msoj-COI-16	*	*	*	*	*	*	*	*	T	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	1
Msoj-COI-17	*	*	*	*	*	*	A	*	*	*	*	*	C	*	*	*	*	*	*	*	*	*	C	*	1
Msoj-COI-19	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	A	A	A	C	*	1
Msoj-COI-20	*	*	*	*	*	*	*	*	*	*	*	*	*	A	*	*	*	*	*	*	*	*	*	*	1
Msoj-COI-21	*	T	*	*	*	C	*	*	*	*	*	*	*	*	*	*	T	G	*	*	*	*	*	*	1
Msoj-COI-22	*	*	*	*	T	*	*	*	*	*	*	*	*	*	C	*	*	*	*	*	*	*	*	C	1
Msoj-COI-23	*	*	*	*	*	*	A	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	C	C	1

**Fig. 3** Single nucleotide polymorphisms (SNPs) and haplotypes identified from this study and previous sequences deposited in Genbank (Arnemann et al. 2016a; Guedes et al. 2017; Vitorio et al. 2019). Consensus SNPs were determined by population majority, corresponding to the mtCOI haplotype Msoj-COI-02. SNP nucleotide positions were numbered according to the *Melanagromyza sojae* mitogenome

(KT597923), corresponding to the mtCOI haplotype Msoj-COI-01. Nucleotide changes identical to the consensus base substitution patterns are indicated by an asterisk. The final column shows the total individuals identified for each mtCOI haplotype (n). Msoj-COI-02 includes the two individuals sampled from Australia

The proportions of the different haplotypes among countries and among states are presented on a haplotype network (Fig. 6) and a haplotype distribution map (Fig. 7). The most prevalent haplotype was Msoj-COI-02, found in all countries except Argentina. Msoj-COI-01 and Msoj-COI-13 follow as second and third most frequent haplotypes, respectively, attesting to their ecological competence and adaptability to South American conditions. Together, haplotypes Msoj-COI-01 and Msoj-COI-02 represent 47% of all specimens sampled in the continent, and 54% of those sampled in the Brazilian state of Rio Grande do Sul. Accordingly, these two haplotypes (Msoj-COI-01 and Msoj-COI-02) occupied central positions in the network distribution. Ten nucleotide substitutions separated the haplotypes most apart from each other (Msoj-COI-14 and Msoj-COI-05; see Fig. 6), but they were located geographically near (Alto Paraná department in Paraguay and Santa Catarina state in Brazil, respectively; see Fig. 7). Furthermore, these two haplotypes diverged with five mutation steps from the major haplotype Msoj-COI-02, more than any other.

**Discussion**

Haplotype patterns

Our study updates information on the genetic diversity and mtDNA COI haplotype distribution of *M. sojae* in the soybean belt of South America. Samples from 14 Brazilian sites were collected and analysed, alongside one sample from Argentina. We combined our mtDNA data with published sequences of *M. sojae* from 21 other South American sites (Table 1), revealing the presence of 22 different haplotypes in the continent, three of which were previously unidentified (Msoj-COI-21, Msoj-COI-22 and Msoj-COI-23, from municipalities of Catuípe, Giruá and Santo Cristo, Rio Grande do Sul state, Brazil). The specimen from Argentina was identified as haplotype Msoj-COI-11, also reported in Paraguay (municipality of San Alberto, Alto Paraná) but not in Bolivia and Brazil. The specimens from Goiás state (Brazil) matched haplotypes Msoj-COI-02 and Msoj-COI-13, the two most frequent haplotypes alongside Msoj-COI-01. Msoj-COI-02 was also the haplotype identified in Australia (Arnemann et al. 2016a) and found overwintering on *T. resupinatum* plants from Southern Brazil (Ferreira et al. 2020), thus appearing to thrive particularly well under South American conditions.

**Table 2** *Melanogromyza sojæ* partial (740 bp) mtDNA COI gene nucleotide diversity ( $\pi$ ), haplotype diversity ( $h$ ), Tajima's D, number of unique and shared haplotypes identified in different states, departments and provinces of Brazil, Paraguay, Bolivia, Argentina and Australia. Diversity analyses were conducted on DNA Sequence Polymorphism (DnaSP) version 5.10.01 (Librado and Rozas 2009)

Country	Population	Number of samples	Nucleotide diversity	Haplotype diversity	Tajima's D <sup>a</sup>		Number of haplotypes	Number of unique haplotypes	Number of shared haplotypes
					D-value	P-value			
Brazil	Rio Grande do Sul (RS)	31	0.00362 ± 0.00041	0.834 ± 0.050	- 0.0857	ns	11	4	7
	Santa Catarina (SC)	12	0.00446 ± 0.00064	0.924 ± 0.057	- 0.0104	ns	8	3	5
	Goiás (GO)	3	0.00270 ± 0.00127	0.667 ± 0.314	-	-	2	0	2
	Brazil (RS + SC + GO)	46	0.00371 ± 0.00033	0.846 ± 0.037	- 0.4257	ns	14	8	6
Paraguay	Alto Paraná (AP)	10	0.00489 ± 0.00080	0.933 ± 0.077	- 1.2277	ns	8	4	4
	Canindeyú (CA)	5	0.00459 ± 0.00092	0.800 ± 0.164	1.241	ns	3	0	3
	Itapúa (IT)	8	0.00396 ± 0.00070	0.929 ± 0.084	0.4034	ns	6	1	5
	Paraguay (AP + CA + IT)	23	0.00433 ± 0.00047	0.897 ± 0.050	- 1.1037	ns	13	5	8
Bolivia	Santa Cruz (SC)	7	0.00386 ± 0.00087	0.810 ± 0.130	- 0.0634	ns	4	2	2
Argentina	Córdoba (CO)	1	-	-	-	-	1	0	1
Australia	New South Wales (NSW)	2	-	-	-	-	1	0	1
Overall	79	0.00391 ± 0.00026	0.870 ± 0.023	-	-	22	-	-	-

<sup>a</sup>Neutrality test conducted for populations with sample size  $\geq 4$ . ns non-significant P-value ( $> 0.10$ ) (Tajima 1989)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	19	20	21	22	23
Msoj-COI-01		0.002	0.003	0.004	0.004	0.003	0.003	0.002	0.003	0.002	0.003	0.001	0.003	0.003	0.002	0.003	0.001	0.003	0.003	0.004	0.004	0.000
Msoj-COI-02	0.003		0.002	0.003	0.003	0.001	0.001	0.003	0.002	0.002	0.002	0.003	0.002	0.003	0.003	0.001	0.003	0.003	0.001	0.003	0.003	0.002
Msoj-COI-03	0.006	0.003		0.003	0.003	0.003	0.002	0.004	0.003	0.003	0.003	0.003	0.003	0.004	0.004	0.002	0.003	0.003	0.003	0.003	0.001	0.003
Msoj-COI-04	0.008	0.006	0.006		0.001	0.003	0.003	0.004	0.002	0.004	0.002	0.004	0.001	0.004	0.004	0.003	0.004	0.004	0.003	0.002	0.003	0.004
Msoj-COI-05	0.010	0.007	0.007	0.001		0.003	0.003	0.004	0.002	0.004	0.002	0.004	0.002	0.005	0.004	0.003	0.004	0.004	0.004	0.002	0.003	0.004
Msoj-COI-06	0.004	0.001	0.004	0.007	0.008		0.002	0.003	0.002	0.003	0.002	0.003	0.003	0.004	0.003	0.002	0.003	0.003	0.003	0.002	0.003	0.003
Msoj-COI-07	0.004	0.001	0.004	0.007	0.008	0.003		0.003	0.002	0.002	0.002	0.003	0.003	0.004	0.003	0.002	0.003	0.003	0.003	0.002	0.003	0.003
Msoj-COI-08	0.003	0.006	0.008	0.008	0.010	0.007	0.007		0.003	0.003	0.003	0.002	0.003	0.003	0.003	0.003	0.002	0.003	0.003	0.004	0.003	0.002
Msoj-COI-09	0.006	0.003	0.006	0.003	0.004	0.004	0.004	0.008		0.003	0.002	0.003	0.001	0.004	0.004	0.002	0.003	0.003	0.003	0.003	0.002	0.003
Msoj-COI-10	0.003	0.003	0.006	0.008	0.010	0.004	0.004	0.006	0.006		0.003	0.002	0.003	0.003	0.003	0.003	0.002	0.003	0.003	0.003	0.003	0.002
Msoj-COI-11	0.006	0.003	0.006	0.003	0.004	0.004	0.004	0.006	0.003	0.006		0.003	0.001	0.004	0.004	0.002	0.003	0.003	0.002	0.002	0.003	0.003
Msoj-COI-12	0.001	0.004	0.007	0.010	0.011	0.006	0.006	0.004	0.007	0.004	0.007		0.003	0.003	0.003	0.003	0.002	0.003	0.003	0.004	0.004	0.001
Msoj-COI-13	0.007	0.004	0.007	0.001	0.003	0.006	0.006	0.007	0.001	0.007	0.001	0.008		0.004	0.004	0.003	0.004	0.004	0.003	0.001	0.003	0.003
Msoj-COI-14	0.004	0.007	0.010	0.013	0.014	0.008	0.008	0.007	0.010	0.007	0.010	0.006	0.011		0.003	0.004	0.003	0.003	0.004	0.004	0.004	0.003
Msoj-COI-15	0.003	0.006	0.008	0.011	0.013	0.007	0.007	0.006	0.008	0.006	0.008	0.004	0.010	0.007		0.003	0.002	0.004	0.003	0.004	0.004	0.002
Msoj-COI-16	0.004	0.001	0.004	0.007	0.008	0.003	0.003	0.007	0.004	0.004	0.004	0.006	0.006	0.008	0.007		0.003	0.003	0.002	0.003	0.003	0.003
Msoj-COI-17	0.001	0.004	0.007	0.010	0.011	0.006	0.006	0.004	0.007	0.004	0.007	0.003	0.008	0.006	0.004	0.006		0.003	0.003	0.004	0.004	0.001
Msoj-COI-19	0.004	0.004	0.007	0.010	0.011	0.006	0.006	0.007	0.007	0.007	0.007	0.006	0.008	0.006	0.007	0.006	0.006		0.003	0.004	0.004	0.003
Msoj-COI-20	0.004	0.001	0.004	0.007	0.008	0.003	0.003	0.007	0.004	0.004	0.004	0.006	0.006	0.008	0.007	0.003	0.006	0.006		0.003	0.003	0.003
Msoj-COI-21	0.008	0.006	0.008	0.003	0.004	0.007	0.007	0.008	0.003	0.008	0.003	0.010	0.001	0.013	0.011	0.007	0.010	0.010	0.007		0.004	0.004
Msoj-COI-22	0.007	0.004	0.001	0.007	0.008	0.006	0.006	0.007	0.007	0.007	0.007	0.008	0.008	0.011	0.010	0.006	0.008	0.008	0.006	0.010		0.004
Msoj-COI-23	0.000	0.003	0.006	0.008	0.010	0.004	0.004	0.003	0.006	0.003	0.006	0.001	0.007	0.004	0.003	0.004	0.001	0.004	0.004	0.008	0.007	

Fig. 4 Pairwise uncorrected (p) nucleotide distances between all *Melanagromyza sojæ* haplotypes. The number of base substitutions per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal and

were obtained by a bootstrap procedure (1000 replicates). This analysis involved 22 nucleotide sequences, with a total of 740 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al. 2018)

	ARG-CO	AUS-NSW	BOL-SC	BRA-GO	BRA-RS	BRA-SC	PAR-AP	PAR-CA	PAR-IT
ARG-CO		0.002	0.001	0.001	0.002	0.002	0.002	0.001	0.002
AUS-NSW	0.003		0.001	0.001	0.001	0.001	0.001	0.001	0.001
BOL-SC	0.003	0.003		0.001	0.001	0.001	0.002	0.001	0.001
BRA-GO	0.002	0.001	0.003		0.001	0.001	0.001	0.001	0.001
BRA-RS	0.004	0.002	0.004	0.003		0.001	0.001	0.001	0.001
BRA-SC	0.004	0.003	0.004	0.003	0.004		0.001	0.001	0.001
PAR-AP	0.005	0.003	0.005	0.004	0.005	0.005		0.001	0.001
PAR-CA	0.004	0.003	0.004	0.003	0.004	0.004	0.005		0.001
PAR-IT	0.004	0.003	0.004	0.003	0.004	0.004	0.004	0.004	

Fig. 5 Pairwise uncorrected (p) nucleotide distances over sequence pairs between *Melanagromyza sojæ* populations from: Córdoba province, Argentina (ARG-CO); New South Wales state, Australia (AUS-NSW); Santa Cruz department, Bolivia (BOL-SC); Goiás state, Brazil (BRA-GO); Rio Grande do Sul state, Brazil (BRA-RS); Santa Catarina state, Brazil (BRA-SC); Alto Paraná department, Paraguay (PAR-AP); Canindeyú department, Paraguay (PAR-CA); Itapúa

department, Paraguay (PAR-IT). The number of base substitutions per site from averaging over all sequence pairs between groups are shown. Standard error estimate(s) are shown above the diagonal and were obtained by a bootstrap procedure (1000 replicates). This analysis involved 79 nucleotide sequences, with a total of 740 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al. 2018)

The mtDNA COI haplotype network (i.e. haplotype clustering patterns) revealed a pattern of genetic variation consistent between regions, with each haplotype (when present in two or more individuals) showing a wide geographic distribution (see Fig. 6). Such pattern is common in species capable of long-range movement (Avise et al. 1987) and has also been detected on mitochondrial DNA analysis of *H. armigera* (Behere et al. 2007). Together with the low among-group haplotype variance (6.03%; see Table 3)

and low nucleotide diversity (0.0037–0.0043; see Table 2) observed between countries, this could be evidence of high mobility and long distance gene flow in this pest species; however, further *F*-statistic analysis are necessary to support this hypothesis. Similar heterogeneous haplotype patterns have also been detected on *H. armigera* populations across the Cone Sul region of South America (i.e. Argentina, Paraguay, Uruguay, and the Southern Brazilian states of Rio Grande do Sul, Santa Catarina and Paraná;

**Table 3** Analysis of molecular variance (AMOVA) reflecting the correlation of *Melanogromyza sojae* mtDNA haplotype diversity at different levels of hierarchical subdivision. Groups correspond to countries and populations within each country. Distance method used was by pairwise difference. The significance of the variance components and fixation indices ( $\Phi$ -statistics) was tested using 1000 permutations. Analyses were conducted in Arlequin 3.5 (Excoffier et al. 2005)

Source of variation	df <sup>a</sup>	Sum of squares	Variance component <sup>b</sup>	% variation	Significance tests (1000 permutations) <sup>c</sup>		$\Phi$ -statistics
					$P$ (rand. > obs.)	$P$ (rand. = obs.)	
Among groups	2	5.056	$\sigma_a^2$ 0.09041	6.03	$\sigma_a^2 \times \Phi_{CT}$ 0.01564	0.01271	$\Phi_{CT} = 0.06027$
Among populations	4	3.268	$\sigma_b^2$ -0.07278	-4.85	$\sigma_b^2 \times \Phi_{SC}$ 0.95699	0.00000	$\Phi_{SC} = -0.05164$
Within populations	69	102.282	$\sigma_c^2$ 1.48234	98.82	$\sigma_c^2 \times \Phi_{ST}$ 0.53959	0.00000	$\Phi_{ST} = 0.01175$
Total	75	110.605	1.49997	100.00			

<sup>a</sup>Degrees of freedom

<sup>b</sup>Negative values indicate excess of heterozygotes and should be interpreted as zero (Schneider et al. 2000)

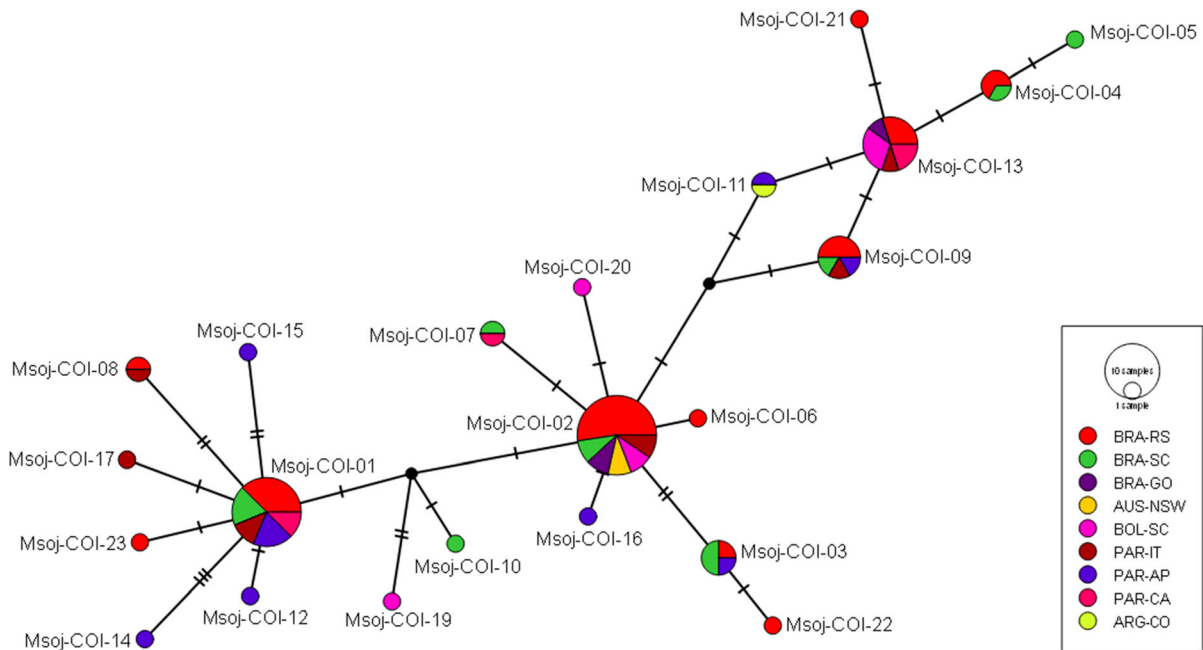
<sup>c</sup>Statistical significance: probability of random value > observed  $\Phi$ -value and of random value = observed  $\Phi$ -value (Excoffier et al. 1992). ns non-significant  $P$ -value (> 0.05)

Arnemann et al. 2019) and Asian citrus psyllid *Diaphorina citri* in Brazil (Guidolin et al. 2014). Both studies suggested multiple introductions of these species as the main factor leading to mtDNA genetic signatures resembling a rapid population expansion.

Long distance migration and gene flow could also explain why the *M. sojae* haplotype clusters observed in the haplotype network (Fig. 6) are seemingly unrelated to the geographical separation between the populations (i.e. country and state boundaries). Alternatively, this could be evidence of a potential selection process occurring over a long timescale and within the species' native range, with the resulting haplotype diversity being introduced in South America through a minimum of two separate introduction events. Although haplotype distribution was not location-specific for South America, whether they might be related to geographic distribution in Asia remained to be investigated. Nonetheless, care should be taken to draw strong evolutionary conclusions based on such short sequences as the mtDNA COI, especially when presenting low number of SNPs. Combined evidence from mtDNA COI and rapidly evolving nuclear non-coding sequences (i.e. intron sequences) will be needed to correctly ascertain the distribution of common and rare *M. sojae* haplotypes among different South American regions.

### Genetic diversity

The overall nucleotide diversity ( $\pi = 0.00391 \pm 0.00026$ ) and haplotype diversity ( $h = 0.870 \pm 0.023$ ) observed in South American *M. sojae* populations were higher than those observed in genetic diversity studies of other dipterans (e.g. *Liriomyza sativae* in China; Du et al. 2014) and similar to other invasive pests that have established in South America (e.g. *Helicoverpa armigera*; Mastrangelo et al. 2014, Arnemann et al. 2016c). The high number of mtDNA haplotypes identified in the relatively small sample sizes within South America resembles the invasive genetic signature of *H. armigera* detected in Brazil (Tay et al. 2013; Mastrangelo et al. 2014) and cannot be explained simply by mutation or divergence of subpopulations, as invasive species require a long evolutionary timeframe to increase their genetic diversity in a new environment (Dlugosch and Parker 2008; Garnas et al. 2016). Mutation rates in the insect mtDNA COI gene are typically around 2% between



**Fig. 6** Haplotype network based on partial mtDNA COI (740 bp) of *Melanagromyza sojae*. Circle sizes indicate approximate proportion of haplotype frequencies. Each mark represents one nucleotide difference. Population acronyms refer to Rio Grande do Sul state, Brazil (BRA-RS); Santa Catarina state, Brazil (BRA-SC); Goiás state, Brazil (BRA-GO); New

South Wales state, Australia (AUS-NSW); Santa Cruz department, Bolivia (BOL-SC); Itapúa department, Paraguay (PAR-IT); Alto Paraná department, Paraguay (PAR-AP); Canindeyú department, Paraguay (PAR-CA); Córdoba province, Argentina (ARG-CO). Analyses were conducted in PopART (Leigh and Bryant 2015)

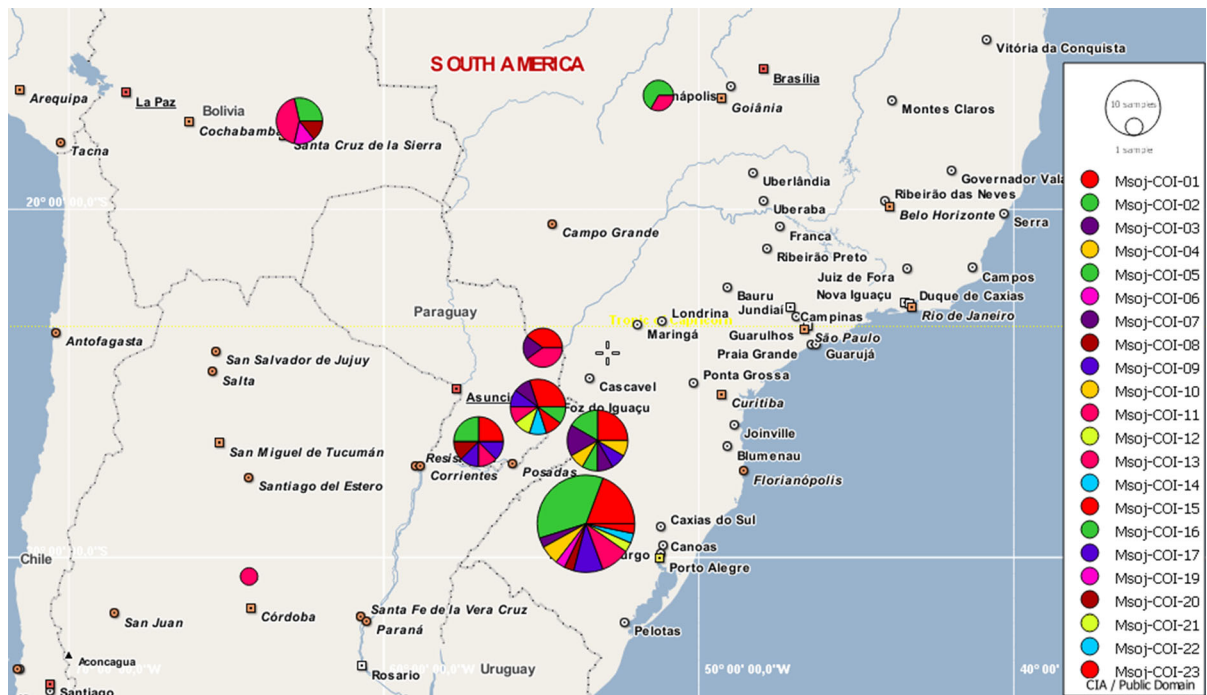
lineages per million years, or approximately 1 mutation per 100,000 years (e.g. Powell 1986; Behere et al. 2007; Papadopoulou et al. 2010).

The rates of nucleotide substitution in *Drosophila* for both mitochondrial DNA and nuclear DNA are similar (Powell 1986) and have been estimated at  $2.8 \times 10^{-9}$  per site per generation (Keightley et al. 2014), including both coding and non-coding regions, and it is reasonable to presume that other dipterans (such as *M. sojae*) would present similar rates. Assuming that *M. sojae* was not present in South America prior to 1983 (Gassen and Schneider 1983), post-introduction mutations and/or population divergence from a single founder could not account for the high number of maternal lineages found in the continent. On the other hand, if multiple introductions of *M. sojae* took place in South America instead of just one, this haplotype diversity can be explained as being the result of high propagule pressure (Simberloff 2009; Simberloff et al. 2013) that ensured successful establishment of the populations, as reported for other invasive pest species in Brazil (e.g. *Bemisia tabaci*

MED, Barbosa et al. 2015; *H. armigera*, Arneemann et al. 2019). The results from Tajima's neutrality test point to the same conclusion.

The positive Tajima's D values found for Canindeyú and Itapúa populations (Paraguay) could be indicative of a recent introduction of different populations (Tay et al. 2020), while the negative D values found for most populations (and for countries as a whole) likely reflect a population expansion after a recent selective sweep or bottleneck process. This discrepancy (i.e. positive values for Canindeyú and Itapúa and negative for the others) is probably linked to the small sample sizes from these two Paraguayan departments, as Tajima's D values are expected to become more negative with increasing number of individuals due to introduction of new mutations occurring in low frequencies or even as singletons (Larsson et al. 2013). Tajima's D estimates are highly sensitive to sample size (Nelson et al. 2012; Larsson et al. 2013) and should be considered with caution when sample size is small (< 50, according to Simonsen et al. 1995 and Marroni et al. 2011),





**Fig. 7** Haplotype distribution of *Melanagromyza sojae* in South America. Localities: Rio Grande do Sul state, Brazil (BRA-RS); Santa Catarina state, Brazil (BRA-SC); Goiás state, Brazil (BRA-GO); Santa Cruz department, Bolivia (BOL-SC); Itapúa department, Paraguay (PAR-IT); Alto Paraná

department, Paraguay (PAR-AP); Canindeyú department, Paraguay (PAR-CA); Córdoba province, Argentina (ARG-CO). The size of pie charts is relative to the number of individuals sequenced at each locality. Analyses were conducted in PopART (Leigh and Bryant 2015)

especially when the associated  $P$ -values are non-significant ( $> 0.10$ ; see Table 2). Thus, a larger number of individuals per population as well as a larger number of loci would be required before firm conclusions are drawn regarding *M. sojae* intra-species diversity in South America.

#### Population structure

The results from the AMOVA test (Table 3) support the hypothesis of multiple recent introductions from different *M. sojae* source populations, associated with limited genetic structure at the invaded range. Almost all haplotype diversity (98.82%) was identified as occurring within each population, rather than among groups or among populations ( $P = 0.53959 \pm 0.01954$ ). Populations among groups were shown to be significantly ( $P = 0.02835 \pm 0.00561$ ) but minimally differentiated, whereas the variation among populations within groups was negligible and not statistically significant ( $P = 0.95699 \pm 0.00592$ ). Locus-by-locus AMOVA test (Supplementary

Table 1) detected non-significant  $P$ -values for all 24 polymorphic loci, except for loci 192 and 603 at the among groups level. Non-significant  $P$ -values are often linked to small sample sizes per group, as a minimum of six total populations and 20 unique permutations are needed to detect  $P < 0.05$  at higher-level population structure (Fitzpatrick 2009). For three groups and seven populations (set in 3–3–1 scheme as in the present study), the minimum number of unique permutations according to the multinomial coefficient (Ross 1998) is 70 and the minimum expected  $P$ -value is 0.0143 (see Table 2 in Fitzpatrick 2009), assuming that the hypothesized nesting maximizes the value of  $F_{CT}$ .

Accordingly, the other possible groupings were tested (schemes 5–1–1, 3–2–2 and 4–2–1) and presented equal or lower  $F_{CT}$  than the proposed grouping (0.00874, 0.06604 and -0.00941, respectively). Therefore, the sample size used is within the minimum range needed to detect hierarchical differentiation with  $\alpha = 0.05$ , supporting the inference that differentiation among groups and populations was not large. Greater

statistical power could be obtained by increasing the number of sites (populations) per group, but a larger sample of individuals per site would probably be uninformative about hierarchical structure. Although a complete  $F_{ST}$  analysis would be needed to correctly assess genetic differentiation between *M. sojae* populations in South America, they do not appear to be distributed according to a clear-cut pattern of geographical separation. Thus, the geographical criteria used to define regional groups and populations (i.e. country and state boundaries) do not accurately represent the species' mtDNA COI diversity and distribution within South America, and could be replaced by an alternate partition of populations or a clustering of all populations into a continental supra-population group.

Identifying incursion pathways into a new landscape, and the routes of dissemination within it, is a key step on mitigating the detrimental impacts of a pest invasion and preventing the occurrence of new ones (Essl et al. 2015). Although the fraction of variation found within *M. sojae* populations from South America was very high (see Table 3) and, apparently, not correlated with the distance of geographical separation between them, any solid conclusion on the matter would depend upon the knowledge of when and where the first introduction(s) occurred. Unfortunately, such knowledge is lacking, as the evidence might have been lost over time when movements assisted by humans and/or natural dispersal took place across the landscape. Further studies should increase sample size and include a genotype-by-sequencing approach (e.g., RAD) to assess regional differentiation more accurately and increase statistical significance.

### Management strategies

The ongoing spread of *M. sojae* across South America poses a considerable challenge for soybean pest management in the continent. Within its native range (i.e. East Asia), *M. sojae* is usually managed by soybean growers through the use of resistant cultivars (Talekar 1980) and sowing dates outside population peaks (Talekar and Chen 1983). The use of parasitoids (e.g. Talekar 1990, van den Berg et al. 1995) and insecticides (e.g. Adak 2012, Jadhav et al. 2013) has also been reported. Curioletti et al. (2018) recommended spraying Chlorantraniliprole as seed

treatment, followed by foliar spraying of Chlorpyrifos until 10 days after plant emergence, and repeated once in an interval shorter than 10 days. On the other hand, control strategies for *M. sojae* in South America remain scarce and poorly tested. Considering the strategies used in other countries, at least two of them are impracticable in Brazil, where soybean cultivars resistant to *M. sojae* are not available and sowing dates can hardly be shifted due tight succession of soybean and maize crops. While Brazilian soybean growers do not employ insecticides targeting specifically at *M. sojae*, populations have probably been indirectly controlled by seed treatments and foliar sprayings aiming at other early-season pests, such as *Sternechus subsignatus*, *Elasmopalpus lignosellus* and *Phyllophaga capillata*.

*Melanagromyza sojae* was officially detected in Paraguay in 2017 (Guedes et al. 2017), but is probably present in the country at least since 2015 (Benitez 2015). The highest infestations have been detected on second-season soybean, in the Southern departments of Alto Paraná, Canindeyú and Itapúa, which represent the main agricultural region of the country. Field monitoring carried out from January, 2020 to March, 2020 in several localities of Itapúa and Alto Paraná detected high presence of *M. sojae* adults in second-season soybean, followed by injury symptoms in the plants, such as leaf yellowing and early dropping of upper leaves. Further investigation revealed an incidence level of 100%, with all sampled plants presenting two to three holes in the main stems and three to five *M. sojae* pupae within each stem. Although control measures for this pest are still under development, the high incidence of adults led soybean growers to fight off infesting populations with sequential sprays of pyrethroids.

In Bolivia, *M. sojae* was first identified in 2019 in the department of Santa Cruz—where almost all the country's soybean is grown (Vitorio et al. 2019). The highest infestations have been recorded during low-precipitation seasons (Aguilar 2018) and when two soybean crops are grown per year instead of just one (Vitorio 2020). Field reports also highlight sowing date as an important factor, with higher incidence of attacked plants reported at early sowings—unlike the Brazilian and Paraguayan scenarios, where high infestation levels are apparently correlated with late sowing (Pozebon et al. 2020). In conditions of low precipitation and early sowing, damage in Bolivian

soybean fields can be severe, with up to 100% of plant incidence and soybean stems tunneled in 70% of their length (Vitorio 2020). Several unidentified parasitoids and one fly species (Diptera: Muscidae) have been observed preying on *M. sojae* adults in Bolivia. Insecticides applied as seed treatment presented better results compared to three foliar applications in the seedling stage (Vitorio 2020).

In Argentina, *M. sojae* was first detected in 2019 in the municipality of Jesús María (north of Córdoba province; Vera et al. 2020). Previously, dipteran larvae of an unidentified species with characteristics similar to those of the genus *Melanagromyza* were detected in several localities of Córdoba province, damaging chickpea plants in 2013 and winter weeds in 2014. During the 2019/20 cropping season, larvae and pupae of *M. sojae* were found in stems of soybean plants in the provinces of Santa Fé (Trossero et al. 2020), Entre Ríos (Saluso 2020) and Tucumán (Murúa et al. 2020). During the 2019 winter cropping season, different chickpea varieties were compared in four field trials, planted in different sites in the north of Córdoba, and preliminarily results reveal 30 to 80% of variation in *M. sojae* incidence among the varieties. Chickpea producers have applied systemic insecticides (e.g. imidacloprid and dimethoate) when observing the presence of *M. sojae* larvae within the stems, but no differences in incidence were observed when comparing with untreated areas.

*Melanagromyza sojae* continues to expand across the Old World as well as the New. It was identified as part of a 3-year (2004–2007) biodiversity study in Spain, being the first and only record of this species from the European continent so far (Gil-Ortiz et al. 2010). The species was also found in Turkey in 2018 in Çukurova region (Özgür et al. 2020), the largest soybean production area in the country (TUIK 2020). Two soybean crops are grown per year in this area, the first sowed in April and the second one in June, after wheat harvesting. Although *M. sojae* occurs in both soybean crops, pest population is higher in the second season, similarly to what occurs in Brazil and Paraguay. Mart (2018) observed that *M. sojae* population established after three weeks of soybean sowing and continued until the tenth week. There are no insecticides registered for *M. sojae* control in Turkey, but infesting populations have probably been indirectly controlled by foliar sprayings of malathion, bifenthrin, novaluron, beta-cyfluthrin, and methomyl

targeted at other pests such as *Bemisia tabaci*, *Nezara viridula*, *Spodoptera exigua* and *Helicoverpa armigera*.

Three of the six lead soybean producers in the world (Brazil, Argentina and Paraguay) are located within the so-called South American soybean belt, with Bolivia following close behind and Uruguay making its way into the group. Caught between high propagule pressure on both country borders (Rio Grande do Sul state in the north and Argentina in the west), Uruguay is on the target for *M. sojae* invasion coming from its neighbouring countries, as similarly occurred with *H. armigera* (Castiglioni et al. 2016; Arnemann et al. 2016c). During our sampling of *M. sojae* specimens across Rio Grande do Sul state, we found that this insect was present at high incidence levels (95–100%) in all soybean fields sowed after December 31<sup>st</sup> (i.e. second-season soybean), with injury often covering the whole length of the soybean stems. If such scenario expands into main-season soybean as well, the main export commodity of these countries would be put at risk, with few alternatives available to manage it. While chemical and biological control alone may not be enough to interrupt the spread of *M. sojae* across the continent, management programs could be strengthened by measures aiming to reduce pest survival in the winter, such as eradication of post-harvest volunteer soybean plants and prohibition of second-season soybean cultivation. The findings from our study contribute to a better understanding of *M. sojae* genetic diversity and population dynamics in this important soybean growing region, and will assist in the development of efficient management measures for this highly damaging invasive pest.

## Conclusions

Single nucleotide polymorphisms (SNPs) within the partial (740 bp) COI gene differentiated *M. sojae* populations from South America into 22 mtDNA haplotypes, based on samples from Brazil, Argentina, Paraguay and Bolivia. Msoj-COI-01 and Msoj-COI-02 were the haplotypes most prevalent in the continent, representing 47% of all individuals sampled across South American countries. Msoj-COI-02 was also the haplotype identified in two individuals sampled from Australia. Three novel haplotypes (Msoj-COI-21, Msoj-COI-22 and Msoj-COI-23) were identified in Rio Grande do Sul state (Brazil). There is

evidence of *M. sojae* introductions involving multiple female founders into South America, but no location-specific genetic structure was detected. Pest management measures are still under development for this pest in the South American soybean belt, and there is paucity of data regarding soybean yield losses due to *M. sojae* attack in the New World. No molecular basis was found for treating country-specific populations of *M. sojae* differently, and they could be grouped together as a continental supra-population for future analysis and pest management purposes.

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**Data availability** The mtDNA sequences generated during and/or analysed during the current study are publicly available in the Genbank repository. Accession numbers to each mtDNA sequence are provided in the text. Other supporting datasets are available from the corresponding author on reasonable request.

#### Compliance with ethical standards

**Conflicts of interest** The authors declare no conflict of interest.

**Human and animals rights** This research did not involve human participants and/or animals.

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## 6 DISCUSSÃO GERAL

A introdução de pragas exóticas representa uma ameaça importante à produção de soja brasileira, setor que domina o cenário agrícola e lidera a exportação de *commodities* no país. Além de reduzir a produtividade da cultura, tais invasões ocasionam aumento no uso de inseticidas pelos produtores, fator que eleva os custos de produção e potencializa impactos ambientais. Exemplos notórios que atestam essa premissa foram as introduções de *B. tabaci* MEAM1, *T. urticae* e *H. armigera* no Brasil, eventos que contribuíram para que o uso de inseticidas na soja triplicasse de 1990 a 2016 (POZEBON et al., 2020). Em média três novas pragas invasivas são detectadas no país por ano, evidenciando a fragilidade das barreiras de biossegurança brasileiras (SUGAYAMA et al., 2015). Somando-se o impacto das pragas invasivas às pragas nativas, a agricultura brasileira perde anualmente 17,7 bilhões de dólares devido ao ataque de artrópodes (OLIVEIRA et al., 2014).

O estudo de invasões passadas pode fornecer critérios que possibilitem avaliar o risco de invasões futuras e, conseqüentemente, evitar perdas econômicas. A identificação de espécies potencialmente invasivas, como passo inicial no desenvolvimento de programas de prevenção, baseia-se no conceito de progressão da curva invasiva: à medida que a praga se dispersa e coloniza novas áreas, os custos para o seu controle aumentam proporcionalmente, enquanto a eficácia de controle diminui (HARVEY; MAZZOTTI, 2014). Portanto, evitar a entrada de uma praga exótica no país é menos oneroso que tentar erradicá-la, contê-la ou manejá-la após seu estabelecimento. Caso a prevenção seja ineficaz e a praga adentre o país, esforços devem ser direcionados para interromper a progressão da curva o mais rápido possível, mitigando assim os efeitos deletérios da invasão.

A análise das pragas invasivas *B. tabaci* MEAM1, *T. urticae* e *H. armigera* (além de outros casos documentados na literatura) aponta para quatro fatores como preponderantes na modulação do potencial invasivo de uma praga: probabilidade de entrada no país, probabilidade de estabelecimento, características biológicas da espécie e disponibilidade de medidas de manejo. Quando esses quatro fatores atuam simultaneamente em favor da praga, seu risco de invasão pode ser considerado alto. Como exemplo, uma praga presente em países geograficamente (ou comercialmente) próximos ao Brasil, adaptada às suas condições climáticas,

dotada de altas taxas de reprodução e com alto nível de resistência a inseticidas satisfaz os quatro critérios e deve, portanto, ser cuidadosamente monitorada para evitar uma possível introdução.

As espécies *A. glycines* e *S. litura* são exemplos de pragas da soja, atualmente ausentes no Brasil, que atendem ao menos três dos quatro critérios citados acima e apresentam alto risco de invadirem o país nos próximos anos. Caso se concretize, tal cenário impactará severamente a produção de soja, a exemplo dos países onde essas duas espécies já se estabeleceram. Já a espécie *M. sojae*, também incluída nesse grupo, encontra-se firmemente estabelecida no Brasil e representa uma das principais ameaças à produção de soja na América do Sul em termos de pragas invasivas, com alto potencial para ampliar sua distribuição geográfica nos próximos anos. O desenvolvimento de programas de manejo eficientes para uma nova praga, como *M. sojae*, é otimizado quando se pauta em uma caracterização genética detalhada das populações invasivas (GUILLEMAUD et al., 2011). Nesse contexto, o estudo da estrutura genética baseada no DNA mitocondrial (mtDNA) refere-se à distribuição da variabilidade genética entre e dentro das populações invasivas, permitindo inferências acerca do fluxo gênico, local de origem e outras relações evolucionárias (SIMON et al., 1994). Dessa forma, torna-se possível minimizar os efeitos deletérios dessa invasão no continente, bem como auxiliar na prevenção de novas introduções nocivas.

Em uma análise envolvendo 79 indivíduos de *M. sojae*, provenientes de diferentes localidades do Rio Grande do Sul, Santa Catarina, Goiás, Paraguai, Bolívia e Argentina, encontrou-se um total de 22 haplótipos diferentes, número considerado elevado para uma amostragem dessa dimensão. Esse padrão é semelhante ao verificado em outras pragas invasivas no Brasil, com *H. armigera*, e pode ser considerada uma evidência de que múltiplas introduções da praga ocorreram na América do Sul. Em outras palavras, significa que pelo menos 22 fêmeas de *M. sojae* foram introduzidas no continente, em um ou mais fluxos invasivos, originando populações que se estabeleceram nos países sul-americanos. Os altos índices de diversidade genética encontrados atestam essa hipótese. Os haplótipos denominados Msoj-COI-01 e Msoj-COI-02 foram os mais frequentes no continente e, portanto, são os que melhor se adaptaram às condições de cultivo da América do Sul. Além disso, três novos haplótipos foram identificados no Rio Grande do Sul, sendo denominados Msoj-COI-21, Msoj-COI-22 e Msoj-COI-23.

Em geral, os haplótipos mais recentes encontram-se tipicamente nas extremidades de uma rede de haplótipos, e os ancestrais, no seu centro (BONATTI et al., 2014; CUNHA; SOLÉ-CAVA, 2012). Assim, o haplótipo Msoj-COI-02 aparenta ser o ancestral dentre as populações de *M. sojae* introduzidas na América do Sul (Figura 6, página 78), embora um estudo comparativo envolvendo amostras de mosca-da-haste provenientes da sua região de origem seja necessário para confirmar essa hipótese. De acordo com a análise de variância molecular (AMOVA), não foi detectada uma estruturação populacional significativa, tampouco uma separação geográfica clara entre as diferentes linhagens de *M. sojae* na América do Sul: mesmo locais separados por grandes distâncias apresentam haplótipos similares entre si. Esse comportamento é um forte indicativo de fluxo genético ocorrendo através de fronteiras, ou seja, trânsito livre de mosca-da-haste de um estado para outro, e de um país para outro (FRANÇOSO et al., 2016).

Se as barreiras fitossanitárias são ineficazes como instrumentos de contenção dessa espécie, é altamente provável que *M. sojae* se disperse cada vez mais, atingindo os demais estados brasileiros e intensificando sua disseminação nos países vizinhos. Ainda, é comum que diferentes linhagens de uma mesma praga apresentem características distintas entre si: preferência por determinados hospedeiros, por exemplo, ou resistência a inseticidas. Assim, dentre as linhagens presentes na América do Sul, é provável que pelo menos uma apresente características favoráveis à colonização da safra principal de soja, e não apenas da segunda safra (ou safrinha), como ocorre até o momento.

Para as condições de cultivo do Rio Grande do Sul, e em especial da metade norte do estado, a flutuação anual de *M. sojae* parece obedecer a uma curva bem definida, representada na Figura 10. Tipicamente, o pico populacional de *M. sojae* no Rio Grande do Sul ocorre entre o final do verão e início do outono, quando as condições climáticas são favoráveis à proliferação da espécie: altas temperaturas e baixa umidade (TALEKAR; CHEN, 1983). Períodos de alta pluviosidade, por outro lado, inibem a alimentação e oviposição das fêmeas adultas (TALEKAR; CHEN, 1985; YADAV et al., 2015). Nos meses de inverno, a população de *M. sojae* decresce, com indivíduos remanescentes sobrevivendo em plantas de soja guaxa, no interior das hastes mortas (na forma de pupa) ou em hospedeiros alternativos (p. ex. *Trifolium resupinatum*; FERREIRA et al., 2020).

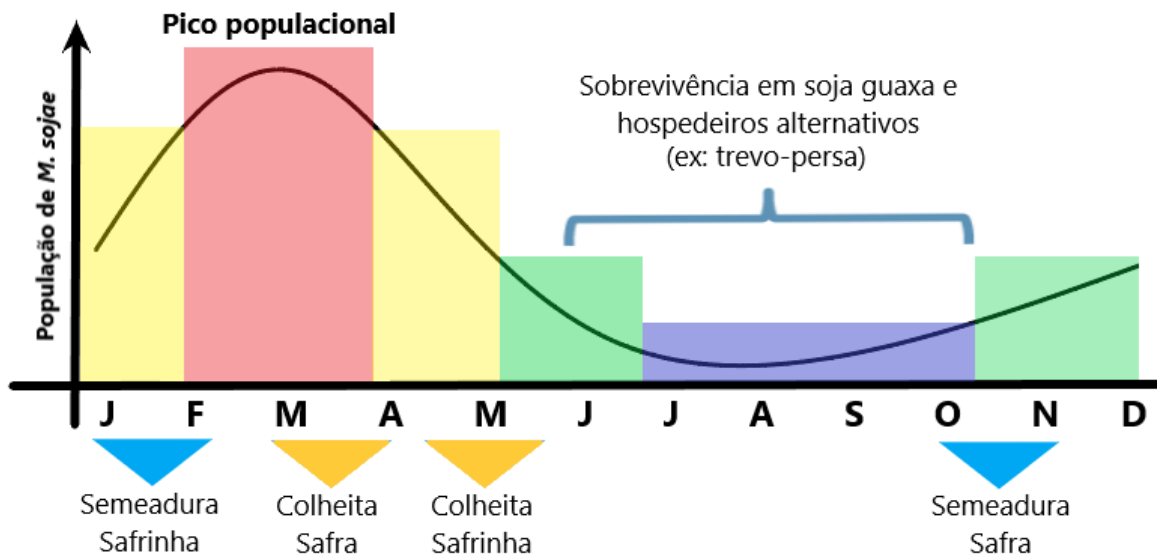


Figura 10 – Representação hipotética da dinâmica populacional anual de *Melanagromyza sojae* na metade norte do Rio Grande do Sul, de acordo com observações no campo, condições de cultivo no estado e comparação com o comportamento da espécie na sua região de origem

Fonte: O Autor

Na semeadura da safra principal (outubro e novembro), a colonização por *M. sojae* é lenta e esparsa, fato auxiliado pelo maior investimento dos produtores em tratamento de sementes (CURIOLETTI et al., 2018). Na semeadura da safrinha, por outro lado, a curva populacional está próxima do pico, indicando que a cultura sofrerá uma alta pressão de propágulo nos seus estádios iniciais de desenvolvimento. Quanto mais cedo as plantas de soja forem infestadas por *M. sojae*, maior será a redução na produtividade de grãos (TALEKAR; CHEN, 1985). As quatro primeiras semanas após a emergência das plantas são a fase mais crítica ao ataque de *M. sojae* (CABI, 2020; TALEKAR; CHEN, 1985).

A duração do ciclo da cultura é outro fator importante, estando diretamente relacionado ao potencial de injúria da praga. Cultivares mais precoces apresentam período vegetativo curto e, portanto, menor capacidade de compensar a injúria sofrida no interior da haste (TALEKAR, 1989). Como a soja semeada a partir de janeiro reduz consideravelmente seu ciclo, devido à influência do fotoperíodo decrescente, os danos por mosca-da-haste tornam-se mais evidentes e impactantes. Nesse sentido, cultivares de ciclo curto representam um alvo em potencial para *M.*

*sojae* caso infestações tornarem-se mais comuns na safra principal. Embora tal cenário pareça improvável à primeira vista, é relativamente comum que altas populações de mosca-da-haste ocorram durante todo o verão em países asiáticos (KWON et al., 1981; SINGH; SINGH, 1990a; SINGH; SINGH, 1990b). Na Bolívia, um monitoramento com armadilhas adesivas amarelas revelou alta presença de adultos de mosca-da-haste ao final do inverno e início do verão, seguido por outro pico ao final do verão (AGUILAR, 2018).

Portanto, a ocorrência de altas infestações de *M. sojae* na safra principal de soja dependerá de uma conjunção de três fatores, relativamente fáceis de ocorrer de forma simultânea: alta sobrevivência invernal das populações infestantes (p. ex. em invernos brandos, com pouca ocorrência de geadas para controlar as plantas de soja voluntárias); condições de alta temperatura e baixa pluviosidade durante o estabelecimento da cultura; e utilização de cultivares de ciclo curto, com baixo investimento em tratamento de sementes por parte dos produtores. Com efeito, relatos de campo da safra 2020/21 no Rio Grande do Sul revelam uma maior percepção da ocorrência de mosca-da-haste por parte dos produtores e técnicos, inclusive durante a safra principal de soja, comparativamente aos anos anteriores. Além de indicar uma disseminação crescente da praga, esse aumento na detecção pode estar associado ao monitoramento mais frequente das lavouras.

Estratégias de manejo visando o controle de *M. sojae* permanecem pouco exploradas na América do Sul. O potencial de dano da mosca-da-haste no Brasil foi estimado em cerca de 1 g planta<sup>-1</sup> de redução da produtividade para cada ponto percentual de haste injuriada (MARQUES et al., 2021), mas o nível de dano econômico para essa praga nas condições brasileiras de cultivo permanece desconhecido. A recomendação atual de manejo inclui monitoramento constante, rotação de cultura com gramíneas, eliminação de plantas voluntárias durante a entressafra visando à supressão populacional, e aplicação de inseticidas via tratamento de sementes e em parte aérea logo após a emergência das plântulas (quatro a cinco semanas), protegendo-as durante o período mais sensível ao ataque de *M. sojae*. Embora não haja atualmente nenhum inseticida registrado para o controle de mosca-da-haste da soja no Brasil (AGROFIT, 2021), alguns ingredientes ativos apresentam eficiência de controle satisfatória para a praga, como clorantropilprole via tratamento de sementes e clorpirifós via pulverização foliar em dez dias após a emergência das plântulas (CURIOLLETTI et al., 2018).

Um estudo preliminar envolvendo 15 cultivares de soja diferentes (safra 2019/20) revelou diferentes graus de suscetibilidade ao ataque de *M. sojae*, semelhante ao verificado em outros países (TALEKAR, 1980). O experimento foi conduzido em uma área pertencente à Sementes Costa Beber (Condor, RS, Brazil). Foram quantificadas as variáveis altura de planta (cm) e comprimento de galeria (cm) resultantes do ataque de *M. sojae*, e a razão entre essas duas variáveis foi expressa como percentagem média de haste injuriada para cada cultivar (Figura 11). As médias foram comparadas pelo teste de Tukey ( $P \leq 0,05$ ). Todas as cultivares apresentaram 100 % de incidência da praga. Embora nenhum material tenha se mostrado altamente resistente à mosca-da-haste, a cultivar BMX FIBRA apresentou o maior índice de tolerância (32,8 % de haste injuriada) (C.V. = 46,4%). Assim, estudos futuros devem ser dedicados à avaliação de uma gama maior de cultivares e determinação dos fatores que conferem tolerância genética ao ataque de *M. sojae*, além de aperfeiçoamento dos programas de monitoramento.

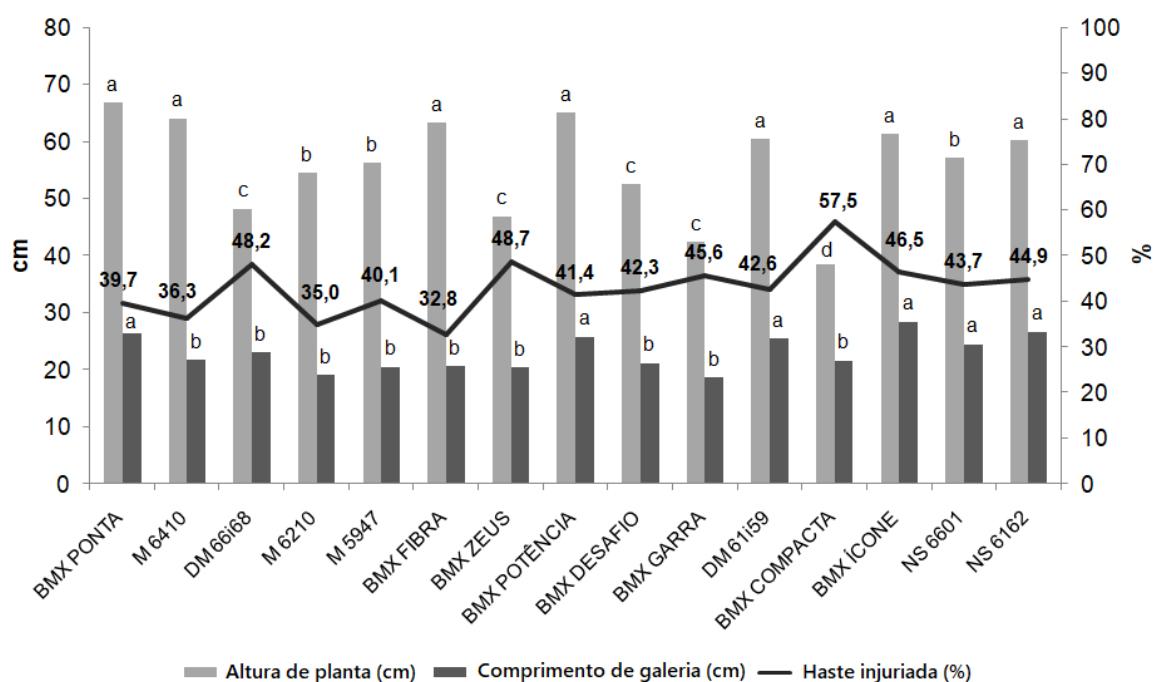


Figura 11 – Altura de planta, comprimento de galeria e porcentagem de haste injuriada por *Melanagromyza sojae* em diferentes cultivares de soja sob condições de campo. Barras acompanhadas pelas mesmas letras não diferem entre si pelo teste de Tukey ( $P \leq 0,05$ )

Fonte: O Autor

O monitoramento de mosca-da-haste envolve a abertura longitudinal da haste principal das plantas de soja, para verificar a presença de larvas, pupas ou galerias de alimentação, já que as plantas praticamente não apresentam sintomas externos (GANGRADE; KOGAN, 1980). Recomenda-se um número mínimo de 20 plantas amostradas por hectare, de forma aleatória; quanto maior a amostragem, maior será a precisão do monitoramento. Com o auxílio de um canivete, efetua-se a remoção do sistema radicular e, em seguida, a abertura da haste principal desde a base até o ápice da planta, incluindo, se possível, as ramificações laterais (Figura 12).

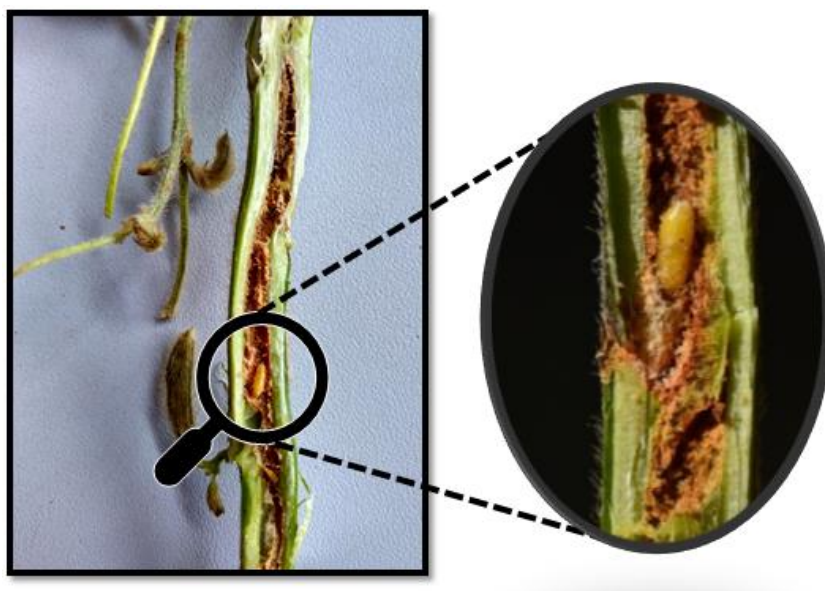


Figura 12 – Galeria de alimentação, de coloração avermelhada típica, e pupa de *Melanagromyza sojae* no interior da haste principal de uma planta de soja.

Fonte: O Autor

O monitoramento deve ser especialmente rigoroso nos estágios iniciais da cultura, quando a sua sensibilidade ao ataque de mosca-da-haste é maior. Por fim, ressalta-se que plantas de soja voluntária (soja guaxa ou tigüera) servem como hospedeiros para o inseto durante o inverno e devem ser eliminadas, evitando-se assim a formação de “pontes verdes” para a praga durante a entressafra (CZEPAK et al., 2018). Portanto, é imprescindível que se realize o monitoramento durante todo o ano para que a safra de soja não se inicie com elevada pressão da praga.





## 7 CONCLUSÃO GERAL

No primeiro artigo, uma revisão histórica dos casos de invasões de artrópodes associadas ao sistema produtivo brasileiro revelou que as introduções das espécies *B. tabaci* MEAM1, *T. urticae* e *H. armigera* no país contribuíram para o aumento expressivo no uso de inseticidas para produção de soja nas últimas décadas. Com base nesses três casos, constatou-se a existência de quatro fatores moduladores do potencial invasivo de uma praga: probabilidade de entrada, probabilidade de estabelecimento, características biológicas e disponibilidade de medidas de manejo. De acordo com esses critérios, as espécies ausentes *A. glycines* e *S. litura* foram identificadas como de alto risco para o Brasil, enquanto *M. sojae* já se encontra estabelecida e apresenta potencial para crescer em ocorrência. No segundo artigo, a caracterização do gene mtCOI em 79 indivíduos de *M. sojae*, provenientes de diferentes localidades do Brasil, Paraguai, Bolívia e Argentina, revelou a presença de 22 haplótipos diferentes de mosca-da-haste no continente. O haplótipo Msoj-COI-02 foi o mais frequente, seguido por haplótipo Msoj-COI-01, e é o provável ancestral a partir do qual as demais linhagens de originaram. Ainda, os haplótipos Msoj-COI-21, Msoj-COI-22 e Msoj-COI-23 foram identificados pela primeira vez no Rio Grande do Sul. A detecção de haplótipos similares em locais geograficamente distantes sugere a ocorrência de fluxo gênico entre as populações de *M. sojae*, enquanto a análise de variância molecular indicou ausência de estruturação populacional significativa para essa espécie na América do Sul. A alta diversidade genética encontrada é uma evidência de que múltiplas introduções de *M. sojae* ocorreram na América do Sul, reiterando a necessidade de reforçarem-se barreiras de biossegurança e desenvolverem-se programas de manejo economicamente eficientes e ambientalmente sustentáveis para essa praga altamente invasiva.



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