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MURIEL CICATTI EMANOELI SOARES

EFEITO DE INSETICIDAS DE ORIGEM BOTÂNICA E SINTÉTICA SOBRE INSETOS SUGADORES E INTERAÇÃO COM GENÓTIPOS DE SOJA

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Tese apresentada à Faculdade de Ciências Agronômicas da Unesp - Câmpus de Botucatu, para obtenção do título de Doutora em Agronomia (Proteção de Plantas).

Orientador: Prof. Dr. Carlos Frederico Wilcken

Prof. Dr. Edson Luiz Lopes Baldin (*in memoriam*)

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Botucatu, 25 de abril de 2024.

A Deus, por iluminar meu caminho, me dando força, sabedoria e paciência durante esta caminhada.

OFEREÇO

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RESUMO

Impulsionados pela grande expansão da cultura da soja nos últimos anos e pelas constantes alterações nos sistemas de cultivo, a mosca-branca *Bemisia tabaci* MEAM1 (Gennadius) (Hemiptera: Aleyrodidae) e o pulgão-da-soja *Aphis glycines* Matsumura (Hemiptera: Aphididae) tem sido apontados como grandes ameaças para diversas regiões produtoras, respectivamente, no Brasil e EUA, com infestações crescentes em todos os estágios de desenvolvimento das plantas, além do difícil manejo. Como medida para o manejo das populações desses sugadores, o controle químico vem sendo o método mais utilizado, incluindo o tratamento de sementes. A associação de genótipos resistentes a *B. tabaci* MEAM1 e inseticidas via tratamento de sementes pode contribuir significativamente para a manutenção das populações de *B. tabaci* durante todo o ciclo da soja, com potencial para reduzir as aplicações de inseticidas sintéticos e seus possíveis efeitos deletérios. Considerando-se a importância da soja para o cenário mundial e o elevado potencial de danos da mosca-branca e do pulgão-da-soja, este estudo teve como objetivo avaliar os efeitos do tratamento de semente com tiametoxam, ciantraniliprole e do derivado botânico à base de anonina (Anosom® 1EC, 10000 mg L^{-1}) associados com dois genótipos de soja (Conquista e IAC 17) sobre *Bemisia tabaci* MEAM1, com infestações realizadas em dois estádios fenológicos (V2 e V4). Inicialmente, foi estimada a colonização por ninfas da mosca-branca, utilizando-se uma escala de notas, visando observar o nível de infestação atingido pelo inseto em plantas tratadas e não tratadas e seus reflexos sobre os componentes de produtividade (número de vagens por planta, número de grãos por vagem e peso médio de grãos). Também foram realizados estudos do comportamento alimentar de *B. tabaci* MEAM1 em soja tratada com os inseticidas, através de análises de EPG, além de análise residual dos ativos em folíolos, bem como análises de colorimetria e quantificação dos tricomas presentes nas folhas. Os ensaios mostraram que as plantas cujas sementes foram tratadas com ciantraniliprole e tiametoxam, no geral, apresentaram menor infestação de *B. tabaci* MEAM1, com parâmetros produtivos mais próximos daqueles apresentados pela testemunha (sem infestação), com destaque para o tratamento constituído pelo inseticida tiametoxam. O genótipo IAC 17 (estádio V2) apresentou maior índice de luminosidade, maior intensidade de verde e amarelo, com maior número de tricomas em V4. Pela análise do EPG, os inseticidas diminuíram o número de caminhamentos estiletares (onda C),

com maior duração dessa fase no genótipo Conquista em estádio V2. Os tratamentos com ciantraniliprole e tiametoxam mostraram tendência de redução do número e da duração de ingestão de seiva do floema (onda E2), enquanto o inseticida à base de anonina aumentou a ingestão em relação ao controle. Também foi verificado que os inseticidas ciantraniliprole e tiametoxam interferiram no ciclo dos insetos e diminuíram a viabilidade das ninfas em relação ao controle em estádio V2. Para o pulgão-da-soja, a bioatividade dos extratos de sementes de *Annona mucosa*, *A. muricata* e do Anosom® 1EC foi analisada em comparação ao inseticida piretroide lambda-cialotrina (Warrior® II). O efeito inseticida foi avaliado em laboratório e as curvas de concentração-resposta foram estimadas (CL_{50} / CL_{90} para *A. muricata* 305,721 $mg.L^{-1}$ / 1290,3 $mg.L^{-1}$ e *A. mucosa* 134,229 $mg.L^{-1}$ / 487.890 $mg.L^{-1}$, respectivamente). No bioensaio de efeito sistêmico foram utilizados os mesmos extratos (100, 500, 1.000, 5.000 e 10.000 $mg.L^{-1}$), mostrando redução no número de insetos. Ambos os extratos (CL_{50} e CL_{90}) foram utilizados em teste de comportamento, indicando efeito repelente, enquanto que Warrior® II foi atrativo para os insetos. As CL's$_{90}$ dos extratos também foram utilizadas no ensaio de tempo letal (TL_{50} em torno de 20 h) e no ensaio de casa de vegetação, onde todos os tratamentos apresentaram taxas de mortalidade acima de 65%, chegando a 100% para Warrior® II, seguido pela CL_{90} de *A. mucosa* (92,1%). Os resultados demonstram que o tratamento de sementes é uma ferramenta auxiliar no controle de *B. tabaci* dentro do MIP durante as fases iniciais da cultura bem como o uso de derivados de *Annona* spp. para o manejo de *A. glycines* na soja e devem ser utilizados em conjunto com outras táticas de controle, para garantir maior eficiência no manejo e atingir maiores índices de produtividade para a cultura.

Palavras-chave: mosca-branca; pulgão-da-soja; tratamento de semente; manejo integrado de pragas; derivados botânicos.

ABSTRACT

Driven by the expansion of soybean crops and constant changes in crop systems, the whitefly *Bemisia tabaci* MEAM1 (Gennadius) (Hemiptera: Aleyrodidae) and the soybean aphid *Aphis glycines* Matsumura (Hemiptera: Aphididae) have been considered as main threats to several producing regions, with rising infestations at all stages of plant development, in addition to difficult management. As a measure for managing sucking insect populations, chemical control has been the most used method, including seed treatment. The association of genotypes resistant to *B. tabaci* MEAM1 and insecticides via seed treatment can significantly contribute to the maintenance of *B. tabaci* populations throughout the soybean cycle, with the potential to reduce the applications of synthetic insecticides and their possible deleterious effects. Considering the worldwide importance of soybeans and the high potential for damage from whiteflies and soybean aphids, this study aimed to evaluate the effects of seed treatment with thiamethoxam, cyantraniliprole and annonin-based insecticide (Anosom® 1EC, 10000 mg L^{-1}) associated with two soybean genotypes (Conquista and IAC 17) on *Bemisia tabaci* MEAM1, with infestations carried out in two phenological stages (V2 and V4). Initially, colonization by whitefly nymphs was estimated, using a visual scale, aiming to observe the level of infestation reached by the insect on treated and untreated plants and its effects on productivity (number of pods per plant, number of grains per pod and average grain weight). Studies were also carried out on the feeding behavior of *B. tabaci* MEAM1 on soybeans treated with insecticides, through EPG analyses, in addition to residual analysis of active ingredients in leaflets as well as colorimetry analyzes and quantification of trichomes present on the leaves. The tests showed that the plants whose seeds were treated with cyantraniliprole and thiamethoxam, in general, presented lower infestation of *B. tabaci* MEAM1, with productive parameters closer to those presented by the uninfested plants, with emphasis on the treatment with thiamethoxam. The IAC 17 genotype at V2 stage showed a higher luminosity index, greater intensity of green and yellow, with a higher number of trichomes at V4 stage. For EPG analysis, insecticides reduced the number of stylet walks (C wave), with a longer duration of this phase in the Conquista genotype at V2 stage. Treatments with cyantraniliprole and thiamethoxam showed a tendency to reduce the number and duration of phloem sap ingestion (wave E2), while annonin-based insecticide increased the ingestion in relation to the control. It was also found

that the insecticides interfered in the insect cycle and reduced the viability of the nymphs in relation to the control at V2 stage. For soybean aphid, the bioactivity of *Annona mucosa* and *A. muricata* seed extracts and Anosom® 1EC was analyzed compared to the pyrethroid insecticide lambda-cyhalothrin (Warrior® II). The insecticidal effect was assessed in vial bioassays and the concentration-response curves were estimated (LC_{50} / LC_{90} for *A. muricata* 305.721 $mg.L^{-1}$ / 1290.3 $mg.L^{-1}$ and *A. mucosa* 134.229 $mg.L^{-1}$ / 487.890 $mg.L^{-1}$, respectively). In the systemic effect bioassay, the same extracts were used (at 100, 500, 1,000, 5,000 and 10,000 $mg.L^{-1}$), showing a reduction in the number of insects on the petioles. Both extracts at LC_{50} and LC_{90} were used in a behavior test, indicating that they are repellent and Warrior II® was attractive for the insects. The LC_{90} rates were also used in lethal time (LT_{50} – around 20h) and in greenhouse bioassays, all treatments showed insect mortality rates above 65%, reaching 100% for Warrior® II, followed by the LC_{90} of *A. mucosa* (92.1%). The results demonstrate that seed treatment is a tool to aid control of *B. tabaci* within the IPM during the initial phases, as well as the *Annona* spp. derivatives to the management of *A. glycines* in soybean, and they should be used in conjunction with other control tactics, to ensure greater management efficiency and achieve higher productivity rates for the crop.

Keywords: whitefly; soybean aphid; seed treatment; integrated pest management, botanical derivatives.

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INTRODUÇÃO GERAL

A cultura da soja

A soja [*Glycine max* L. (Merrill) (Fabaceae)] é a leguminosa mais cultivada em todo o mundo e representa uma importante fonte de proteína e óleo na alimentação humana e animal (Koester *et al.*, 2014), sendo a principal oleaginosa cultivada no Brasil e considerada a principal *commodity* de exportação brasileira (Conab, 2023).

Os ganhos no rendimento da cultura têm aumentado substancialmente ao longo dos anos, sendo principalmente atribuídos aos avanços no melhoramento genético das cultivares, bem como nas tecnologias de cultivo (Koester *et al.*, 2014). Apesar do elevado potencial da cultura, a produtividade da soja é frequentemente reduzida devido ao ataque de inúmeras pragas. Como destaque, podemos citar a mosca-branca *Bemisia tabaci* MEAM1(Gennadius) (Hemiptera: Aleyrodidae), que tem sido apontada como uma das principais ameaças para diversas regiões produtoras do Brasil, com infestações crescentes em todos os estádios de desenvolvimento das plantas (Vieira *et al.*, 2011) e o pulgão-da-soja, *Aphis glycines* Matsumura (Hemiptera: Aphididae), sendo uma praga de grande importância para a América do Norte, principalmente no Centro-Oeste dos Estados Unidos (EUA) (Hurley; Mitchell, 2017).

Bemisia tabaci MEAM1

Considerada uma das principais pragas para várias regiões produtoras de soja no Brasil, a mosca-branca, *B. tabaci* MEAM1 compromete a produtividade das plantas e eleva os custos de produção nas lavouras (Vieira *et al.*, 2011). Pertencente à ordem Hemiptera, subordem Sternorrhyncha e família Aleyrodidae (Evans *et al.*, 2008), a mosca-branca é um inseto pequeno, seus adultos medem entre 1 e 2 mm de comprimento e 0,36 a 0,51 mm de largura, sendo as fêmeas maiores que os machos. O dorso possui coloração amarelo-pálida e as asas são de cor branca (Walker *et al.*, 2010; Souza; Vendramim, 2001).

A taxonomia de *B. tabaci* foi discutida por décadas, com inúmeras descrições de espécies, depois biótipos ou raças. Porém, a proposta mais recente a define como um complexo de espécies crípticas, com um total de 43 espécies identificadas, morfologicamente idênticas, porém geneticamente distintas (De Barro *et al.*, 2011; Tay *et al.*, 2017). No Brasil, há pelo menos quatro espécies do complexo relatadas, baseadas na análise do gene I do citocromo oxidase mitocondrial (mtCOI) (Diinsdale

et al., 2010; De Barro *et al.*, 2011), sendo as espécies nativas New World e New World 2 (NW2) (biótipo A) (Marubayashi *et al.*, 2013) e as espécies invasivas Middle East-Asia Minor 1 – MEAM1 (biótipo B) e a mais recentemente detectada espécie críptica Mediterranean (biótpo Q) (Barbosa *et al.*, 2015).

Bemisia tabaci MEAM1 é uma espécie extremamente polífaga e pode ocasionar danos diretos e indiretos às plantas (Inbar; Gerling, 2008; Lourenção *et al.*, 2015). Os danos diretos estão relacionados com o hábito de ninfas e adultos em se alimentarem da seiva do floema e em injetarem toxinas durante a alimentação, afetando os processos fisiológicos da planta (Hoffmann-Campo *et al.*, 2000; Oliveira *et al.*, 2001; Villas Bôas, 2005). Os danos indiretos se devem à sua capacidade de transmitir mais de 200 tipos de vírus (Jones, 2003; Navas-Castillo *et al.*, 2011; Polston *et al.*, 2014; Krause-Sakate *et al.*, 2020). Na cultura da soja, *B. tabaci* MEAM1 age como vetora do vírus causador da necrose da haste (*Cowpea mild mottle virus* - CpMMV), pertencente ao gênero *Carlavirus* (Almeida *et al.*, 2005; Marubayashi *et al.*, 2010). Outro dano indireto importante é decorrente da intensa excreção de "honeydew", que favorece o desenvolvimento de fumagina (*Capnodium* sp.), podendo comprometer a capacidade fotossintética das plantas (Musa; Ren, 2005; Naranjo; Legg, 2010; Cameron *et al.*, 2013).

***Aphis glycines* Matsumura**

Aphis glycines é um inseto pequeno, com, aproximadamente, 0,16 mm de comprimento, e corpo oval. Apresenta coloração verde-amarelada, com olhos escuros, com um par de cornículos escuros localizados no sexto segmento dorsal-abdominal (Voegtlin *et al.* 2004). As ninfas são menores e mais leves quando comparadas aos adultos (Wu *et al.*, 2004). Passam por quatro ínstares ninfais e o desenvolvimento até adulto ocorre em aproximadamente 7 dias (Heimpel; Shelly, 2004).

Nos sistemas de cultivo de soja no Centro-Norte dos EUA, *A. glycines* apresenta grande importância (Heimpel; Shelly, 2004; Myers *et al.*, 2005; McCornack; Ragsdale, 2006; Ragsdale *et al.*, 2007). Desde sua introdução nos EUA na década de 2000 (RAGSDALE *et al.*, 2007), o inseto se espalhou através desta região até o sul do Canadá (Venette; Ragsdale, 2004). Danos ocasionados pela alimentação dos insetos podem resultar em uma redução da taxa fotossintética, altura da planta,

número de vagens por planta, sementes por vagem, qualidade do tegumento, peso da semente e aumento do abortamento da vagem (Macedo *et al.* 2003, Beckendorf *et al.* 2008). Em altas densidades, as populações de *A. glycines* reduzem o rendimento da cultura entre 40 e 50% (Ragsdale *et al.*, 2007).

O pulgão-da-soja é um inseto que se alimenta do floema e pode reduzir a produtividade da soja significativamente (Ragsdale *et al.*, 2007). O inseto pode transmitir doenças virais à soja, incluindo o vírus do mosaico da soja (*Soybean mosaic virus* - SMV) e o vírus do mosaico da alfafa (*Alfafa mosaic virus* - AMV) (Hill *et al.*, 2001), causando distorção das folhas. O rendimento é geralmente reduzido pela diminuição do número de sementes e pela redução do conjunto de vagens (Hartman; Hill, 2010).

Métodos de controle dos insetos sugadores na soja

Controle químico

Como medida para o manejo das populações de *B. tabaci* MEAM1, o controle químico, por meio de pulverizações com inseticidas, vem sendo o método mais utilizado pelos produtores (Viera *et al.*, 2013; Padilha *et al.*, 2021). Entretanto, diversas mudanças no sistema de cultivo brasileiro, como plantio sucessivo de diversas culturas hospedeiras, além da proximidade de áreas contendo plantas em diferentes estágios fenológicos (Nagoshi, 2009; Barros *et al.*, 2010), vêm alterando o *status* dessa praga na soja. Devido a essas mudanças no cultivo, o inseto tem encontrado alimento em abundância durante todo o ano. Em consequência, seus danos vêm sendo crescentemente relatados em todos os estágios de cultivo da soja, desde os estádios iniciais das plantas até o período de maturação.

Algumas características biológicas e comportamentais de *B. tabaci* MEAM1 (Ahmad *et al.*, 2002), tais como rápido desenvolvimento, alta fecundidade e grande capacidade de dispersão são fatores que aumentam a probabilidade de aparecimento de resistência aos inseticidas comerciais, sendo que já há relatos de populações de *B. tabaci* resistentes a vários grupos de inseticidas sintéticos, incluindo organofosforados, carbamatos, piretroides, reguladores de crescimento, hidrocarbonetos clorados, neonicotinoides, além de derivados do ácido tetrônico (Roditakis *et al.*, 2005; Naveen *et al.*, 2017; Dângelo *et al.*, 2018).

Quando empregados via tratamento de sementes (TS), os inseticidas representam uma importante opção de manejo de diversas pragas durante os estágios iniciais das culturas (Elbert *et al.*, 1991; 2008) por possibilitarem redução do número de aplicações foliares, que, muitas vezes, precisam ser iniciadas logo após a emergência das plântulas (Menten, 2005). Dentre as opções recomendadas contra insetos sugadores em soja, o neonicotinoide tiametoxam tem se tornado um importante aliado na proteção de plantas durante o início do cultivo, incluindo sua potente ação contra *B. tabaci* MEAM1 (Carvalho *et al.*, 2011). Outro inseticida que tem ganhado espaço no mercado é a antranilamida ciantraniliprole (Maluta *et al.*, 2020; 2021; Agrofit, 2023).

Informações quanto à eficiência de controle, bem como o período residual dos produtos utilizados nas sementes são ainda limitadas na literatura, dificultando a padronização de recomendação aos produtores. Nesse sentido, são necessários estudos adicionais, a fim de avaliar a eficiência de controle dos inseticidas, além de elucidar discrepâncias quanto ao período residual dos produtos no tratamento de sementes de soja. Além dos estudos de performance biológica do inseto em plantas que recebem tratamento de sementes, o monitoramento do comportamento alimentar da mosca-branca em plantas tratadas pode ser utilizado como um potente bioindicador.

Com relação ao pulgão-da-soja, existem várias ferramentas de manejo disponíveis, como resistência da planta hospedeira e controle biológico, mas o uso de inseticidas também é a principal ferramenta de manejo. O uso de inseticidas sintéticos aumentou significativamente em resposta à infestação por *A. glicines* desde seu primeiro registro nos EUA (Ragsdale *et al.*, 2011; Coupe; Capel, 2016). No entanto, sabe-se que o uso continuado desses produtos resulta na seleção de populações resistentes, como já relatado para organofosforados (Wang *et al.*, 2011, 2012), neonicotinoides (Ribeiro *et al.*, 2018) e piretroides (Koch *et al.*, 2018), além de desequilíbrios ambientais e problemas relacionados à saúde humana.

Inseticidas botânicos

Existem diversas famílias botânicas promissoras como fonte de compostos inseticidas, com destaque para Asteraceae, Annonaceae, Canellaceae, Lamiaceae, Meliaceae, Piperaceae e Rutaceae (Zabel *et al.*, 2002; Céspedes *et al.*, 2004; Tamm

et al., 2004). O Brasil apresenta-se como um dos países com maior potencial para estudos nessa área, devido à sua ampla biodiversidade (cerca de 20% do total de espécies da flora do planeta), além de comportar a maior floresta equatorial e tropical úmida do mundo (Pinto *et al.*, 2002; Simões; Schenkel, 2002).

Dentre as famílias botânicas com potencial inseticida/insetistático, a família Annonaceae apresenta-se como uma das principais fontes de compostos naturais bioativos frente a artrópodes-praga (Krinski *et al.*, 2014; Ribeiro *et al.*, 2023).

Entre as anonáceas de interesse comercial se destacam a cherimoia (*Annona cherimola* Mill.), a pinha ou fruta-do-conde (*Annona squamosa* L.), atemoia (cruzamento *A. squamosa* x *A. cherimola*) e a graviola (*Annona muricata* L.) (Lorenzi; Matos, 2002; Ribeiro *et al.*, 2023).

Os estudos com espécies dessa família têm se tornando cada vez mais frequentes, principalmente a partir da descoberta dos alcaloides benzilisoquinolínicos e das acetogeninas (ACG's), com grande potencial para a medicina (Bermejo *et al.*, 2005; Rainer, 2007), causando efeitos antitumorais, anti-parasíticos, pesticidas, antimicrobianos e imunossupressivos (Ratnayake *et al.*, 1993; McLaughlin *et al.*, 1997; Matsumoto *et al.*, 2010; Ferreira *et al.*, 2013).As ACG's são exclusivas de alguns gêneros da família Anonnaceae e podem ser encontradas em cascas de troncos, ramos, raízes e, principalmente, nas sementes (Bermejo *et al.*, 2005; Castillo-Sânchez *et al.*, 2010).

Como modo de ação sobre artrópodes, as ACG's apresentam potente inibição do complexo I (NADH - ubiquinona oxidoredutase) na cadeia transportadora de elétrons mitocondrial, levando à redução das taxas respiratórias e cardíaca e à morte celular programada (Degli Esposti *et al.*, 1994; Gallardo *et al.*, 2000).

Em diversos estudos há relatos do potencial de controle de derivados obtidos de diversas espécies de Annonaceae sobre insetos de importância agrícola (Ansante *et al.*, 2015; Ribeiro *et al.*, 2015, 2016; Souza *et al.,* 2017, 2019), incluindo resultados satisfatórios sobre ninfas e adultos de *B. tabaci* MEAM1 (Soares *et al.*, 2021). No entanto, não existem relatos sobre a eficácia de derivados de *Annona* spp. sobre *B. tabaci* MEAM1 quando utilizados no tratamento de sementes, tampouco sobre *A. glycines*.

Resistência de plantas a insetos

Com o advento do Manejo Integrado de Pragas (MIP), a possibilidade do uso de genótipos resistentes associado a outras práticas pode ser uma estratégia valiosa na manutenção das populações de insetos-praga nas culturas (Auclair, 1989). Segundo Painter (1951), variedade resistente é aquela que devido às suas características hereditárias, é capaz de apresentar maior capacidade de produção, comparativamente a outras variedades em igualdade de condições de infestação do inseto. Além de apresentar reconhecida eficiência e ser compatível com outras táticas de controle, esse método possui outras características desejáveis, tais como especificidade para uma ou várias pragas, efeito cumulativo e persistência. Por fim, variedades resistentes possibilitam a manutenção da população de insetos abaixo do nível de dano econômico, tornando a cultura mais rentável para o produtor (Painter, 1951; Kogan, 1982; Smith, 2005; Vendramim; Guzzo, 2009).

A resistência de uma planta frente ao ataque de um inseto pode se revelar através de três categorias: antixenose, antibiose e tolerância (Painter, 1951). Na antixenose, o comportamento de colonização e alimentação do inseto é afetado negativamente pela presença de fatores químicos ou morfológicos das plantas (Lara, 1991). Para a cultura da soja, alguns genótipos já tiveram essa categoria de resistência caracterizada sobre *B. tabaci* MEAM1 (Valle; Lourenção, 2002; Valle *et al.*, 2012; Silva *et al.*, 2012).

A antibiose ocorre quando o inseto tem seu desempenho biológico afetado negativamente, porém sem interferir no comportamento de alimentação, oviposição e abrigo (Painter, 1951). Elevados índices de mortalidade na fase jovem, reduções de peso, além de prolongamento de ciclo e baixa viabilidade estão entre os efeitos mais comuns dessa categoria (Lara, 1991; Panda; Khush, 1995). Em soja, poucos estudos caracterizaram a expressão de antibiose sobre a mosca-branca (Vieira *et al.*, 2016; Cruz; Baldin, 2017). Por sua vez, a tolerância é descrita como a capacidade da planta em resistir ou recuperar-se de um dano causado por um inseto-praga, sem que a biologia ou o comportamento do mesmo sejam afetados (Painter, 1951; Smith, 2005). Essa categoria foi descrita no genótipo de soja KS4202 sobre *B. tabaci* MEAM1 (Cruz *et al.*, 2016).

As causas da resistência de plantas, principalmente antixenóticas e antibióticas, estão presentes entre as diferentes camadas de tecidos existentes entre a superfície

da epiderme e o floema e influenciam no processo de alimentação de insetos sugadores. Plantas que expressam antixenose podem apresentar diferentes fatores (modificações da epiderme, tricomas, etc) que inibem ou impedem a alimentação do inseto sobre a planta, reduzindo o risco de serem atacadas por pulgões e moscas-brancas (Smith, 2005). Já as plantas portadoras de antibiose podem liberar compostos químicos deletérios nos vasos condutores, prejudicando a performance biológica dos insetos (Luski *et al.*, 2013).

***Electrical Penetration Graph* (EPG)**

Dentre as técnicas promissoras na caracterização de resistência das plantas sobre a mosca-branca, destaca-se o uso do *Electrical Penetration Graph* (EPG). A partir dos padrões de ondas gerados, é possível correlacionar o comportamento de alimentação do inseto com a expressão de diferentes categorias de resistência (antibiose ou antixenose). O uso da técnica de EPG também pode auxiliar no dimensionamento dos efeitos residuais de inseticidas utilizados via tratamento de sementes.

A técnica de EPG foi inicialmente desenvolvida em 1964 (McLean; Kinsey, 1964) e modificada posteriormente em 1978 (Tjallingii, 1978). Tem como objetivo amplificar os sinais elétricos provenientes da interação inseto-planta, permitindo o conhecimento de distintos aspectos do comportamento alimentar de insetos sugadores, por meio de diferentes padrões de ondas. O EPG é baseado em um circuito elétrico composto por um amplificador com uma fonte de voltagem conectada a um resistor e dois fios, um ligado à entrada (resistor) e o outro à saída (fonte de voltagem) da caixa. O fio de saída possui dois eletrodos em sua extremidade, um ligando-se ao solo ou à planta e outro ligado no inseto. O eletrodo do inseto é conectado a um fio de ouro (10 a 20 µm) e fixado no dorso do mesmo, com auxílio de uma cola condutora (tintura de prata).

Quando o inseto insere os estiletes no tecido vegetal, o circuito é fechado, e uma variação de voltagem é obtida e gravada em um computador, sendo possível acompanhar as ações realizadas (Walker, 2000). Alterações na voltagem do sistema correspondentes aos componentes de resistência (R) e de força eletromotriz (fem) ocorrem por conta das atividades estiletares efetivadas. Os sinais elétricos gerados (ondas) são ampliados e registrados em um gráfico. Distintas formas de onda estão sendo apresentadas através de estudos de correlação entre a atividade de insetos

sugadores e a localização do estilete no tecido da planta. O EPG pode ser separado em dois momentos: o primeiro representa a prova (penetração do estilete) e o segundo a não-prova (np). O período de prova possui três fases distintas: caminhamento do estilete “C” (contendo a queda de potencial – pd), fase de xilema (onda G) e a fase do floema (ondas E1 e E2) (Tjallingii, 1988).

A técnica do EPG pode auxiliar em diferentes tipos de estudos de transmissão de vírus (Prado; Tjallingii, 1994; Fereres; Collar, 2001), caracterização de resistência de plantas (Van Helden; Tjallingii, 1993; Jiang *et al.*, 2001; Diaz-Montano *et al.*, 2007; Zhu *et al.*, 2011; Todd *et al.*, 2016), no comportamento de insetos vetores e na dinâmica de inseticidas (Nisbet *et al.*, 1993). Essa técnica também pode contribuir no dimensionamento de efeitos residuais de inseticidas a partir de tratamento de sementes (Stamm *et al.*, 2013).

Objetivos

Considerando-se a importância da soja para o Brasil e para o mundo, e o elevado potencial de danos que esses insetos apresentam para a cultura, ressalta-se a importância do uso associado de práticas de controle, em consonância com os preceitos do MIP. Diante do exposto, este estudo teve como objetivo avaliar os efeitos do tratamento de semente com inseticidas à base de tiametoxam, ciantraniliprole e do derivado botânico à base de anonina (Anosom® 1EC) associados com genótipos resistentes e suscetíveis sobre *B. tabaci* MEAM1 em soja. Também foram realizados estudos do comportamento alimentar de soja tratada com os inseticidas, através de análises de EPG, além de análise cromatográficas para determinação dos resíduos de inseticidas ao longo do tempo e de análises de tricomas e de colorimetria.

A bioatividade dos extratos de sementes de *Annona mucosa*, *A. muricata* e do inseticida à base de anonina também foi avaliada para *A. glycines.*

Os resultados provenientes desse estudo poderão auxiliar no delineamento de estratégias integradas de manejo de *B. tabaci* MEAM1 e de *A. glycines* na cultura da soja, além de gerar subsídios para programas de melhoramento com foco em resistência à mosca-branca.

De modo a cumprir com os objetivos elencados, a referida tese foi dividida em três capítulos, sendo o primeiro intitulado “Infestation of *Bemisia tabaci* MEAM1 and

its effect on yield of soybean genotypes under different seed treatment and in different level of susceptibility", o segundo "Soybean seed treatment with insecticides: effects on feeding behavior and development of *Bemisia tabaci* MEAM1 and residual content analysis" e o terceiro "Efficacy of ethanolic seed extracts of *Annona* spp. against *Aphis glycines*", sendo este último redigido e já publicado de acordo com as normas da revista Crop Protection.

CHAPTER 1

INFESTATION OF *Bemisia tabaci* MEAM1 AND ITS EFFECT ON YIELD OF TWO SOYBEAN GENOTYPES UNDER DIFFERENT SEED TREATMENT

RESUMO

O tratamento de sementes (TS) é uma prática importante na prevenção de infestações de pragas iniciais na cultura da soja. Dentro de um programa de manejo integrado de pragas (MIP), o TS destaca-se pela sua seletividade ecológica frente aos inimigos naturais e facilidade operacional. Neste estudo, foi avaliada a ação do TS com dois inseticidas sintéticos (ciantraniliprole e tiametoxam) e um inseticida botânico à base de anonina (acetogenina) na colonização da mosca-branca *Bemisa tabaci* MEAM1 e sua influência nos componentes produtivos da soja. Para isso, em casa de vegetação, duas cultivares (Conquista e IAC 17) foram infestadas em dois estádios distintos do desenvolvimento vegetativo da soja (V2 e V4). Quantificação de tricomas e análises colorimétricas também foram realizadas. Em geral, os resultados obtidos indicaram que plantas cujas sementes foram tratadas com ciantraniliprole e tiametoxam apresentaram menor infestação de *B. tabaci* MEAM1, com parâmetros produtivos mais próximos daqueles apresentados pela testemunha (sem infestação), com destaque para o tratamento constituído pelo tiametoxam. O genótipo IAC 17 em estádio V2 apresentou maior índice de luminosidade, maior intensidade de verde e amarelo, com o aumento do número de tricomas com o desenvolvimento das plantas. O tratamento com anonina não apresentou resultados positivos quando utilizado via TS. É possível concluir que os inseticidas tiametoxam e ciantraniliprole se apresentam como uma ferramenta de auxílio no controle da mosca-branca dentro do MIP durante as fases iniciais da cultura da soja.

Palavras-chave: mosca-branca; ciantraniliprole; tiametoxam; colorimetria; resistência de plantas; inseticida botânico

ABSTRACT

Seed treatment (ST) is an important practice in preventing inicial pest infestations in soybean crops. Within an integrated pest management program (IPM), ST stands out for its ecological selectivity in relation to natural enemies. In this study, the action of ST was evaluated with two synthetic insecticides (cyantraniliprole and thiamethoxam) and a botanical insecticide based on annonin (acetogenin) on the colonization of the whitefly *Bemisa tabaci* MEAM1 and its influence on the crop productivity parameters. For this, in a greenhouse, two cultivars (Conquista and IAC 17) were infested at two stages (V2 and V4) of soybean vegetative development. Trichome quantification and colorimetric analyzes were also carried out. In general, the results obtained indicated that plants whose seeds were treated with cyantraniliprole and thiamethoxam showed lower infestation of *B. tabaci* MEAM1, with productivity parameters components closer to those presented by the control (without infestation), with emphasis on the treatment consisting of thiamethoxam. The IAC 17 genotype at V2 stage showed a higher luminosity index, greater intensity of green and yellow, increasing the number of trichomes throughout the plant developmet. The treatment with annonin didn't present positive results when applied via ST. It is possible to conclude that thiamethoxam and cyantraniprole present themselves as a tool to control whiteflies within IPM during the initial phases of soybean cultivation.

Keywords: Whitefly; cyantraniliprole; thiamethoxam; colorimetry; host plant resistance; botanical insecticide

1.1 INTRODUCTION

Soybean [*Glycine max* (L.) Merrill (Fabaceae)] is one of the most cultivated plants worldwide and represents an important source of protein and oil to human and animal nutrition (Usda, 2022). In addition, it is the main oilseed cultivated in Brazil, being considered the largest exporter of this commodity (Conab, 2023). Despite of its socioeconomic importance, soybean productivity is often reduced due to the attack of several pests and diseases. Currently, the whitefly *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae) Middle East-Asia Minor 1 (MEAM1) is one of the main threats in several soybean-producing regions of Brazil, with increasing infestations at all stages of plant

development, resulting in extensive losses in crop yield as well as increasing the production costs (Tamai *et al.*, 2006; Vieira *et al.*, 2011; Padilha *et al.*, 2021).

Considered a polyphagous pest, *B. tabaci* MEAM1 causes direct and indirect damage to a large number of cultivated plants due to feeding of nymphs and adults in the phloem and through the injection of toxins, which can affect the vegetative and reproductive development of plants (Yee *et al.*, 1996; Lin *et al.*, 1999a, 1999b; Tamai *et al.*, 2006). In addition, it is an important vector of viruses to different plant species (Polston *et al.*, 2014; Baldin *et al.*, 2017). In soybean crops, *B. tabaci* MEAM1 can transmits the Cowpea Mild Mottle Virus (CPMMV), causal agent of the soybean stem necrosis, a disease already recorded in all major soybean-producing areas in Brazil (Inoue-Nagata *et al.*, 2016; Silva *et al.*, 2020). Moreover, the direct damage of *B. tabaci* MEAM1 favoring the development of grey mold (*Capnodium* sp.), resulting from the intense excretion of honeydew (Musa; Ren, 2005; Naranjo; Legg, 2010; Cameron *et al.*, 2013).

Among the most commonly widespread strategy for *B. tabaci* MEAM1 management, the application of synthetic insecticides is still the main method used, with more than 85 products registered in Brazil (Agrofit, 2024). However, some biological and behavioral characteristics of *B. tabaci* MEAM1, including its rapid development, high fecundity and great dispersal capacity (Ahmad *et al.*, 2002), increase the probability of rising resistance to commercial insecticides. To date, there are already reports of species of *B. tabaci* complex resistant to several groups of conventional insecticides, including organophosphates, carbamates, pyrethroids, growth regulators, chlorinated hydrocarbons, neonicotinoids, in addition to tetronic acid derivatives (Roditakis *et al.*, 2005; Silva *et al.*, 2009; Naveen *et al.*, 2017; Dângelo *et al.*, 2018). Therefore, it becomes increasingly necessary to search for more sustainable control alternatives within an Integrated Pest Management (IPM) program (Baldin *et al.*, 2017) as well as validate the existing technologies.

When applied via seed treatment (ST), insecticides represent an important option for managing several pests during the initial stages of crops by reducing the number of foliar applications at the beginning of the crop cycle, preserving the action of natural enemies and biological balance. In ST, the seed is the carrier of the pesticide, which will solubilize in the soil and eventually be absorbed by the plant's root system and translocated to its canopy, providing control of sucking insects and sometimes

early defoliators during the initial stages of growth plant development (Lamichhane, 2020; Vojvodic; Bazok, 2021). This pattern of insecticide use is often considered ecologically and economically justifiable because it reduces pesticide applications, decrease costs with insect control and the impact to non-target organisms and promotes the reduction of health risk to farmers (Lamichhane, 2020; Vojvodic; Bazok, 2021).

In ligh of this context, research associated with the use of plant extracts in ST modality has also been carried out (Carvalho *et al.*, 2022), but no specific study to check their efficiency on *B. tabaci* MEAM1. Among the botanical families with insecticidal/insectistactic potential, the Annonaceae family is one of the main sources of bioactive natural compounds against arthropod pests (Krinski *et al.*, 2014). Acetogenins stand out among the classes of secondary metabolites predominant in *Annona* species, which cause a series of biological activities, including action against helminths, microorganisms in general and protozoa, in addition to being toxic to tumor cells and exhibiting insecticidal activities (McLaughlin *et al.*, 1997; Ocampo; Ocampo, 2006). In several studies, there are evidence of the great potential control of derivatives obtained from different Annonaceae species on insects of agricultural importance, including satisfactory results on nymphs and adults of *B. tabaci* MEAM1 by foliar spraying (Soares *et al.*, 2021 a; b). However, there are no reports on the effect of *Annona* spp. derivatives on *B. tabaci* MEAM1 when used in ST modality.

Resistant genotypes associated with other practices can also be a valuable strategy in managing insect pest populations within an IPM program (Sulistyo; Inayati, 2016). The association of chemical control via ST with resistant genotypes can significantly contribute to reduction of *B. tabaci* MEAM1 populations throughout the soybean cycle and has the potential to decrease successive applications of synthetic insecticides and their possible deleterious effects (Smith; Clement, 2012).

Considering the importance of soybean crops for Brazil and the high potential of damage that whiteflies represent, the importance of an alternative control is highlighted, in line with IPM precepts. Furthermore, due to the biological characteristics of the insect, previously, it is necessary to verify whether the doses recommended of registered insecticides are still in agreement and control the insect. Accordingly, this study aimed to evaluate the effects of ST with thiamethoxam, cyantraniliprole and a

botanical insecticide based on annonin (acetogenin) in two soybean genotypes against *Bemisia tabaci* MEAM1.

1.2 MATERIAL AND METHODS

The study was carried out in the Insect Plant Resistance and Insecticidal Plants Laboratory (LARESPI) – Department of Plant Protection/School of Agriculture, Sao Paulo State University (FCA/UNESP), in Botucatu, SP, Brazil (22°85'09 "S and 48°43'16" W) between 2019 and 2022.

1.2.1 Choice of soybean genotypes and seed treatment procedure

The soybean genotypes used in the experiments, their respective genealogies and criteria for choice (*B. tabaci* MEAM1 resistance history) are described in Table 1.

Table 1. Soybean genotypes used in the experiments, their respective genealogies and criteria for choice.

Genotypes	Genealogy/Origin	Historical of resistance	References
Conquista	Lo76-4484² × Numbaíra	Susceptible to *B. tabaci* MEAM1 (previously described as biotype B)	Silva *et al.* (2012)
IAC 17	D72-9601-1 × IAC 8 (IAC/Campinas)	Antixenosis to *Bemisia tabaci* MEAM1	Valle; Lourenção (2002); Silva *et al.* (2012); Cruz; Baldin (2017)

The seeds of the respective materials were treated in the laboratory with the neonicotinoid insecticide Cruiser® 350 FS (thiamethoxam, 350g.L^{-1}), at a dose of 200 mL 100 kg^{-1} of seeds, the anthranilamide insecticide Fortenza® 600 FS (cyantraniliprole, 600g.L^{-1}), at a dose of 160 mL 100 kg^{-1} of seeds and with the

botanical insecticide Anosom® 1 EC (based on acetogenins, annonin 10,000 mg L^{-1}), at a dose of 200 mL 100 kg^{-1} of seeds. To ensure homogeneity in the distribution of the insecticides, seeds from each genotype and insecticides were put inside a glass tube with a lid and were shaken manually for 2 minutes.

1.2.2 Rearing of *B. tabaci* MEAM1

A colony of *B. tabaci* MEAM1 was kept in a greenhouse (2.5 × 2.5 × 2 m), with a roof covered by glass and shade (30%) and closed on the sides with glass. To maintain the insects, collard green plants [*Brassica oleracea* var. *acephala* L. (Brassicaceae)] were offered, kept in plastic pots with a capacity of 2.5 L. The plants were irrigated daily and replaced periodically, in order to maintain the nutritional quality of the food and also the vigor of whitefly populations. Before and during the experiment, molecular analyzes were carried out to confirm the whitefly cryptic species (Walsh *et al.*, 1991; Simon *et al.*, 1994; De Barro *et al.*, 2011).

Bioassays

Plants from treated and untreated seeds were grown in plastic pots (3 L), filled with autoclaved substrate, containing a mixture of soil, sand and farmyard manure in a 1:1:1 ratio (v^{-1} v^{-1} v^{-1}) and fertilized as recommended for the crop (Cantarella *et al.*, 2022). The plants obtained were kept in a greenhouse, free from insect infestation, until the appropriate stage of development for carrying out the bioassays.

1.2.3 Effects on *B. tabaci* MEAM1 colonization in soybeans treated with thiamethoxam, cyantraniliprole and annonin

In this trial, soybean plants in growth stages V2 and V4 (Fehr; Caviness, 1977) of the Conquista genotype were used, obtained from seeds treated with thiamethoxam, cyantraniliprole and the annonin-based botanical insecticide.

The plants were individualized in metal cages (35 cm in diameter × 55 cm in height), covered with voil fabric. The plants were infested with 25 pairs of whiteflies, which were collected from colony using a mouth aspirator (4 cm in diameter ×11 cm in height), giving preference to pairs of whiteflies, once insect couples usually stay paired (Byrne; Bellows Junior, 1991).

At 21 days after infestation (DAI) (corresponding to 36 days after emergence), three leaflets were removed from each plant (upper, middle and lower extract), which were taken to the laboratory to count the number of eggs and nymphs, with the aid of a stereoscopic microscope (magnification of 40 x). The plants remained individualized in the cages and kept in a greenhouse for the continuity of the cycle and subsequent evaluation of crop productivity components.

A completely randomized design was adopted in a 2 × 4 factorial scheme [2 soybean stages (V2 and V4) or 2 soybean genotypes (Conquista and IAC 17) × 4 treatments: 1- plants from seeds treated with thiamethoxam and infested, 2- plants from seeds treated with cyantraniliprole and infested, 3- plants from seeds treated with annonin and infested, 4- plants from untreated seeds and infested]. A fifth treatment was conducted as a control (plants from untreated plants and without insect infestation), but for this first step, this data was not included on the statistical analysis for having null variance. However, for productivity parameters (2.4 item), the control treatment was included in the factorial scheme 2 × 5 (2 soybean stages × 5 treatments). For each treatment level, we used 8 repetitions, with each cage containing a plant representing one repetition (n = 8).

1.2.4 Determination of the level of infestation and effects on crop productivity

After the evaluations carried out in the previous test (2.3), plants were kept in a greenhouse and were monitored until the end of the cycle, aiming to observe the reached level of infestation by the insect and its effects on productivity components. At approximately 65 DAI (plants in R2-R3), colonization by whitefly nymphs was estimated, using a visual scale of scores from 1 to 5, with: score 1 = leaf with few nymphs; up to grade 5 = leaf completely colonized by nymphs (BALDIN *et al.*, 2017). To this end, 3 leaflets were removed from the middle third of each genotype, and the final grade of the plot resulted from the mean of the leaflets, obtained by 3 different evaluators (VALLE; LOURENÇÃO, 2002; COELHO *et al.*, 2009). The infestation level was analyzed with the following productivity parameters to be determined: number of pods per plant, number of grains per pod and mean grain weight. The experimental design followed the same scheme described previously.

1.2.5 Effects on *B. tabaci* MEAM1 colonization in soybeans treated with thiamethoxam, cyantraniliprole and annonin-based botanical insecticide in resistant and susceptible genotypes

Based on the results of previous tests (2.3 and 2.4), soybean plants at V2 stage of the Conquista and IAC 17 genotypes were used, originating from seeds treated or not with thiamethoxam, cyantraniliprole and an annonin-based botanical insecticide. The plants were individualized in metal cages (35 cm in diameter × 55 cm in height), covered with voile fabric. The plants were infested with 25 pairs of whiteflies (individuals from colony), which were collected using a mouth aspirator (4 cm in diameter × 11 cm in height), giving preference to whitefly pairs, and the same procedures carried out in items 2.3 and 2.4 were followed.

1.2.6 Colorimetric analysis of leaves

In order to evaluate the colorimetry, three additional plants were planted on the colonization test (2.5 item) for each genotype. To this end, two detached leaflets of each plant (6 in total for each genotype) were stored in plastic containers containing moistened cotton and paper towels to prevent leaf degradation. Subsequently, the color evaluation of the genotypes was carried out in 3 distinct regions of each leaflet, through reflectance in the CIELab color space (Minolta Colorimeter, CR 300), which determines the parameters L* (brightness), a* (green intensity) and b * (yellow intensity). The value of L* can range from 0 to 100, where 0 is black and white is 100. The value of a* is represented by positive numbers when the object is red, and by negative numbers when the object is green. The value of b* is positive when the object is yellow and negative when it is blue (Takatsui *et al.*, 2012).

1.2.7 Trichome analysis

To complement the observations from the studies described previously, quantification of trichomes in soybean plants at stages V2 and V4 were carried out for both genotypes (Conquista and IAC 17).

To quantify the density of trichomes, two distinct areas of 1 cm^2 in the middle of the leaflet were analyzed on the abaxial surface of each leaflet evaluated (lower and upper). Trichome counting was carried out using a transparent mold and a

stereoscopic microscope at 40 × magnification (Channarayappa *et al.*, 1992; Glas *et al.*, 2012).

1.2.8 Statistical analysis

For data analysis, normality of residues with the Shapiro-Wilk test (Shapiro; Wilk, 1965) and the homogeneity of variances with the Bartlett test (Bartlett, 1937) were firstly assessed. When the assumptions were satisfied, the data were subjected to the analysis of variance (ANOVA), and the means were compared by the Tukey test ($p < 0.05$), using the PROC GLIMMIX procedure in SAS 9.4.

1.3 RESULTS

Significant interaction was observed between seed treatments × soybean genotypes when considering the number of eggs in all leaflets evaluated [upper ($F = 45.8$; $df = 3$; $p < 0.0001$), middle ($F = 4.91$; $df = 3$; $p = 0.0043$), and lower ($F = 0.15$; $df = 3$; $p = 0.093$) (Table 2). For Conquista genotype, untreated plants presented the highest number of eggs in lower and middle leaflets and did not differ statistically from annonin-based botanical insecticide. In upper leaflet, untreated plants kept the highest number of eggs, but not differing from treatment constituted by thiamethoxam (Table 2). Conversely, for IAC 17 genotype, cyantraniliprole showed a significative difference from no seed treatment, with the lowest average number of eggs in upper and middle leaflets.

Considering the differences between genotypes, in the lower leaflet, Conquista genotype presenting a higher number of eggs than in genotype IAC 17 in the cyantraniliprole treatment. In the middle and upper leaflets, plants treated with annonin and untreated plants showed significant differences between genotypes.

For nymphs, there was no significant interaction observed among treatments and soybean genotypes in lower ($F = 2.01$; $df = 3$; $p = 0.123$) and upper leaflets ($F = 1.51$; $df = 3$; $p = 0.2222$) (Table 2). Significant interaction was observed between seed treatments × soybean genotypes when considering the number of nymphs in middle leaflets ($F = 0.06$; $df = 3$; $p = 0.06$). In middle leaflets, the lowest number of nymphs was recorded in plants treated with thiamethoxam in Conquista genotype, which

differed statistically from the other treatments and in upper leaflets, there was no difference among treatments within each genotype. However, there was a reduction in the number of nymphs in untreated plants in IAC 17 genotype, when compared to Conquista genotype.

Table 2. Mean (± SE) of eggs and nymphs of *Bemisia tabaci* MEAM1 on leaflets of two soybean genotypes submitted to different seed treatments (21 days after infestation).

UPPER LEAFLET	**EGGS[1]**		**NYMPHS[1]**	
Treatment	**CONQUISTA**	**IAC 17**	**CONQUISTA**	**IAC 17**
Cyantraniliprole	29.25 ± 1.58 cA	27.63 ± 13.76 cA	51.88 ± 36.09	1.38 ± 1.24 aA
Thiamethoxam	95.63 ± 5.00 aA	79.50 ± 5.67 bcA	32.38 ± 14.74	12.00 ± 6.12 aA
Annonin	56.63 ± 5.53 bB	117.50 ± 14.59 bA	13.00 ± 6.51	6.00 ± 3.17 aA
No seed treatment	97.75 ± 6.89 aB	271.75 ± 50.83 aA	70.13 ± 23.69	0.25 ± 0.25 aB
Genotypes (G)	*F* = 72.11; df = 1; p < 0.0001		*F* = 10.03; df = 1; p = 0.0025	
Treatments (T)	*F* = 102.70; df = 3; p < 0.0001		*F* = 0.85; df = 3; p = 0.4734	
Interaction (G x T)	*F* = 45.8; df = 3; p < 0.0001		*F* = 1.51; df = 3; p = 0.2221	
MIDDLE LEAFLET				
Cyantraniliprole	16.88 ± 2.40 bA	15.13 ± 2.20 bA	163.88 ± 37.94 abA	72.75 ± 15.24 aB
Thiamethoxam	27.63 ± 2.48 bA	34.50 ± 4.16 abA	92.00 ± 12.45 bA	89.63 ± 12.53 aA
Annonin	77.25 ± 10.66 aA	45.00 ± 7.32 aB	222.38 ± 31.74 aA	78.50 ± 15.54 aB
No seed treatment	89.5 ± 9.72 aA	56.50 ± 7.76 aB	169.75 ± 44.12 abA	124.38 ± 22.69 aA
Genotypes (G)	*F* = 9.74; df = 1; p = 0.0029		*F* = 14.04; df = 1; p = 0.0004	
Treatments (T)	*F* = 30.60; df = 3; p <0.0001		*F* = 2.19; df = 3; p = 0.0995	
Interaction (G x T)	*F* = 4.91; df = 3; p = 0.0043		*F* = 0.06; df = 3; p = 0.0614	
LOWER LEAFLET				
Cyantraniliprole	6.63 ± 1.07 bA	1.88 ± 1.61 aB	112.75 ± 35.79 aA	78.00 ± 21.76 aA
Thiamethoxam	7.00 ± 2.93 bA	15.50 ± 5.95 aA	129.75 ± 25.25 aA	267.63 ± 75.51 aA
Annonin	35.38 ± 6.83 abA	29.63 ± 11.25 abA	151.63 ± 50.81 aA	147.75 ± 17.60 aA
No seed treatment	43.00 ± 13.43 aA	46.25 ± 29.24 bA	112.38 ± 22.27 aA	268.25 ± 85.23 aA
Genotypes (G)	*F* = 0.08; df = 1; p = 0.9720		*F* = 3.47; df = 1; p = 0.0677	
Treatments (T)	*F* = 4.45; df = 3; p = 0.0071		*F* = 1.90; df = 3; p = 0.1395	
Interaction (G x T)	*F* = 0.15; df = 3; p = 0.9315		*F* = 2.01; df = 3; p = 0.1230	

[1]Data (mean ± SE) followed by the same letters, lowercase to compare the different treatments within each soybean genotype and plant part and uppercase letters to compare soybean genotypes within each seed treatment and plant part, do not differ significantly by the Tukey's LSD test (p > 0.05).

The nymphal colonization rating scale present significant differences among treatments in Conquista genotype (F = 8.49; df = 1; p = 0.0052). Seed treatment with

thiamethoxam resulted in a lower number of nymphs per leaflet, with the lowest scale between treatments (Table 3). Although untreated plants demonstrated a tendency of the highest number of nymphs, it did not differ from cyantraniliprole and annonin-based botanical insecticide. The same tendency was observed for genotype IAC 17; however, there was no difference between treatments.

Table 3. Mean (±SE) of visual scale[1] used to assess the nymphal colonization of *Bemisia tabaci* MEAM1 at 65 days after infestation (DAI) of two soybean genotypes in a greenhouse trial.

TREATMENT	**CONQUISTA[2]**	**IAC 17[2]**
Cyantraniliprole	1.96 ± 0.31 aA	2.45 ± 0.46 aA
Thiamethoxam	1.03 ± 0.12 bB	1.92 ± 0.29 aA
Annonin	1.58 ± 0.27 abA	1.92 ± 0.28 aA
No seed treatment	2.00 ± 0.21 aB	2.63 ± 0.19 aA
Genotype (G)	F = 8.49; df = 1; p = 0.0052	
Treatment (T)	F = 2.93; df = 4; p = 0.0289	
Interaction (T x G)	F = 0.32; df = 3, 55; p = 0.8110	

[1] Visual scale of colonization of up 1 to five, where the initial score 1 was a leaflet with a small nymphs and exuviae and reached the score 5 when a leaflet was totally colonized by nymphs and exuviae. The scores 2 to 4 represented a gradual increase in the colonization by nymphs, as demonstrated to Baldin *et al.* (2017).

[2] Data followed by the same letters, lowercase to compare the different treatments within each soybean genotype and uppercase letters to compare soybean genotypes within each seed treatment, do not differ significantly by the Tukey's LSD test (p > 0.05).

According to the crop yield components data, there was no significant difference between treatments in the IAC 17 genotype for all parameters, with the exception of the number of empty pods/plant, showing that the highest number was obtained by the thiamethoxam treatment, which did not differ statistically from cyantraniliprole but differed from annonin-based botanical insecticide and untreated plants (Table 4). For the Conquista genotype, the highest number of pods was obtained in the cyantraniliprole treatment, that differed from thiamethoxam, untreated and non-infested plants and annonin. The highest number of empty pods was also obtained by plants treated with cyantraniliprole, followed by thiamethoxam and both differed from

annonin. The highest number of grains per plant, total grain weight and average grain weight were observed in plants treated with cyantraniliprole. On the other hand, annonin had the lowest average for these parameters.

Table 4. Means (± SE) of productive parameters of two soybean genotypes submitted to different seed treatments and infestation of *Bemisia tabaci* MEAM1.

	nº pods/plant		nº empty pods/ plant		nº total of grains	
Treatments	**Conquista**	**IAC 17**	**Conquista**	**IAC 17**	**Conquista**	**IAC 17**
Cyantraniliprole	16.38 ± 2.28 aA	17.88 ± 1.77 aA	10.38 ± 0.925 aA	5.63 ± 0.703 abB	32.00 ± 4.44 aA	38.75 ± 4.65 aA
Thiamethoxam	7.25 ± 1.33 bcB	23.38 ± 2.45 aA	7.50 ± 0.732 abA	6.88 ± 0.944 aA	14.75 ± 2.81 bcB	49.00 ± 5.20 aA
Annonin	2.13 ± 0.72 cB	15.75± 3.70 aA	3.88 ± 0.915 cA	3.88 ± 0.854 bA	4.00 ± 1.51 cB	33.00 ± 7.71 aA
No seed treatment	13.25 ± 2.46 abA	16.50 ± 1.80 aA	6.00 ± 0.707 bcA	3.00 ± 0.50 bB	26.63 ± 4.71 abA	30.75 ± 4.77 aA
Control	6.75 ± 2.58 bcA	14.00 ± 4.04 aA	5.63 ± 0.844 bcA	3.88 ± 0.515 bA	14.63 ± 5.97 bcA	29.75 ± 9.08 aA
Genotypes (G)	*F* = 27.85; df = 1; p < 0.0001		*F* = 14.39; df = 1; p = 0.0003		*F* = 3.65; df = 1; p = <0.0001	
Treatments (T)	*F* = 3.89; df = 5; p = 0.0065		*F* = 11.81; df = 5; p <0.0001		*F* = 2.51; df = 5; p = 0.0184	
Interaction (G x T)	*F* = 3.24; df = 4; p = 0.0169		*F* = 3.49; df = 4; p = 0.0117		*F* = 2.70; df = 4; p = 0.0256	
	nº grain/pod		**Total weight of grains (g)**		**Average grain weight (g)**	
Treatments	**Conquista**	**IAC 17**	**Conquista**	**IAC 17**	**Conquista**	**IAC 17**
Cyantraniliprole	1.95 ± 0.04 aA	2.13 ± 0.12 aA	3.83 ± 0.93 aA	2.67 ± 0.46 aA	0.12 ± 0.01 aA	0.07 ± 0.01 aB
Thiamethoxam	1.78 ± 0.27 aA	2.10 ± 0.04 aA	1.45 ± 0.35 bcB	3.46 ± 0.59 aA	0.08 ± 0.02 abA	0.07 ± 0.01 aA
Annonin	1.38 ± 0.33 aB	2.12 ± 0.09 aA	0.27 ± 0.12 cB	2.31 ± 0.54 aA	0.05 ± 0.01 bA	0.07 ± 0.01 aA
No seed treatment	1.79 ± 0.27 aA	1.83 ± 0.13 aA	2.49 ± 0.54 abA	2.13 ± 0.39 aA	0.08 ± 0.01 abA	0.07 ± 0.01 aA
Control	1.58 ± 0.35 aA	1.81 ± 0.27 aA	1.70 ± 0.80 abcA	2.91 ± 1.18 aA	0.08 ± 0.02 abA	0.07 ± 0.01 aA
Genotypes (G)	*F* = 5.38; df = 1; p = 0.0592		*F* = 3.65; df = 1; p = 0.0602		*F* = 2.37; df = 1; p = 0.1283	
Treatments (T)	*F* = 2.78; df = 5; p = 0.0231		*F* = 2.51; df = 5; p <0.0497		*F* = 1.67; df = 5; p = 0.1659	
Interaction (G x T)	*F* = 0.96; df = 4; p = 0.4332		*F* = 2.70; df = 4; p = 0.0375		*F* = 1.96; df = 4; p = 0.1099	

[1] Data (mean ± SE) followed by the same letters, lowercase to compare the different treatments within soybean genotypes and uppercase letters to compare soybean genotypes within each seed treatment, do not differ significantly by the Tukey's LSD test (P > 0.05).

When plants from Conquista genotype treated with the different insecticides were subjected to *B. tabaci* MEAM1 infestation at different stages of development (V2 and V4), it was possible to observe that annonin stimulated the oviposition at V4 stage in the middle leaflet ($F = 9.61$; $df = 3$; $p < 0.0001$) (Table 5). Regardless of the phenological phase in which the infestation occurred (V2 and V4) and the treatment used (annonin, thiamethoxam, cyantraniliprole, untreated plants), there were no significant differences for the number of nymphs in leaflets in the lower ($F = 0.4$; $df = 3$; $p = 0.756$), middle ($F = 0.89$; $df = 3$; $p = 0.453$) and upper ($F = 0.39$; $df = 3$; $p = 0.762$) plant parts and for the number of eggs in the lower ($F = 0.78$; $df = 3$; $p = 0.50$) and upper leaflets ($F = 1.00$; $df = 3$; $p = 0.399$).

The nymphal colonization rating scale at R2-R3 stage showed no significant differences between the two phenological stages infested. However, when plants were infested in V2, there was a greater number of nymphs in untreated plants when compared to the others ($F = 12.79$; $df = 4$; $p < 0.0001$) (Table 6), with the same tendency at V4 stage.

For Conquista genotype at V2 stage, the highest number of pods was obtained in non-infested plants and in plants whose seeds were treated with thiamethoxam and cyantraniliprole, which differed statistically from the annonin-based treatment and in untreated and infested plants (Table 7). At V4 stage, only plants treated with thiamethoxam produced pods with grains, in addition to the control and these treatments did not differ statistically from each other. Thus, other parameters evaluated [total number of grains, number of grains per pod, total grain weight and average grain weight also followed this pattern (Table 7).

Table 5. Means (± SE) of eggs and nymphs of *Bemisia tabaci* MEAM1 on leaflets from Conquista soybean genotype with two stages of infestation (V2 and V4) and submitted to different seed treatments.

UPPER LEAFLET	**Eggs[1]**		**Nymphs[1]**	
TREATMENT	**V2**	**V4**	**V2**	**V4**
Cyantraniliprole	31.25 ± 7.65	30.38 ± 8.74	47.38 ± 23.32	51.38 ± 19.84
Thiamethoxam	55.00 ± 8.65	45.75 ± 12.68	46.88 ± 28.32	32.75 ± 18.01
Annonin	43.38 ± 25.34	39.38 ± 12.99	26.43 ± 9.34	3.00 ± 0.96
No seed treatment	18.25 ± 4.82	50.63 ± 14.76	17.50 ± 10.61	31.88 ± 19.46
Phenological stage (Ps)	*F* = 0.23; df = 1; p = 0.6304		*F* = 0.10; df = 1; p = 0.7582	
Treatment (T)	*F* = 0.83; df = 3; p = 0.4805		*F* = 1.57; df = 3; p = 0.2072	
Interaction (Ps x T)	*F* = 1.00; df = 3; p = 0.3996		*F* = 0.39; df = 3; p = 0.7629	
MIDDLE LEAFLET				
Cyantraniliprole	30.63 ± 9.85 aA	16.75 ± 6.00 bA	121.75 ± 29.67	128.75 ± 36.28
Thiamethoxam	35.00 ± 5.90 aA	3.25 ± 2.85 bB	215.63 ± 60.53	138.75 ± 43.76
Annonin	11.00 ± 2.15 aB	57.75 ± 14.55 aA	207.75 ± 39.34	139.38 ± 22.42
No seed treatment	13.63 ± 6.90 aA	12.75 ± 5.46 bA	130.63 ± 25.33	167.38 ± 60.54
Phenological stage (Ps)	*F* = 0.00; df = 1; p = 0.9908		*F* = 0.73; df = 1; p = 0.3969	
Treatment (T)	*F* = 2.73; df = 3; p = 0.0523		*F* = 0.66; df = 3; p = 0.5795	
Interaction (Ps x T)	*F* = 9.61; df = 3; p < 0.0001		*F* = 0.89; df = 3; p = 0.4532	
LOWER LEAFLET				
Cyantraniliprole	25.88 ± 14.70	6.25 ± 3.12	94.50 ± 31.54	30.38 ± 9.33
Thiamethoxam	21.25 ± 8.46	30.00 ± 27.89	136.38 ± 27.93	72.00 ± 47.86
Annonin	0.00 ± 0.00	14.25 ± 6.39	97.75 ± 43.77	55.75 ± 22.77
No seed treatment	3.88 ± 2.62	4.63 ± 1.88	73.75 ± 22.68	68.13 ± 25.35
Phenological stage (Ps)	*F* = 0.02; df = 1; p = 0.9026		*F* = 4.01; df = 1; p = 0.0502	
Treatment (T)	*F* = 1.32; df = 3; p = 0.2759		*F* = 0.67; df = 3; p = 0.5718	
Interaction (Ps x T)	*F* = 0,78; df = 3; p = 0.5088		*F* = 0.40; df = 3; p = 0.7567	

[1] Data (mean ± SE) followed by the same letters, lowercase to compare the different treatments within each phenological stages and uppercase letters to compare soybean genotypes within each seed treatment, do not differ significantly by the Tukey's LSD test (p > 0.05)

Table 6. Means (± SE) of visual scale[1] of nymphal colonization of *Bemisia tabaci* MEAM1 on soybean leaflets in two stages of infestation and under different seed treatments at 65 days after infestation (DAI) in a greenhouse trial.

TREATMENT	CONQUISTA V2[2]	CONQUISTA V4[2]
Cyantraniliprole	0.75 ± 0.22 b	0.75 ± 0.14 a
Thiamethoxam	1.33 ± 0.30 b	1.50 ± 0.39 a
Annonin	0.92 ± 0.15 b	1.32 ± 0.43 a
No seed treatment	2.42 ± 0.26 a	2.46 ± 0.28 a
Phenological stage (Ps)	F = 0.60; df = 1; p = 0.4435	
Treatment (T)	F = 12.79; df = 4; p < 0.0001	
Interaction (Ps x T)	F = 0.22; df = 3; p = 0.8854	

[1] Visual scale of colonization of up 1 to five, where the initial score 1 was a leaflet with a small nymphs and exuviae and reached the score 5 when a leaflet was totally colonized by nymphs and exuviae. The scores 2 to 4 represented a gradual increase in the colonization by nymphs, as demonstrated to Baldin *et al.* (2017).

[2] Data followed by the same letters, lowercase to compare the different treatments within each stage of infestation and uppercase letters to compare each seed treatment within different stages of infestation, do not differ significantly by the Tukey's LSD test (p > 0.05)

Table 7. Means (± SP) of productive parameters of soybean plants submitted to different seed treatments and under infestation of *Bemisia tabaci* MEAM1.

	nº pods/plant[1]		nº empty pods/plant[1]		nº total of grains[1]	
TREATMENT	**Conquista V2**	**Conquista V4**	**Conquista V2**	**Conquista V4**	**Conquista V2**	**Conquista V4**
Cyantraniliprole	12.13 ± 4.11 aA	0.00 ± 0.00 bB	7.88 ± 2.29 aA	10.38 ± 3.13 aA	24.75 ±8.46 aA	0.00 ± 0.00 bB
Thiamethoxam	18.63 ± 3.20 aA	16.88 ± 2.84 aA	4.13 ± 1.08 aB	13.63 ± 3.04 aA	37.5 ± 6.50 aA	36.25 ± 6.48 aA
Annonin	0.38 ± 0.38 bA	0.00 ± 0.00 bA	11.75 ± 4.23 aA	15.38 ± 4.21 aA	1.00 ± 1.00 bA	0.00 ± 0.00 bA
No seed treatment	1.25 ± 0.84 bA	0.00 ± 0.00 bA	5.38 ± 2.21 aA	4.65 ± 3.12 aA	2.75 ± 1.84 bA	0.00 ± 0.00 bA
Control	18.38 ± 1.19 a	18.38 ± 1.19 a	3.75 ± 1.00 b	3.75 ± 1.00 b	40.25 ± 3.38 a	40.25 ± 3.38 a
Phenological stage (Ps)	*F* = 6.18; df = 1; p = 0.0153		*F* = 2.04; df = 1; p = 0.0157		*F* = 4.84; df = 1; p = 0.0312	
Treatment (T)	*F* = 41.14; df = 5; p <0.0001		*F* = 3.91; df = 5; p = 0.0064		*F* = 40.04; df = 5; p <0.0001	
Interaction (Ps x T)	*F* = 3.33; df = 4; p = 0.0148		*F* = 1.22; df = 4; p = 0.3094		*F* = 3.04; df = 4; p = 0.0225	
	nº grain/pod		**Total weight of grains (g)**		**Average grain weight (g)**	
	Conquista V2	**Conquista V4**	**Conquista V2**	**Conquista V4**	**Conquista V2**	**Conquista V4**
Cyantraniliprole	1.28 ± 0.38 abcA	0.00 ± 0.00 bB	2.71 ± 1.00 bcA	0.00 ± 0.00 bB	0.07 ± 0.02 bA	0.00 ± 0.00 bA
Thiamethoxam	1.76 ± 0.26 abA	2.11 ± 0.11 aA	5.23 ± 0.92 abA	4.06 ± 0.89 aA	0.124 ± 0.02 aA	0.11 ± 0.14 aA
Annonin	0.33 ± 0.33 cA	0.00 ± 0.00 bA	0.05 ± 0.05 dA	0.00 ± 0.00 bA	0.01 ± 0.01 cA	0.00 ± 0.00 bA
No seed treatment	0.55 ± 0.36 bcA	0.00 ± 0.00 bA	0.18 ± 0.12 cdA	0.00 ± 0.00 bA	0.07 ± 0.07 bcA	0.00 ± 0.00 bA
Control	2.17 ± 0.08 a	2.17 ± 0.08 a	5.77 ± 0.48 a	5.77 ± 0.48 a	0.146 ± 0.01 a	0.146 ± 0.01 a
Phenological stage (Ps)	*F* = 4.87; df = 1; p = 0.036		*F = 5.41;* df = 1; p = 0.023		*F* = 7.52; df = 1; p = 0.0078	
Treatment (T)	*F* = 36.16; df = 5; p <0.0001		*F* = 45.86; df = 5; p <0.0001		*F* = 70.94; df = 5; p <0.0001	
Interaction (Ps x T)	*F* = 3.13; df = 4; p = 0.02		*F* = 2.15; df = 4; p = 0.0837		*F* = 2.77; df = 4; p = 0.034	

[1] Data followed by the same letters, lowercase to compare the different treatments within each phenological stage and uppercase letters to compare soybean genotypes within each seed treatment, do not differ significantly by the Tukey's LSD test (p > 0.05)

The IAC 17 genotype at V2 stage presented the highest luminosity index (L*) (46.19), highest green intensity (-a*) [(-) 20.04] and highest yellow intensity (b*) (30.31), differing from Conquista genotype in different phenological phases (Table 8). On the other hand, Conquista genotype at V4 stage presented the lowest value for each of the variables (L* = 39.28; (-) a* = (-) 15.38 and b* = 22.24), also differing from other treatments (Table 8).

The number of trichomes reduced more than 40% with the development of plants in the Conquista genotype and more than doubled in IAC 17 genotype, within this same period, with significant differences between the variables analyzed (F = 127.97; df = 1; p < 0.0001) (Table 9).

Table 8. Means (± SE) of colorimetric parameters on adaxial surface of leaves from two soybean genotypes at two phenological stages.

	L*[1]	**(-) a*[1]**	**B*[1]**
IAC17 (V2)	46.19 ± 0.39 a	(-) 20.04 ± 0.33 a	30.31 ± 0.83 a
IAC17 (V4)	43.61 ± 0.31 b	(-) 17.90 ± 0.32 b	25.52 ± 0.56 b
Conquista (V2)	42.31 ± 0.79 b	(-) 17.38± 0.45 b	25.46 ± 0.53 b
Conquista (V4)	39.28 ± 0.51c	(-) 15.38 ± 0.40c	22.24 ± 0.81 c
F	29.19	25.86	22.73
Df	3, 20	3, 20	3, 20
P value	<0.001	<0.001	<0.001

L*= luminosity, a* = (-) green, b* = (+) yellow.

[1]Data followed by the same letters within each assessed parameter, do not differ significantly by the Tukey's LSD test (p > 0.05)

Table 9. Means (± SE) of number of trichomes (trichomes per cm²) on abaxial leaf surface of Conquista and IAC 17 soybean genotypes at V2 and V4 phenological stages.

Genotype	Phenological stage	
	V2	V4
Conquista	428.67 ± 24.13 Aa	227.667 ± 19.79 Ba
IAC 17	267.93 ± 16.53 Ab	552.92 ± 24.39 Bb
Genotypes (G)	*F* = 14.62; df = 1; p = 0.0011	
Phenological stages (Ps)	*F* = 3.81; df = 1; p = 0.0652	
Interaction (G × Ps)	*F* = 127.97; df = 1; p <0.0001	

[1]Data followed by the same letters, lowercase to compare the soybean genotypes within each phenological stage and uppercase letters to compare phenological stage within each genotype, do not differ significantly by the Tukey's LSD test (p > 0.05).

1.4 DISCUSSION

The present study demonstrated that the use of cyantraniliprole and thiamethoxam in ST can be a tool for the management of *Bemisia tabaci* MEAM1 in soybean crops. However, when leaflets infested in two phenological stages (V2 and V4) with adults of *B. tabaci* MEAM1 were evaluated at 21 days after infestation, it was observed that, in general, there were no significant differences in the number of eggs and nymphs between the periods of infestation nor between treatments within each infested phenological phase. On the other hand, when a new evaluation was made at R2-R3 stage, plants that received seed treatment and were infested at V2 stage showed lower infestation than the untreated plants and at V4 stage, there was the same tendency, although with no more statistical difference between treatments. A similar result was obtained in a study carried out with cotton plants, offered aphid suppression, and the most prolonged effect was with thiamethoxam, that also restricted the infestation of adults and nymphs of *B. tabaci* MEAM1 on the leaves (Campos, 2022).

Although it has already been reported the long-lasting residual effect of these products when the treatment is directed via soil (in ST or close to the root system) (Chen *et al.*, 2015; Adams *et al.*, 2016; Schmidt-Jeffris; Nault, 2016; Selby *et al.*, 2017; Sánchez-Bayo; Tennekes, 2020), other authors verified that the residues of cyantraniliprole and thiamethoxam were significantly reduced between 12 and 22 days after emergence (DAE) on cotton leaves and, after 32 days of infestation of *B. tabaci*, it was no longer possible to detect residual products present in the leaves (Campos, 2022). It can be the reason that, in our study, the lower productivity was observed in treated plants which were infested at V4 stage when compared to V2 stage, especially in cyantraniliprole treated plants.

When Conquista and IAC 17 genotypes were compared when infestation of *B. tabaci* occurred at V2 stage, a lower number of eggs was observed in the treatments with thiamethoxam and cyantraniliprole. Regarding the number of nymphs, only the middle leaflet in the Conquista genotype showed this difference. When the rating scale evaluation was carried out at 65 DAI, the IAC 17 genotype showed greater colonization by nymphs compared to Conquista. In the Conquista genotype, thiamethoxam presented the lowest number of nymphs and cyantraniliprole was the closest to that obtained with untreated plants.

Some previous studies show variable results in relation to the data obtained here. The IAC 17 genotype was considered one of the least attractive and least preferred for whitefly oviposition, with antixenosis-type resistance when compared to other soybean genotypes (Valle; Lourenção, 2002; Silva *et al.*, 2012; Cruz; Baldin, 2017). In a similar study, Conquista genotype showed an intermediate level of preference for oviposition when compared to the IAC 17 and IAC 19 genotypes (taken as a resistance standard) and the Holambra Stwart genotype (taken as a susceptibility standard) (Vieira *et al.*, 2011). In another experiment, Conquista genotype showed an increase in the cycle of *B. tabaci*, indicating a certain degree of antibiosis, as well as IAC 17 (Silva *et al.*, 2012).

This data could be justified by the result of colorimetric analysis of the leaflets of each genotype, where it was possible to observe that IAC 17 genotype presented greater luminosity, a greater shade of green and a greater shade of yellow compared to Conquista genotype, since some authors observed that *B. tabaci* MEAM1 have a preference for surfaces that reflect yellow, which stimulate colonization behavior on the host plant (Berlinger, 1986; Moreau; Isman 2011), while the green tonality

influences the landing of *B. tabaci* and its orientation (Isaacs *et al.*, 1999). The positive correlation between the intensity of green and colonization by whiteflies has been verified in cotton genotypes, where whiteflies were more attracted to genotypes with higher levels of this color (Prado *et al.* 2015). In eggplant genotypes, the greater intensity of the green color has also been related to the attraction of adults and the preference for whitefly oviposition (Hasanuzzaman *et al.*, 2016). In cabbage genotypes, it was observed that the lower whitefly oviposition was due to low light and green tone (variation from red to green) (Domingos *et al.*, 2018).

The trichome quantification test on leaves was carried out in order to establish possible inferences between number of trichomes and colonization of insects on leaflets, since the type and density of trichomes can play an important role in the resistance of plants to insects and can influence the preference for feeding and/or oviposition of *B. tabaci* MEAM1 (Oriani; Lara, 2000; Oriani *et al.*, 2005, Oriani; Vendramim, 2010). In this way, it would be possible to observe whether there was interference due to physical resistance factors or whether the low feeding activity was caused by the presence of insecticides in the sap. According to Silva *et al.* (2012), the greater quantity of trichomes is related to greater oviposition/colonization of insects on soybean leaves and, thus, we can infer that Conquista genotype could attract more adults of *B. tabaci* in the beginning of development (V2) then IAC-17 (Table 9).

In general, the seed treatment with thiamethoxam was more efficient than the other treatments. This could also be the fact that it is considered a product with a bioactivating effect, acting on the expression of genes responsible for the synthesis and activation of metabolic enzymes, related to plant growth, altering the production of aminoacid precursors of plant hormones and activating reactions such as the expression of proteins (Castro, 2006a), promoting greater vigor and root and shoot development (Castro, 2006b). Tavares *et al.* (2008) observed that soybean seed treatment with thiamethoxam promoted an increase in leaf and root area and greater plant height.

Currently, neonicotinoids are the main target of environmental concerns among insecticide classes (Guedes *et al.*, 2016; Frank; Tooker, 2020) and then, new insecticides, with characteristics more compatible with IPM, are an alternative face to broad-spectrum insecticides, such as neonicotinods (Satphaty *et al.*, 2020). As an example, diamides have good mobility and absorption in the plant, good control efficiency and being considered to have low impact on beneficial and predatory insects

(Mandal, 2012; Singh *et al.*, 2016, Machado *et al.*, 2019; Wang *et al.*, 2019) and according to our study, it proved to be also a viable option for whitefly control.

The seed application of the botanical insecticide based on annonin (acetogenin) did not promote benefits on soybean plants or favor whitefly control. Although there is no studies on the use of *Annona squamosa* L. (Annonaceae) extract in seed treatment, the result obtained was expected, since much higher concentrations of the product were dropped via soil into tomato pots and it was not possible to verify the systemic effect of the product on the control of whiteflies present in leaflets (Soares *et al.*, 2021a). The process of product translocation in plants is complex and poorly understood due to the large number of related variables. Factors such as the variability of plant morphology and physiology, as well as the physical-chemical properties of the molecules, are some of these factors that make understanding difficult (Trapp, 2004) and, therefore, application of the products must be monitored, and foliar spraying may be recommended to complement the application via root.

In general, the results obtained indicated that plants whose seeds were treated with cyantraniliprole and thiamethoxam showed lower infestation of *B. tabaci* MEAM1 when compared to untreated plants, with productivity parameters components closer to those presented by uninfested plants, with emphasis on the treatment consisting of thiamethoxam. Therefore, the treatment of seeds with these two insecticides can be considered a tool for controlling whiteflies within the IPM during the initial phases of soybean cultivation, however, they must be used together with other control tactics to ensure greater efficiency in management and achieve higher levels of crop productivity.

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CHAPTER 2

SOYBEAN SEED TREATMENT WITH INSECTICIDES: EFFECTS ON FEEDING BEHAVIOR AND DEVELOPMENT OF *Bemisia tabaci* MEAM1 AND RESIDUAL CONTENT ANALYSIS

RESUMO

O tratamento de sementes (TS) é uma prática usual entre os produtores de grãos visando prevenir o ataque de pragas iniciais, com reduzido impacto ambiental e destacada seletividade ecológica. A técnica EPG (*Electrical Penetration Graph*) é utilizada para avaliar o comportamento alimentar de insetos sugadores, podendo ser uma ferramenta valiosa para verificar o efeito de inseticidas no manejo de *Bemisia tabaci* MEAM1. Assim, este estudo avaliou os efeitos dos inseticidas sintéticos à base de tiametoxam (Cruiser® 350 FS) e ciantraniliprole (Fortenza® 600 FS) e do inseticida botânico à base de acetogeninas (Anosom® 1 EC, anonina) aplicados via TS, sobre o comportamento alimentar e aspectos biológicos de *B. tabaci* em plantas de soja (cultivares Conquista e IAC 17), nos estádios de desenvolvimento V2 e V4. Os inseticidas em teste diminuíram o número de caminhamentos estiletares nas folhas (onda C), com maior duração dessa fase no genótipo Conquista em estádio V2. Os tratamentos com ciantraniliprole e tiametoxam mostraram, ainda, tendência de redução do número de inserções no floema e da duração de ingestão de seiva do floema (onda E2), enquanto o inseticida à base de anonina aumentou a ingestão em comparação ao controle. Em V2, também foi verificado que os inseticidas sintéticos à base de tiametoxam, ciantraniliprole e o inseticida botânico à base de anonina interferiram na duração do período ninfal. Além disso, tiametoxam e ciantraniliprole diminuíram a viabilidade das ninfas de *B. tabaci* MEAM1. Porém, tais efeitos não foram verificados quando as infestações de *B. tabaci* ocorreram no estádio V4, com exceção para o tiametoxam. Análises cromatográficas indicaram redução significativa nos teores foliares dos inseticidas testados do estádio V2 para o V4, com maior ação residual do tiametoxam em relação ao ciantraniliprole. Em conclusão, o TS com inseticidas à base de ciantraniliprole e tiametoxam afetam negativamente o comportamento alimentar dos adultos e o desenvolvimento de ninfas de *B. tabaci* MEAM1, podendo contribuir para o seu manejo em soja. O composto à base de

acetogeninas, quando aplicado via TS, não apresenta benefícios para o manejo do inseto.

Palavras-chave: mosca-branca; electrical penetration graph; tiametoxam; ciantraniliprole; acetogeninas

ABSTRACT

Seed treatment (ST) is a common practice among grain producers to prevent initial pest attacks, with reduced environmental impact and outstanding ecological selectivity. The EPG (Electrical Penetration Graph) technique is used to evaluate the feeding behavior of sucking insects and can be a valuable tool to verify the effect of insecticides in the management of *Bemisia tabaci* MEAM1. Thus, this study evaluates the effects of synthetic insecticides based on thiamethoxam (Cruiser® 350 FS) and cyantraniliprole (Fortenza® 600 FS) and the botanical insecticide based on acetogenins (Anosom® 1 EC, annonin) applied via (ST), on the feeding behavior of *B. tabaci* on soybean plants of Conquista and IAC 17 genotypes at V2 and V4 stages. The insecticides tested reduced the number of stylet walks (C wave), with a longer duration of this phase in the Conquista genotype at V2 stage. Treatments with cyantraniliprole and thiamethoxam also showed a tendency to reduce the number of insertions in the phloem and the duration of phloem sap ingestion (wave E2), while the annonin-based insecticide increased ingestion compared to the control. At V2 stage, it was also found that the synthetic insecticides based on thiamethoxam, cyantraniliprole and the botanical insecticide based on annonin interfered on the duration of the nymphal period. In addition, thiamethoxam and cyantraniliprole decreased the viability of the nymphs of *B. tabaci* MEAM1. However, these results were not observed when *B. tabaci* infestations occurred at V4 stage, with the exception of thiamethoxam. Chromatographic analyzes indicated a significative reduction in the foliar levels of the insecticides tested from stage V2 to V4, with greater persistence of thiamethoxam in relation to cyantraniliprole. In conclusion, ST with the insecticides based on cyantraniliprole and thiamethoxam negatively affect the feeding behavior of adults and the development of nymphs of *B. tabaci* MEAM1 and may contribute to its the management in soybean. The acetogenin-based compound, when applied via TS, does not present benefits for whitefly management.

Keywords: whitefly; electrical penetration graph; thiamethoxam; cyantraniliprole; acetogenins

2.1 INTRODUCTION

Currently, soybean cultivars have shown good agronomic characteristics, reflecting years of genetic improvement (Ritter *et al.*, 2022). However, the intensification of soybean crops along of mostly of Brazilian regions has been increasing the phytosanitary problems in light of stablished “green bridge”. Among the arthropod pests that threaten soybean production, the whitefly *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae) is classified as the second most widespread and economically important arthropod pest in the world, feeding on 36 genera of plants and with reported resistant populations to 56 different insecticides in 165 countries (Willis, 2017). The increasing occurrence of *B. tabaci* outbreaks in Brazilian soybean fields has been associated with the expansion of planted areas (Arnemann, 2018) and favorable climatic conditions during the soybean growing season [e.g., hot and dry weather] (Sharma *et al.* al., 2013), in addition to the dispersion of *B. tabaci* populations from neighboring crops (Araújo, 2022), resulting in greater damage by the insect and reduced control by insecticides currently used in Brazil (Arnemann *et al.*, 2019).

Bemisia tabaci comprises a complex of cryptic species, currently with more than 40 recognized species (De Barro *et al.* 2011; Brown *et al.* 2023). In Brazil, there are two species of agricultural importance, *B. tabaci* MEAM1, introduced in the 1990s (Lourenção; Nagai, 1994), and *B. tabaci* MED, founded for the first time in the country in 2013 (Barbosa *et al.*, 2015). *Bemisia tabaci* MEAM1 has spread widely throughout the main agricultural regions of Brazil, and currently remains predominant in monocultures such as soybeans, cotton and tomatoes (Fernandes *et al.*, 2024).

Bemisia tabaci can also be found on remaining weeds and on alternative hosts present in native or commercial crop areas (Sacilotto *et al.*, 2023), keeping the whitefly population high in the off-season (Villas-Boas; Castelo-Branco, 2009). One of the reasons for increasing in the whitefly population is the use of non-selective and broad-spectrum insecticides carried out at the beginning of vegetative crop cycle. These insecticides, when applied incorrectly, can cause ecological imbalances, favoring the increase of *B. tabaci* and reducing the population of its natural enemies (Sosa-Gomes; Silva, 2010; Turchen *et al.*, 2016). Therefore, it is important to carry out integrated

whitefly management programs in soybean crops to maintain their population at levels that do not cause economic damage to the crop (Padilha *et al.*, 2021).

Seed treatment (ST) with insecticides is a practice that promote the reduction in the number of insecticide applications after plant emergence, reducing yield costs for the farmers, controlling initial crop pests, preventing loss of the desired stand and ensuring establishment seedlings, in addition to reducing risks for agricultural pesticide applicators and natural enemies of insect pests (Lamichhane, 2020; Vojvodic; Bazok, 2021).

With the advent of Integrated Pest Management (IPM), the possibility of using resistant genotypes associated with other practices can also be a valuable strategy in maintaining insect pest populations below of economic thresholds (Sulistyo; Inayati, 2016). The association of chemical control via ST with resistant genotypes can significantly contribute to the reduce of *B. tabaci* populations throughout the soybean cycle, with the potential to reduce successive applications of synthetic insecticides and their possible deleterious effects.

Considering the importance of soybeans for Brazil and the high potential for damage that whiteflies present to the crop, the importance of the associated use of control practices is highlighted, in line with the IPM precepts. Thus, this study aimed to evaluate the effects of ST with thiamethoxam, cyantraniliprole and an annonin-based botanical insecticide associated with two soybean genotypes on *Bemisia tabaci* MEAM1. For this purpose, we used the Electrical Penetration Graph (EPG) technique for assess the feeding behavior of *B. tabaci* MEAM1 in treated plants. Moreover, we analyzed the residual level of insecticides, the nymphal viability and duration of nymphal period.

2.2 MATERIAL AND METHODS

The study was carried out in the Insect Plant Resistance and Insecticidal Plants Laboratory (LARESPI) of the Plant Protection Department/School of Agriculture, Sao Paulo State University (FCA/UNESP), in Botucatu, SP, Brazil (22°85’09 “S and 48°43’16” W) between 2021 and 2022.

2.2.1 Genotypes and seed treatment

The soybean genotypes used in the experiments, their respective genealogies and criteria for choice (resistance history) are described in Table 1.

Table 1. Soybean genotypes used in the experiments, their respective genealogies and criteria for choice.

Genotypes	Genealogy/ Origin	Historical of resistance	References
Conquista	Lo76-4484 × Numbaíra	Susceptible to *B. tabaci* MEAM1 (previously described as biotype B)	SILVA *et al.* (2012)
IAC 17	D72-9601-1 × IAC 8 (IAC/Campinas)	Antixenosis to *Bemisia tabaci* MEAM1	VALLE; LOURENÇÃO (2002); SILVA *et al.* (2012); CRUZ; BALDIN (2017)

The seeds of the respective materials were treated in the laboratory with a) neonicotinoid insecticide Cruiser® 350 FS (thiamethoxam, 350g.L^{-1}), at a dose of 200 mL 100 kg^{-1} of seeds, b) anthranilamide insecticide Fortenza® 600 FS (cyantraniliprole, 600g.L^{-1}), at a dose of 160 mL 100 kg^{-1} of seeds and c) the botanical insecticide Anosom® 1 EC (based on acetogenins, annonin 10,000 mg L^{-1}), at a dose of 200 mL 100 kg^{-1} of seeds. To ensure homogeneity in the distribution of the insecticides, seeds from each genotype and insecticides were put inside a glass tube with a lid and were shaken manually for 2 minutes.

Plants from treated and untreated seeds were grown in plastic pots (3 L), filled with autoclaved substrate, containing a mixture of soil, sand and farmyard manure in a 1:1:1 (v^{-1} v^{-1} v^{-1}) ratio and fertilized as recommended for the crop (Cantarella *et al.*, 2022). The plants obtained were kept in a greenhouse, free from insect infestation, until the appropriate stage of development for carrying out the bioassays.

2.2.2 Rearing of *B. tabaci* MEAM1

A colony of *B. tabaci* MEAM1 was kept in a greenhouse (2.5 × 2.5 × 2 m), with a roof covered by glass and shade (30%) and closed on the sides with glass. To maintain the insects, collard green plants [*Brassica oleracea* var. *acephala* L. (Brassicaceae)] were offered, kept in plastic pots with a capacity of 2.5 L. The plants were irrigated daily and replaced periodically, as needed, in order to maintain the nutritional quality of the food and also the vigor of whitefly populations. Before and

during the experiment, molecular analyzes were carried out, aiming to confirm the cryptic insect species (Walsh *et al.*, 1991; Simon *et al.*, 1994; De Barro *et al.*, 2011).

2.2.3 Feeding behavior of *Bemisia tabaci* MEAM1 on Conquista soybean treated with thiamethoxam, cyantraniliprole and an annonin-based botanical insecticide

The feeding behavior of *B. tabaci* MEAM1 on Conquista soybean genotype plants treated or not with thiamethoxam, cyantraniliprole and annonin was evaluated under controlled conditions (T= 25 ± 2º C, RH= 70 ± 10% and 12 h of photophase), using the *Electrical Penetration Graph* (EPG) technique. The plants used were grown in 3 L pots, and were evaluated at phenological stages V2 and V4, according to Fehr and Caviness (1977).

Before starting the tests, newly emerged whitefly adults (24 h old) were transferred from the collard green plants to soybean plants, aiming to conditioned the insects on soybean plants for 48 h. After that, the insects were placed inside glass tubes (1 cm in diameter × 3 cm in height) and placed on containers with ice. Under a stereoscopic microscope, the insects were kept at 4º C for 3-5 min in order to reduce their activity and facilitate their attachment (Jiang *et al.*, 2001). Next, the insects were connected to a gold wire (electrode) (≈ 12 µm in diameter × 1-2 cm in length), which was fixed to the pronotum with water-based silver glue. Another copper wire (2 mm in diameter × 10 cm in length), which serves as an electrode for the plant, was inserted into the soil of the pot with the test plant. Both electrodes were connected to a signal amplifier (Giga-8 DC EPG), with a resistance of 109 Ω and an adjustable voltage unit (Diaz-Montano *et al.*, 2007). More detailed descriptions of the use of this technique (Tjallingii, 1988, 1990; Walker, 2000) and adaptations for studies with whiteflies are documented in the literature (Jiang *et al.*, 1999, 2000; Rodríguez-Lopes *et al.*, 2011, 2012; Moreno-Delafuente *et al.*, 2013).

After a one-hour fasting period, the insects were placed on the abaxial side of the leaflets. The monitoring process was carried out inside a Faraday cage, under natural lighting (Tjallingii, 2006). The records were made at the same time on treated and untreated plants.

The main parameters of feeding behavior were evaluated, including the number of potential drops (Pd), number of walking phases (C wave), duration of walking phases, number of xylem phases (G), duration of xylem phases, number of phloem phases (E), duration of phloem phases and duration of phases with no probe (Np)

(Diaz-Montano *et al.*, 2007). EPG waveforms were recorded for 12 h per whitefly. A total of 15 insects were evaluated per treatment and each one was placed on a plant alone. Each insect represented one repetition (15 per treatment) disposed in a completely randomized design. The software PROBE 3.0 (Windows) (Wageningen Agricultural University) was used to characterize the waves.

2.2.4 Interaction between two genotypes associated with seed treatment on the feeding behavior of *Bemisia tabaci* MEAM1

The feeding behavior of *B. tabaci* MEAM1 in soybean genotypes Conquista and IAC 17, at stage V2, treated or not with thiamethoxam, cyantraniliprole and annonin-based botanical insecticide were evaluated under controlled conditions (T= 25 ± 2º C, RH= 70 ± 10% and 12 h of photophase), using the EPG technique and following the same method described previously (item 2.3).

The option to compare genotypes at stage V2 was determined by observing the period of greatest effect of ST on insects (Soares *et al.*, In preparation).

2.2.5 Residual content

To complement the observations from the studies described previously, residual levels of insecticides (thiamethoxam and cyantraniliprole) in soybean plants at stages V2 and V4 were carried out for both genotypes (Conquista and IAC 17).

To carry out this study, five plants were planted for each treatment. All leaves from these plants were collected, processed and analyzed by a system consisting of a high-efficiency liquid chromatograph and a triple quadrupole mass spectrometer (LC-MS/MS), composed of a High-Efficiency Liquid Chromatograph (HPLC, Shimadzu, model Proeminence UFLC, which combines ultra-fast analysis and excellent separation performance), with high reliability of results; equipped with two LC-20AD pumps, SIL-20AC auto-injector, DGU-20A5 degasser, CBM-20A controller system (allows automated operation) and CTO-20AC oven (for column temperature control). Coupled to the HPLC is the 3200 Q TRAP mass spectrometer, triple quadrupole hybrid, where Q1 and Q3 were used as mass filters and Q2 is a collision cell where intact molecules and fragments of Q1 are broken into fragments of smaller masses.

2.2.6 Effect on post-embryonic development

To evaluate the effect of the insecticides applied via ST on post-embryonic development of *B. tabaci* MEAM1, a trial was conducted in a greenhouse.

Plants from treated and untreated seeds were grown in plastic pots, filled with autoclaved substrate, as described previously. The plants obtained were kept in a greenhouse, free from insect infestation, until reaching the appropriate development stage for carrying out the bioassays (V2 or V4).

For each plant, a leaflet was individualized, which was isolated with small cages (15 × 8 cm) made of organdy fabric and infested with 20 insect couples. The infestation was maintained for 6 h, and, after that, the adults were removed. Thirty viable eggs were maintained on each leaflet until the nymphs hatched. During the test, the plants were only irrigated with water, when necessary, in order to supply their water needs.

We carried out one experiment, but for statistical analysis we split the data in two parts, to facilitate comparisons. First, we compared the Conquista genotype in two phenological stages (V2 and V4). After that, we compared the two genotypes (Conquista and IAC 17) at V2 stage, which we observed more activity by the insects. So, a completely randomized design was adopted in a 2 × 4 factorial scheme [2 genotypes (IAC 17 and Conquista) or Conquista genotype in two stages of development (V2 and V4) and 4 treatments: 1- with insect infestation and treated with thiamethoxam, 2- with insect infestation and treated with cyantraniliprole, 3- with insect infestation and treated with annonin-based botanical insecticide, 4- with insect infestation and untreated. Six repetitions were performed for each combination, with each cage containing a plant representing one repetition (total of 6 per treatment).

2.2.7 Statistical analysis

For the analysis of data obtained from greenhouse and laboratory bioassays, normality of residues with the Shapiro-Wilk test (Shapiro; Wilk, 1965) and the homogeneity of variances with the Bartlett test (Bartlett, 1937) were checked. When the assumptions were satisfied, the data were subjected to the analysis of variance (ANOVA), and the means were compared by the Tukey test ($p < 0.05$), using the PROC GLIMMIX procedure in SAS 9.4 (SAS Institute, 2001).

EPG recordings were analyzed during the 12-h recording, and the evaluated parameters were estimated using an Excel spreadsheet described by Sarria *et al.* (2009), which allows the automatic calculation of around 125 variables. To obtain answers regarding the effects of treatments on the feeding behavior of *B. tabaci*

MEAM1, non-sequence variables were selected (wave np, C, pd, E1, E2 and G) and non-sequential variables (mean ± EP) were calculated and compared between treatments, as described by Backus *et al.* (2007): NWEI - number of events of a given wave per insect; WDI - duration of a wave or sequential variable per insect and WDE - wave duration per event. The data were analyzed for normality using the Shapiro-Wilk test and homogeneity using the Levene test (Levene, 1960). Since the data did not follow a normal distribution, the Kruskal-Wallis non-parametric ANOVA was used, followed by a Tukey's test to identify differences between treatments ($p < 0.05$). All data were analyzed using Statistica 7.0 software (Statsoft, 2004).

2.3 RESULTS

Effects of seed treatment on feeding behavior of *Bemisia tabaci* MEAM1

The analysis of non-sequential variables per insect revealed differences in the feeding behavior of whiteflies on plants treated with the different insecticides via ST in the two genotypes evaluated (Table 2, Table 3). In the Conquista soybean genotype (V2 stage), this whitefly showed lower number of stylet walking by each insect (NWEI – C wave; $H = 9.023$; $p = 0.003$) and lower non-probe activity (NWEI – NP wave; $H = 10.134$; $p = 0.02$) in plants treated with thiamethoxam, differing from to untreated plants. However, no significant differences were observed among treatments for the Conquista genotype at stage V4 (NWEI C wave; $H = 2.594$; $p = 0.46$) (Table 2). A significant difference was found among treatments related to wave duration per insect (WDI – wave C), with a shorter duration of stylet walking in untreated plants ($H = 19.160$; $p<0.001$), but with longer durations of no probe ($H = 15,884$; $p = 0.001$) for the Conquista genotype at V2 stage (Table 2).

For the IAC 17 genotype, annonin-based botanical insecticide showed the shortest stylet walking duration, followed by cyantraniliprole (WDI-C, $H = 12.109$; $p = 0.01$), with the shortest no-probe durations for untreated plants (WDI-np, $H = 10.554$; $p = 0.001$) (Table 3). For the non-sequential variables per event (WDE), differences were found among treatments only in two of the non-phloem events (wave C and np). For the duration of event C, whiteflies that fed on plants treated with annonin, thiamethoxam and cyantraniliprole presented the highest means, differing from the control in the Conquista genotype ($H = 21.152$; $p < 0.001$). Regarding the duration of the np event, thiamethoxam presented the highest mean in the Conquista genotype ($H = 8.857$; $p = 0.03$).

Regarding pre-phloem parameters, no differences were observed between treatments in the Conquista genotype at V2 stage (H = 2.849; p = 0.42); V4 (H = 9.386; p = 0.05) and IAC 17 (H = 7.823; p = 0.05), with only the plants treated with annonin-based botanical insecticide differing from the other treatments in terms of the duration of each salivation ingestion (WDI-E2), showing greater mean in the Conquista genotype at stage V2; thiamethoxam and cyantraniliprole, although without differing from the control, presented the shortest ingestion times (H = 10.319; p = 0.02).

For the xylem phase, there were no differences between treatments for the Conquista genotype at V2 (H = 5.616; p = 0.13) and V4 (H = 7.660; p = 0.05) stages (Table 2). For IAC 17, annonin-based botanical insecticide caused the lowest number of insect accesses to the xylem (NWEI-G, H = 10.317; p = 0.02) and the shortest wave duration per insect (WDI-G, H = 9.907; p = 0.02), followed by cyantraniliprole and thiamethoxam.

Table 2. Probing and feeding behavior of *Bemisia tabaci* MEAM1 on Conquista soybean genotype with its seeds treated with thiamethoxam, cyantraniliprole and annonin and infested at V2 and V4 stages.

Treatments	NWEI C		NWEI NP		NWEI PD	
	Conquista V2	**Conquista V4**	**Conquista V2**	**Conquista V4**	**Conquista V2**	**Conquista V4**
Control	125.8 ± 21.00 Aa	44.00 ± 11.21 Ba	120.47 ± 20.87 Aa	35.20 ± 11.57 Ba	16.27 ± 3.14	18.13 ± 5.27
Thiamethoxam	50.33 ± 8.37 Ab	45.60 ± 9.60 Aa	39.67 ± 7.08 Ab	43.20 ± 9.07 Aa	22.33 ± 4.41	26.40 ± 6.83
Cyantraniliprole	87.87 ± 16.08 Aab	59.87 ± 14.60 Aa	70.07 ± 16.60 Aab	54.20 ± 14.85 Aa	43.47 ± 10.90	26.20 ± 8.25
Annonin	76.2 ±12.45 Aab	35.33 ± 10.61 Ba	54.33 ± 11.38 Aab	26.87 ± 7.00 Aa	36.73 ± 22.97	14.80 ± 5.51
Treatment (T)	*F* = 13.18; df = 1; p < 0.0001		*F* = 5.59; df = 1; p = 0.005		*F* = 2.37; df = 1; p = 0.127	
Phenological stage (Ps)	*F* = 2.92; df = 3; p = 0.037		*F* = 3.79; df = 3; p = 0.012		*F* = 1.80; df = 3; p = 0.152	
Interaction (T × Ps)	*F* = 2.86; df = 3; p = 0.04		*F* = 4.52; df = 3; p = 0.005		*F* = 1.49; df = 3; p = 0.221	
Treatments	**WDI C (min)**		**WDI NP (min)**		**WDI PD (min)**	
Control	88.43 ± 14.38 Aa	177.62 ± 35.82 Ba	**357.14** ± 19.54 Aa	554. 19 ± 284.66 Aa	1.96 ± 0.49	5.93 ± 2.78
Thiamethoxam	163.99 ± 29.99 Aab	208.89 ± 44.77 Aa	294.60 ± 36.63 Aab	302.82 ± 31.53 Aa	2.14 ± 0.58	2.51 ± 0.76
Cyantraniliprole	275.32 ± 30.26 Ab	105.01 ± 20.40 Ba	193.54 ± 27.78 Ab	341.19 ± 29.39 Ba	3.25 ± 0.81	3.31 ± 1.17
Annonin	197.24 ± 26.31 Ab	89.70 ± 23.34 Ba	171.77 ± 37.37 Ab	358.37 ± 33.39 Ba	3.58 ± 1.00	5.53 ± 4.14
Treatment (T)	*F* = 2.77; df = 1; p = 0.0598		*F* = 3.06; df = 1; p = 0.049		*F* = 1.28; df = 1; p = 0.2608	
Phenological stage (Ps)	*F* = 1.84; df = 3; p = 0.014		*F* = 1.35; df = 3; p = 0.012		*F* = 0.47; df = 3; p = 0.7038	
Interaction (T × Ps)	*F* = 8.11; df = 3; p < 0.0001		*F* = 0.32; df = 3; p = 0.8120		*F* = 0.42; df = 3; p = 0.7397	
Treatments	**WDE C (min)**		**WDE NP (min)**		**WDE PD (min)**	
Control	0.88 ± 0.17 Aa	8.09 ± 2.54 Ba	4.86 ± 0.88 Aab	34.98 ± 18.07 Aa	0.11 ± 0.02	3.21 ± 2.81
Thiamethoxam	7.40 ± 4.14 Ab	11.84 ± 7.10 Aa	15.28 ± 4.14 Ab	12.40 ± 2.11 Aa	0.08 ± 0.01	0.09 ± 0.02
Cyantraniliprole	6.75 ± 2.73 Ab	2.00 ± 0.48 Aa	8.38 ± 2.23 Aab	73.63 ± 41.26 Aa	0.07 ± 0.00	0.09 ± 0.03
Annonin	2.96 ± 0.51 Ab	24.48 ± 21.35 Aa	7.12 ± 3.16 Aa	96.38 ± 40.13 Aa	0.09 ± 0.01	0.31 ± 0.26
Treatment (T)	*F* = 1.40; df = 1; p = 0.0239		*F* = 8.39; df = 1; p = 0.0045		*F* = 1.31; df = 1; p = 0.2543	
Phenological stage (Ps)	*F* = 0.56; df = 3; p = 0.6411		*F* = 1.28; df = 3; p = 0.2844		*F* = 1.11; df = 3; p = 0.3474	
Interaction (T × Ps)	*F* = 0.82; df = 3; p = 0.4841		*F* = 1.65; df = 3; p = 0.1814		*F* = 1.07; df = 3; p = 0.3657	

[1]Data (mean ± SE) followed by the same letters, lowercase to compare the different treatments within phenological stage and uppercase letters to compare soybean genotypes within each seed treatment, do not differ significantly by the Tukey's LSD test (p > 0.05).

NWEI - number of events of a given wave per insect; WDI - duration of a wave or sequential variable per insect and WDE - wave duration per event.

Table 2... continuation

Treatments	NWEI E1		NWEI E2		NWEI G	
	Conquista V2	Conquista V4	Conquista V2	Conquista V4	Conquista V2	Conquista V4
Control	57.07 ± 27.97	36.87 ± 10.99	50.47 ± 26.44	25.53 ± 8.64	3.6 ± 1.34 Aa	1.73 ± 0.68 Aa
Thiamethoxam	45.40 ± 27.05	8.87 ± 2.16	33.00 ± 25.16	4.60 ± 1.32	3.4 ± 2.22 Aa	1.27 ± 0.60 Aa
Cyantraniliprole	46.33 ± 10.71	56.40 ± 24.98	31.33 ± 8.73	49.33 ± 23.86	5.07 ± 1.27 Aa	0.20 ± 0.10 Ba
Annonin	74.67 ± 20.60	39.20 ± 22.54	74.67 ± 20.60	27.73 ± 19.43	2.07 ± 0.58 Aa	1.80 ± 0.93 Aa
Treatment (T)	*F* = 2.71; df = 1; p = 0.1027		*F* = 2.21; df = 1; p = 0.1398		*F* = 7.49; df = 1; p = 0.0072	
Phenological stage (Ps)	*F* = 1.22; df = 3; p = 0.3063		*F* = 0.95; df = 3; p = 0.4192		*F* = 0.17; df = 3; p = 0.9188	
Interaction (T × Ps)	*F* = 1.02; df = 3; p = 0.3876		*F* = 0.99; df = 3; p = 0.4021		*F* = 1.31; df = 3; p = 0.2748	
Treatments	**WDI E1 (min)**		**WDI E2 (min)**		**WDI G (min)**	
Control	24.10 ± 11.76	56.27 ± 21.83	9.66 ± 5.10 ab	12.23 ± 4.89	1.82 ± 1.05	1.30 ± 0.58
Thiamethoxam	22.65 ± 7.66	9.79 ± 3.06	2.93 ± 1.43 a	1.12 ± 0.49	10.43 ± 8.62	0.74 ± 0.34
Cyantraniliprole	21.35 ± 4.80	19.12 ± 11.68	6.53 ± 2.13 ab	8.49 ± 4.35	2.46 ± 0.55	0.10 ± 0.06
Annonin	39.56 ± 8.01	20.03 ± 10.81	33.22 ± 11.99 b	16.67 ± 14.03	1.10 ± 0.30	5.07 ± 4.50
Treatment (T)	*F* = 0.01; df = 1; p = 0.9408		*F* = 0.43; df = 1; p = 0.5134		*F* = 0.72; df = 1; p = 0.3996	
Phenological stage (Ps)	*F* = 1.68; df = 3; p = 0.1754		*F* = 3.44; df = 3; p = 0.0194		*F* = 0.60; df = 3; p = 0.6146	
Interaction (T × Ps)	*F* = 1.94; df = 3; p = 0.1268		*F* = 0.72; df = 3; p = 0.5431		*F* = 1.25; df = 3; p = 0.2946	
Treatments	**WDE E1 (min)**		**WDE E2 (min)**		**WDE G (min)**	
Control	0.48 ± 0.15	5.44 ± 3.32	0.12 ± 0.03 a	0.78 ± 0.49	0.26 ± 0.11	0.57 ± 0.26
Thiamethoxam	1.61 ± 0.70	0.80 ± 0.18	0.16 ± 0.05 ab	0.19 ± 0.05	0.80 ± 0.41	0.23 ± 0.09
Cyantraniliprole	0.66 ± 0.21	0.28 ± 0.07	0.17 ± 0.04 ab	0.14 ± 0.05	0.49 ± 0.11	0.10 ± 0.06
Annonin	0.67 ± 0.18	0.82 ± 0.53	0.98 ± 0.61 b	0.24 ± 0.08	0.35 ± 0,08	0.68 ± 0.56
Treatment (T)	*F* = 1.19; df = 1; p = 0.2770		*F* = 0.01; df = 1; p = 0.9194		*F* = 0.16; df = 1; p = 0.6945	
Phenological stage (Ps)	*F* = 1.57; df = 3; p = 0.2007		*F* = 1.12; df = 3; p = 0.0344		*F* = 0.28; df = 3; p = 0.8366	
Interaction (T × Ps)	*F* = 12.25; df = 3; p = 0.0860		*F* = 1.92; df = 3; p = 0.1307		*F* = 1.37; df = 3; p = 0.2557	

[1]Data (mean ± SE) followed by the same letters, lowercase to compare the different treatments within phenological stage and uppercase letters to compare soybean genotypes within each seed treatment, do not differ significantly by the Tukey's LSD test (p > 0.05)

NWEI - number of events of a given wave per insect; WDI - duration of a wave or sequential variable per insect and WDE - wave duration per event.

Table 3. Probing and feeding behavior of *Bemisia tabaci* MEAM1 of Conquista and IAC 17 soybean genotypes with their seeds treated with thiamethoxam, cyantraniliprole and annonin at V2 phenological stage.

Treatments	NWEI C		NWEI NP		NWEI PD	
	Conquista	**IAC 17**	**Conquista**	**IAC 17**	**Conquista**	**IAC 17**
Control	125.8 ± 21.00 Aa	56.20 ± 15.50 Ba	120.47 ± 20.87 Aa	36.20 ± 10.61 Ba	16.27 ± 3.14	14.67 ± 5.44
Thiamethoxam	50.33 ± 8.37 Ab	46.40 ± 8.48 Aa	39.67 ± 7.08 Ab	41.13 ± 6.79 Aa	22.33 ± 4.41	30.60 ± 9.87
Cyantraniliprole	87.87 ± 16.08 Aab	49.53 ± 8.22 Aa	70.07 ± 16.60 Aab	40.20 ± 7.68 Aa	43.47 ± 10.90	23.13 ± 6.34
Annonin	76.2 ±12.45 Aab	52.27 ± 5.28 Aa	54.33 ± 11.38 Aab	45.53 ± 4.15 Aa	36.73 ± 22.97	21.07 ± 4.53
Genotypes (G)	*F* = 12.08; df = 1; p = 0.0007		*F* = 12.23; df = 1; p = 0.0007		*F* = 1.77; df = 1; p = 0.1857	
Treatments (T)	*F* = 3.48; df = 3; p = 0.0183		*F* = 3.45; df = 3; p = 0.0191		*F* = 1.90; df = 3; p = 0.1337	
Interaction (G x T)	*F* = 2.12; df = 3; p = 0.1011		*F* = 4.85; df = 3; p = 0.0033		*F* = 1.41; df = 3; p = 0.2425	
Treatments	**WDI C (min)**		**WDI NP (min)**		**WDI PD (min)**	
Control	88.43 ± 14.38 Aa	251.54 ± 36.28 Bab	**357.14** ± 19.54 Aa	140.82 ± 40.18 Ba	1.96 ± 0.49	2.61 ± 0.81
Thiamethoxam	163.99 ± 29.99 Aab	262.22 ± 39.20 Aa	294.60 ± 36.63 Aab	183.02 ± 26.62 Bab	2.14 ± 0.58	3.08 ± 0.98
Cyantraniliprole	275.32 ± 30.26 Ab	136.68 ± 23.82 Bab	193.54 ± 27.78 Ab	252.81 ± 21.06 Aab	3.25 ± 0.81	2.83 ± 0.82
Annonin	197.24 ± 26.31 Ab	132.82 ± 27.70 Ab	171.77 ± 37.37 Ab	299.48 ± 35.26 Bb	3.58 ± 1.00	2.59 ± 0.61
Genotypes (G)	*F* = 0.46; df = 1; p = 0.4994		*F* = 2.00; df = 1; p = 0.1596		*F* = 0.01; df = 1; p = 0.9407	
Treatments (T)	*F* = 1.30; df = 3; p = 0.014		*F* = 0.18; df = 3; p = 0.9080		*F* = 0.44; df = 3; p = 0.7216	
Interaction (G x T)	*F* = 10.60; df = 3; p < 0.0001		*F* = 9.97; df = 3; p < 0.0001		*F* = 0.62; df = 3; p = 0.6004	
Treatments	**WDE C (min)**		**WDE NP (min)**		**WDE PD (min)**	
Control	0.88 ± 0.17 Aa	25.03 ± 14,21 Aa	4.86 ± 0.88 ab	8.55 ± 2.90	0.11 ± 0.02	0.28 ± 0.10
Thiamethoxam	7.40 ± 4.14 Ab	28.64 ± 14.20 Aab	15.28 ± 4.14 b	8.85 ± 2.65	0.08 ± 0.01	0.10 ± 0.01
Cyantraniliprole	6.75 ± 2.73 Ab	2.88 ± 0.46 Ac	8.38 ± 2.23 ab	16.87 ± 6.26	0.07 ± 0.00	0.11 ± 0.22
Annonin	2.96 ± 0.51 Ab	3.50 ± 1.23 Aabc	7.12 ± 3.16 a	7.14 ± 0.96	0.09 ± 0.01	0.11 ± 0.02
Genotypes (G)	*F* = 3.84; df = 1; p = 0.052		*F* = 0.35; df = 1; p = 0.0455		*F* = 4.80; df = 1; p = 0.305	
Treatments (T)	*F* = 1.68; df = 3; p = 0.1745		*F* = 1.67; df = 3; p = 0.1771		*F* = 3.45; df = 3; p = 0.190	
Interaction (G x T)	*F* = 1.76; df = 3; p = 0.1596		*F* = 1.67; df = 3; p = 0.1776		*F* = 1.81; df = 3; p = 0.1490	

[1]Data (mean ± SE) followed by the same letters, lowercase to compare the different treatments within each soybean genotypes and uppercase letters to compare soybean genotypes within each seed treatment, do not differ significantly by the Tukey's LSD test (p > 0.05).

NWEI - number of events of a given wave per insect; WDI - duration of a wave or sequential variable per insect and WDE - wave duration per event.

Table 3. continuation

Treatments	NWEI E1		NWEI E2		NWEI G	
	Conquista	**IAC 17**	**Conquista**	**IAC 17**	**Conquista**	**IAC 17**
Control	57.07 ± 27.97	71.89 ± 20.19	50.47 ± 26.44	52.13 ± 15.56	3.6 ± 1.34 Aa	4.93 ± 1.50 Aa
Thiamethoxam	45.40 ± 27.05	24.53 ± 10.63	33.00 ± 25.16	14.13 ± 6.79	3.4 ± 2.22 Aa	1.53 ± 0.48 Aab
Cyantraniliprole	46.33 ± 10.71	43.60 ± 16.33	31.33 ± 8.73	35.27 ± 14.80	5.07 ± 1.27 Ba	1.27 ± 0.41 Aab
Annonin	74.67 ± 20.60	44.07 ± 15.88	74.67 ± 20.60	33.40 ± 12.73	2.07 ± 0.58 Ba	0.40 ± 0.18 Ab
Genotypes (G)	*F* = 0.94; df = 1; p = 0.3338		*F* = 1.25; df = 1; p = 0.2665		*F* = 2.96; df = 1; p = 0.058	
Treatments (T)	*F* = 1.35; df = 3; p = 0.2619		*F* = 1.32; df = 3; p = 0.2714		*F* = 2.13; df = 3; p = 0.010	
Interaction (G x T)	*F* = 1.02; df = 3; p = 0.3857		*F* = 0.61; df = 3; p = 0.6110		*F* = 1.48; df = 3; p = 0.2242	
Treatments	**WDI E1 (min)**		**WDI E2 (min)**		**WDI G (min)**	
Control	24.10 ± 11.76	57.02 ± 18.05	9.66 ± 5.10 Aab	20.92 ± 7.32 Aa	1.82 ± 1.05 Aa	9.69 ± 6.69 Aa
Thiamethoxam	22.65 ± 7.66	13.54 ± 5.87	2.93 ± 1.43 Aa	17.59 ± 8.94 Aa	10.43 ± 8.62 Aa	0.50 ± 0.12 Aab
Cyantraniliprole	21.35 ± 4.80	34.99 ± 8.13	6.53 ± 2.13 Aab	29.09 ± 7.75 Ba	2.46 ± 0.55 Aa	1.14 ± 0.47 Aab
Annonin	39.56 ± 8.01	22.77 ± 8.80	33.22 ± 11.99 Ab	21.66 ± 9.91 Aa	1.10 ± 0.30 Aa	0.16 ± 0.10 Bb
Genotypes (G)	*F* = 0.51; df = 1; p = 0.4786		*F* = 2.72; df = 1; p = 0.010		*F* = 0.14; df = 1; p = 0.034	
Treatments (T)	*F* = 1.62; df = 3; p = 0.1880		*F* = 1.66; df = 3; p = 0.017		*F* = .83; df = 3; p = 0.048	
Interaction (G x T)	*F* = 2.41; df = 3; p = 0.0703		*F* = 1.71; df = 3; p = 0.1689		*F* = 1.63; df = 3; p = 0.1856	
Treatments	**WDE E1 (min)**		**WDE E2 (min)**		W**DE G (min)**	
Control	0.48 ± 0.15	10.32 ± 8.95	0.12 ± 0.03 Aa	0.26 ± 0.05 Ba	0.26 ± 0.11	7.32 ± 6.77
Thiamethoxam	1.61 ± 0.70	0.71 ± 0.20	0.16 ± 0.05 Aab	0.37 ± 0.13 Aa	0.80 ± 0.41	0.27 ± 0.08
Cyantraniliprole	0.66 ± 0.21	1.92 ± 0.58	0.17 ± 0.04 Aab	1.25 ± 0.44 Ba	0.49 ± 0.11	0.37 ± 0.15
Annonin	0.67 ± 0.18	0.45 ± 0.17	0.98 ± 0.61 Ab	0.38 ± 0.12 Aa	0.35 ± 0,08	0.09 ± 0.05
Genotypes (G)	*F* = 1.15; df = 1; p = 0.2867		*F* = 1.07; df = 1; p = 0.3022		*F* = 0.77; df = 1; p = 0.3928	
Treatments (T)	*F* = 0.91; df = 3; p = 0.4386		*F* = 1.79; df = 3; p = 0.0153		*F* = 0.94; df = 3; p = 0.4239	
Interaction (G x T)	*F* = 1.14; df = 3; p = 0.3352		*F* = 2.87; df = 3; p = 0.0396		*F* = 1.10; df = 3; p = 0.3529	

[1]Data (mean ± SE) followed by the same letters, lowercase to compare the different treatments within each soybean genotypes and uppercase letters to compare soybean genotypes within each seed treatment, do not differ significantly by the Tukey's LSD test (p > 0.05).

Insecticides residual content

Considering the residual of thiamethoxam (F = 9.97; df = 1; p < 0.0061) and cyantraniliprole (F = 5.20; df = 1; p < 0.0366) insecticides, it is possible to observe that both the Conquista and IAC 17 genotypes showed a significant reduction of more than 75% in the thiamethoxam content when comparing stages V2 and V4 (Table 4). Although without statistical differences between the stages, cyantraniliprole showed the same trend.

Effect on post-embryonic development

Regarding the post-embryonic development of the whitefly, it was possible to observe that for Conquista genotype at V4 stage, there were no differences among treatments in the duration of nymphal period (Table 5). At V2 stage, there was a longer duration of the nymphal period of insects that fed on untreated plants, differing from cyantraniliprole and annonin in Conquista genotype and from thiamethoxam and cyantraniliprole in IAC 17 genotype (Table 5). Regarding nymphal viability, the lowest averages were found with thiamethoxam seed treatment, in both genotypes and in the two phenological stages evaluated and with cyantraniliprole at V2 stage in both genotypes (Tables 5 and 6).

Table 4. Residual level (ng/g) in leaflets (V2 and V4 phenological stages) of cyantraniliprole and thiamethoxam applied on seed treatment of Conquista and IAC 17 soybean genotypes.

Genotypes	Cyantraniliprole[1]		Thiamethoxam[1]	
	V2	V4	V2	V4
Conquista	19.68 ± 11.33 Aa	3.43 ± 2.29 Aa	1143.90 ± 331.71 Ab	238.86 ± 96.12 Ba
IAC 17	13.41 ± 5.36 Aa	8.30 ± 3.59 Aa	2523.00 ± 290.14 Aa	190.80 ± 29.71 Ba
Genotype (G)	*F* = 3.29; df = 1; p = 0.0887		*F* = 8.67; df = 1; p = 0.095	
Phenological stage (Ps)	*F* = 1.14; df = 1; p < 0.3013		*F* = 51.29; df = 1; p < 0.0095	
Interaction (G × Ps)	*F* = 5.20; df = 1; p < 0.0366		*F* = 9.97; df = 1; p < 0.0061	

[1]Data (mean ± SE) followed by the same letters, lowercase to compare the soybean genotypes and uppercase letters to compare each phenological stage, do not differ significantly by the Tukey's LSD test (p > 0.05).

Table 5. Means (± SE) of nymphal period duration and nymphal viability of *Bemisia tabaci* MEAM1 on Conquista soybean genotype treated with different insecticides via seed treatment, at V2 and V4 phenological stages.

Treatment	Nymphal period (days)		Nymphal viability (%)	
	V2	V4	V2	V4
Control	12.28 ± 0.66 Aa	10.58 ± 0.27 Ba	97.22 ± 1.34 Aa	95.78 ± 3.69 Aa
Thiamethoxam	11.62 ± 0.13 Bab	10.39 ± 0.41 Aa	82.22 ± 2.05 Ab	86.11 ± 4.07 Ab
Cyantraniliprole	10.02 ± 0.17 Ac	11.36 ± 0.45 Ba	85.56 ± 4.36 Bb	98.5 ± 1.03 Aa
Annonin	10.74 ± 0.26 Abc	11.00 ± 0.27 Aa	87.22 ± 3.15 Aa	92.22 ± 1.86 Aa
Treatments (T)	*F* = 1.50; df = 1; p = 0.2282		*F* = 10.53; df = 1; p = <0.001	
Phenological stages (Ps)	*F* = 1.64; df = 3; p = 0.2074		*F* = 1.68; df = 3; p = 0.2026	
Interaction (T × Ps)	*F* = 7.35; df = 3; p = 0.0005		*F* = 4.95; df = 3; p = 0.0052	

[1]Data followed by the same letters, lowercase to compare seed treatments and uppercase letters to compare each phenological stage (individually by insect parameter), do not differ significantly by the Tukey's LSD test ($p > 0.05$).

Table 6. Means (± SE) of nymphal period duration and nymphal viability of *B. tabaci* MEAM1 on Conquista and IAC 17 soybean genotypes at V2 stage treated with different insecticides via seed treatment.

Treatment	Nymphal period (days)		Nymphal viability (%)	
	Conquista	IAC 17	Conquista	IAC 17
Control	12.28 ± 0.66 Aa	12.63 ± 0.52 Aa	97.22 ± 1.34 Aa	91.11 ± 3.30 Aa
Thiamethoxam	11.62 ± 0.13 Aab	10.75 ± 0.52 Ab	82.22 ± 2.05 Ab	80.00 ± 4.39 Ab
Cyantraniliprole	10.02 ± 0.17 Ac	10.44 ± 0.09 Ab	85.56 ± 4.36 Ab	72.22 ± 1.86 Ab
Annonin	10.74 ± 0.26 Abc	11.39 ± 0.23 Aab	87.22 ± 3.15 Aa	90.56 ± 3.59 Aa
Treatments (T)	$F = 0.28$; df = 1; $p = 0.5978$		$F = 9.24$; df = 1; $p = 0.0042$	
Phenological stages (Ps)	$F = 11.78$; df = 3; $p < 0.0001$		$F = 14.46$; df = 3; $p < 0.0001$	
Interaction (T × Ps)	$F = 1.66$; df = 3; $p = 0.1918$		$F = 3.91$; df = 3; $p = 0.0153$	

[1]Data followed by the same letters, lowercase to compare seed treatments and uppercase letters to compare soybean genotypes within each treatment, do not differ significantly by the Tukey's LSD test ($p > 0.05$).

2.4 DISCUSSION

The analysis of the feeding behavior of *B. tabaci* MEAM1 revealed that the insecticides cyantraniliprole and thiamethoxam, in addition to annonin-based botanical insecticide, reduced the number of events in the intracellular stylet walking phase (C). However, with long periods in this activity, also with shorter periods of no-probe (np) when compared with untreated plants for the Conquista genotype at V2 stage. Additionally, thiamethoxam and cyantraniliprole showed a tendency in reducing the duration in the phloem sap ingestion phase (E2) by target insect, with more significant values for thiamethoxam.

Insects that reached the E1 phase, there was a reduction of 25 to 30% of those that reached the E2 phase in treated plants. These effects can be attributed to the rapid solubility, translocation of insecticides within plants and their effect on insects (Selby *et al.*, 2013; Barry *et al.*, 2015; Selby *et al.*, 2017). Several studies have demonstrated that during insect salivation in the phloem (E1) some types of viruses

can be inoculated into the plant, and through ingestion of phloem sap (E2), viruses are acquired by insects (Jiang *et al.*, 2000; He *et al.*, 2011; Civolani *et al.*, 2014; Maluta *et al.*, 2021). This result present significant findings to different crops that are greatly affected by viruses transmitted by the *B. tabaci* complex, including soybeans (Inoue-Nagata *et al.*, 2016). Civolani *et al.* (2014) observed that plants treated with cyantraniliprole via soil negatively interfered in the feeding behavior of *B. tabaci* MED in tomato, where the insects were able to reach the phloem, but in smaller quantities than the control, in accordance with the results obtained in this study.

It is important to highlight that, in the case of insect vectors such as *B. tabaci* MEAM1, the effect of reducing the ingestion of phloem sap by insects may minimize the acquisition of viruses and, consequently, reduce their high rates of transmission to plants (Civolani *et al.*, 2014; Maluta *et al.*, 2021). The reduction in transmission efficiency depends mainly on how much the active ingredient acts to prevent any penetration into the phloem and, above all, to prevent the repetition of this behavior for long periods before the death of the insect (Jiang *et al.*, 2000; Maluta *et al.*, 2021).

In our study, waves of the phloem phase (including E1 and E2) were verified by *B. tabaci* MEAM1, indicating that insects need to taste and feed on the treated plants, even if for shorter periods, as observed for cyantraniliprole and thiamethoxam, with emphasis on thiamethoxam in Conquista genotype at V2 stage. Although there were no significant differences between untreated and treated plants, the IAC 17 genotype also showed a tendency towards shorter phloem phase duration when treat with thiamethoxam.

Some possible explanations for these reductions in insect feeding activities include the fact that neonicotinoids not only interfere with the central nervous system of insects but can also cause injury to midgut cells and epidermal tissues (Wang *et al.*, 2015). These effects result in a reduction in the ability to coordinate the stylet, the pharyngeal valve, or dilator muscles (Jeschke, nauen, 2008, Cui *et al.*, 2010), thus influencing the digestion and transport of food (Dow, 1986; Terra, 1988; Dittbrenner *et al.*, 2011). On the other hand, annonin-based insecticide stimulated the insects to ingest the phloem sap, maybe for having some compound that favor the insect feeding, but this inference should be investigated.

The residual analysis of thiamethoxam and cyantraniliprole insecticides proves their lesser effect when the treated plants are infested at V4 stage, showing a decrease in the concentration of active ingredients with plant development. These results were

also observed in cotton crops, where cyantraniliprole and thiamethoxam residues were significantly reduced between 12 and 22 days after emergence (Campos, 2022). The author also verified that after 32 days of *B. tabaci* infestation, it was no possible to detect residuals of these insecticides in the leaves.

Regarding the nymphal period duration of the whitefly, it was possible to observe that it was longer to insects that fed on untreated plants, differing from the other treatments for both Conquista and IAC 17 genotypes at V2 stage, showing that the treatments interfered on the insect cycle. For the Conquista genotype at stage V4, there were no differences between treatments, possibly due to the low residual of the products present in the soybean sap. The lowest means of nymphal viability were found with the cyantraniliprole and thiamethoxan treatments, in both genotypes at V2 stage and also for the Conquista genotype at V4 stage for thiamethoxam, confirming and corroborating the results obtained in our previous tests.

In conclusion, the seed treatment is an important practice to farmers, being one more strategy to protect plants, altering insect cycle and reducing nymphal viability of *B. tabaci* MEAM1. Our results also showed that insecticide residuals reduce considerably even at the initial stages of plant development and, therefore, other practices within the IPM must be adopted throughout the cycle to ensure greater crop productivity. Moreover, previous studies (Li *et al.* 2011, Caballero *et al.* 2013) showed that cyantraniliprole does not present cross-resistance with neonicotinoids, which makes them important tools within integrated resistance and integrated pest management programs.

The EPG technique is an important tool for understanding the feeding behavior of insects and, in this case, it made us infer that the use of thiamethoxam and cyantraniliprole reduce the probability of acquiring viral transmission by reducing insect access to the phloem, proving the importance for crops through the correct management of insecticides.

Regarding the anonin, it was possible to conclude that this molecule does not have efficacy in controlling *B. tabaci* when was applied via seed treatment.

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CHAPTER 3

EFFICACY OF ETHANOLIC SEED EXTRACTS OF *Annona* spp. AGAINST *Aphis glycines*[1]

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RESUMO

O pulgão-da-soja, *Aphis glycines* Matsumura (Hemiptera: Aphididae), é uma importante praga que pode causar danos econômicos significativos à soja. Atualmente, os inseticidas sintéticos são empregados como principal estratégia de manejo do inseto; no entanto, o uso excessivo destes produtos tem estimulado o desenvolvimento de métodos alternativos de manejo, incluindo o uso de derivados botânicos. Neste estudo, a bioatividade dos extratos de sementes de *Annona mucosa* e *A. muricata* e de uma formulação à base de acetogeninas (Anosom® 1 EC, 10.000 $mg.L^{-1}$) em comparação ao inseticida piretroide lambda-cialotrina (Warrior® II) em *A. glycines* foi avaliada. Inicialmente, o efeito inseticida foi avaliado em laboratório e as curvas de concentração-resposta foram estimadas (CL_{50} / CL_{90} para *A. muricata* 305,721 $mg.L^{-1}$ / 1290,3 $mg.L^{-1}$ e *A. mucosa* 134,229 $mg.L^{-1}$ / 487.890 $mg.L^{-1}$, respectivamente). No bioensaio de efeito sistêmico foram utilizados os mesmos extratos (100, 500, 1.000, 5.000 e 10.000 $mg.L^{-1}$), mostrando redução no número de insetos nos pecíolos. Ambos os extratos (CL_{50} e CL_{90}) foram utilizados em teste de comportamento, indicando efeito repelente, enquanto que Warrior® II foi atrativo para os insetos.

[1]Capítulo redigido de acordo com as normas do periódico **Crop Protection**

As CL's 90 dos extratos também foram utilizadas no ensaio de tempo letal (TL) e no ensaio de casa de vegetação. A TL_{50} para os extratos de *A. mucosa* e *A. muricata* foi em torno de 20 h. Todos os tratamentos apresentaram taxas de mortalidade de insetos acima de 65%, chegando a 100% para Warrior® II, seguido pela CL_{90} de *A. mucosa* (92,1%). Os resultados demonstram o potencial dos derivados de *Annona* spp. para o manejo de *A. glicines* na soja, e esses extratos derivados de sementes de Annonaceae podem servir como uma ferramenta adicional no MIP para melhorar as estratégias de controle de *A. glicines* em sistemas de cultivo orgânico.

Palavras-chave: derivados botânicos, aleloquímicos, pulgão-da-soja, acetogeninas.

ABSTRACT

Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is an important pest that can cause significant economic damage to soybean. Synthetic insecticides are currently employed as the main management strategy to control *A. glycines*; however, over-use of these products has stimulated research in the development of alternative management methods, including botanical derivatives. In this study, we evaluated the bioactivity *Annona mucosa* and *A. muricata* seed extracts and a formulation based on acetogenins (Anosom® 1EC, 10,000 $mg.L^{-1}$) compared to the pyrethroid insecticide lambda-cyhalothrin (Warrior II®) on *A. glycines*. Initially, the insecticidal effect was assessed in vial bioassays and the concentration-response curves were estimated (LC_{50} / LC_{90} for *A. muricata* 305.721 $mg.L^{-1}$ / 1290.3 $mg.L^{-1}$ and *A. mucosa* 134.229 $mg.L^{-1}$ / 487.890 $mg.L^{-1}$, respectively). In the systemic effect bioassay, the same extracts were used (at 100, 500, 1,000, 5,000 and 10,000 $mg.L^{-1}$), showing a reduction in the number of insects on the petioles. Both extracts at LC_{50} and LC_{90} were used in a behavior test, indicating that they are repellent and Warrior II® was attractive for the insects. The LC_{90} rates were also used in lethal time (LT) and in greenhouse bioassays. The LT_{50} for *A. mucosa* and *A. muricata* extracts was around 20 h. All treatments showed insect mortality rates above 65%, reaching 100% for Warrior II®, followed by the LC_{90} of *A. mucosa* (92.1%). The results demonstrate the potential of *Annona* spp. derivatives for the management of *A. glycines* in soybean, and these Annonaceae-derived seed extracts could likely serve as an additional IPM tool to improve *A. glycines* control strategies in organic farming systems.

Keywords: botanical derivatives, allelochemicals, soybean aphid, acetogenins.

3.1 Introduction

Soybean [*Glycine max* (L.) Merr.] is one of the most important crops worldwide and top-traded commodities, with several applications, including biofuels and processed products for animal feed and human food (Hartman *et al.*, 2011). The leading soybean producers are Brazil (37.81%), United States of America (28.44%) and Argentina (14.36%) (USDA, 2022). Among the factors that can cause a reduction in soybean productivity, the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphidade), is a major yield limiting pest, mainly in the Upper Midwest of the United States (Hurley and Mitchell, 2017). Since its discovery in the USA in 2000 (Ragsdale *et al.*, 2007), the insect has spread throughout this region and southern Canada (Venette and Ragsdale, 2004). Soybean aphid is a phloem-feeding insect that can reduce soybean yield by up to 40% (Ragsdale *et al.*, 2007). The insect can transmit viruses that cause important diseases to soybean plants, including *Soybean mosaic virus* (SMV) and *Alfalfa mosaic virus* (AMV) (Hill *et al.*, 2001; Domier *et al.*, 2003), causing leaf distortion. Yield is generally reduced by decreasing the number of seeds and reducing pod set (Hartman *et al.*, 2001; Hill *et al.*, 2004).

There are several pest management tools available to manage soybean aphid, such as host-plant resistance and biological control, but insecticide use is the primary management tool and has increased dramatically in response to *A. glycines* infestation since its first record in the US (Ragsdale *et al.*, 2011; Coupe and Capel, 2016). However, the continued use of these products results in selection of insecticide resistance, as already reported for organophosphates (Wang *et al.*, 2011, 2012), neonicotinoids (Ribeiro *et al.*, 2018), and pyrethroids (Koch *et al.*, 2018; Menger *et al.*, 2022; Valmorbida *et al.*, 2022 a, b), in addition to environmental impacts and human health issues.

This scenario has stimulated the search for alternative control methods, as well as increased options for organic soybean production in the US (Hartman *et al.*, 2016), where chemical use is more restricted. The use of botanical insecticides could be a valuable strategy to manage soybean aphids and be more sustainable and secure for humans and the environment (Isman *et al.*, 2011). Ethanolic extracts have demonstrated similar or superior efficacy compared to synthetic insecticides with

important arthropod pests in soybean (Souza *et al.*, 2019), tomato (Baldin *et al.*, 2015; Soares *et al.*, 2021a), citrus (Ribeiro *et al.*, 2015) and other crops.

Among promising plant families with insecticidal properties, Annonaceae has proved to be one of the main sources of bioactive natural compounds (Krinski *et al.*, 2014), particularly after the discovery of acetogenins (ACGs) (Bermejo *et al.*, 2005; Rainer, 2007). ACGs are exclusive to the Annonaceae family, found in mainly in seeds as well as branches and roots (Bermejo *et al.*, 2005; Castillo-Sánchez *et al.*, 2010). ACGs show potent activity on insects by inhibiting the complex I (NADH - ubiquinone oxidoreductase) in the mitochondrial electron transport chain (Degli Esposti *et al.*, 1994; Gallardo *et al.*, 2000). Its potential has been documented for several species of economic importance, such as *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) (Ansante *et al.*, 2015), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) (Ribeiro *et al.*, 2015), *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) (Ribeiro *et al.*, 2014a), *Bemisia tabaci* (Gennadius) MEAM1 (Hemiptera: Aleyrodidae) (Soares *et al.*, 2021 a, b), *Plutella xylostella* L. (Lepidoptera: Plutellidae) (Trindade *et al.*, 2011), and *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Brito *et al.*, 2020).

Faced with the difficulties in managing *A. glycines* and the growing demand for effective control methods that are at the same time less harmful to the environment**,** the objective of this study was to evaluate the bioactivity of ethanolic extracts from seeds of *Annona muricata* and *A. mucosa* compared to a commercial bioinsecticide based on ACGs (Anosom® 1 EC, annonin 10,000 mg.L^{-1}) and a pyrethroid (Warrior II®), assessing both the lethal and sublethal effects of these compounds to *A. glycines* on soybean plants.

3.2 Material and Methods

3.2.1 *Aphis glycines* colony

For this study, we used insects from a colony established from aphids collected on soybean plants during the 2011 crop season at the University of Nebraska Haskell Agricultural Laboratory, Concord, NE and maintained at the Department of Entomology – University of Nebraska-Lincoln, Lincoln, Nebraska, USA.

For the experiments, wingless aphids, consisting in a mix of nymphs (4^{th} instar) and non-alate adults, were used. Soybean aphids were maintained on pesticide-free V2 soybean (Fehr *et al.*, 1971), cv KS4202, in a growth chamber at 25 ± 3°C, 70 ± 5% relative humidity, and photoperiod of 14:10h (L:D). New non-infested seedlings were

supplied every week to maintain a fresh food source for the aphids (Marchi-Werle *et al.*, 2017).

3.2.2 Annonaceae species and preparing plant extracts

Information about the *Annona* species used in the study is shown in Table 1.

Table 1 *Annona* species used in the study: collection data

Species	Used part	Local of collection	Date of collection	Number of Voucher*
A. muricata L.	Seeds	Piracicaba municipality, SP State, Brazil (22º42'25,4''S; 47º37'43,9''W; elevation: 576 m)	16/03/2017	121892
A. mucosa Jacq.	Seeds	Piracicaba municipality, SP State, Brazil (22º42'28,5''S; 47º37'59,6''W; elevation: 534 m)	16/03/2017	120985

* Deposited in the herbarium of the Department of Biological Sciences at "Luiz de Queiroz" College of Agriculture/University of Sao Paulo.

Voucher specimens, previously identified by Prof. Dr. Renato Mello-Silva [Department of Botany, Biosciences Institute/University of São Paulo (BI/USP)], were deposited in the herbarium of the Department of Biological Sciences at "Luiz de Queiroz" College of Agriculture/University of São Paulo, in Piracicaba, Sao Paulo, Brazil.

To obtain the organic extracts, seeds from ripe fruits were dehydrated in an oven with forced air circulation at 40 °C for a period of 72 h. After drying, seeds were ground in a knife mill, and the powder obtained was stored in airtight glass containers and kept in a freezer (-10 °C) until use. The seed powder was subsequently submerged in ethanol [analytical degree (99.5%)] at a 1:5 ratio (seed powder mass (g): volume of solvent (mL)]. The mixture (seed powder + ethanol) was stirred for 10 min and stored for 72 h. Subsequently, solids were removed using filter paper. This procedure was repeated in triplicate. The remaining solvent (ethanol) was then eliminated in a rotary evaporator (temperature: 50 °C; pressure: - 600 mmHg), and the extract yield was determined.

3.2.3 Commercial Insecticides

As positive controls, the synthetic pyrethroid lambda-cyhalothrin (Warrior II®, Syngenta, US) and a commercial ethanolic extract based on acetogenin: annonin 10,000 mg.L^{-1} (Anosom® 1 CE, AgriLife, Hyderabad, India) were used.

3.2.4 Plant Material

Five soybean seeds (cv KS 4202) were sown in potting media (34% peat, 31% perlite, 31% vermiculite and 4% soil mix) in 1 L round plastic pot. Plants were grown in a greenhouse under 400W high-intensity lamps, 23 ± 3°C, 60 ± 10% RH, and a photoperiod of 16:8 L:D. The V2 plants served as host plants for the aphid colonies, and were replaced weekly, whereas V3–V4 were used for the systemic detached leaf and greenhouse bioassays.

3.2.5 Screening - Vial Bioassay

Six treatments were evaluated: ethanolic seed extracts of *A. mucosa* at 500, 1,000 and 5,000 mg.L^{-1} and *A. muricata* at 500, 1,000 and 5,000 mg.L^{-1}, in addition to negative (deionized water and solvent solution water:acetone 1:1 (v/v)) and positive controls (Anosom® 1 EC and Warrior II®, in commercial doses of 2,000 mg.L^{-1} and 1.28 oz/acre, respectively). Ethanolic seed extracts were diluted in acetone solution [water:acetone 1:1 (v/v)], whereas Anosom® 1 EC and Warrior II® were diluted in distilled water. An 0.5 mL-aliquot of each concentration was placed into 20 mL scintillation glass vials.

To provide a uniform coating on the inside of the vial and to optimize the process, vials were dried using a commercial hot dog roller grill (model 8045SXW NEMCO Food Equipment, Hicksville, OH). After complete evaporation, 20 insects were transferred per vial and each vial was covered with a plastic lid, in order to prevent escape of aphids. The vial with 20 wingless aphids was considered a replication per treatment (5 in total, n=100) in a completely randomized design. Mortality was evaluated at 24 hours after infestation (Ribeiro *et al.*, 2018).

3.2.6 Concentration-response curves of ethanolic seed extracts of *A. mucosa* and *A. muricata*

Based on the previous results, the ethanolic seed extracts of *A. mucosa* and *A. muricata* were evaluated again to estimate their LC_{50} and LC_{90}. Five concentrations of

each extract were used in addition to the control (range: 0–5000 mg.L^{-1}) and were defined based on the formula proposed by Finney (1971).

The same experimental procedures described in the screening bioassay were used here; however, four replicates were performed per concentration, with each replicate consisting of a vial with 15 wingless aphids (n=60). Mortality was evaluated 24 hours after infestation.

3.2.7 Lethal time bioassay

Based on the results of the concentration-response curve test, the ethanolic seed extracts of *A. mucosa* and *A. muricata* at LC_{90} were evaluated to estimate the LT_{50} for *A. glycines*. In addition to treatments, negative (deionized water and solvent solution water:acetone 1:1 (v/v)) and positive controls (Anosom® 1 EC and Warrior II®) were used.

The same experimental procedures described in the screening bioassay were used here. However, each vial with 15 wingless aphids was considered a replication (5 in total, n=75) in a completely randomized design. Mortality was evaluated at 1, 3, 6 and 24 h after aphid infestation.

3.2.8 Detached Leaf Bioassay

The petioles of excised V3–V4 soybean trifoliates were immersed in 5 mL glass tubes containing different treatment concentrations. Each glass tube containing a trifoliate was kept individually in a cell tray. The trifoliates were held for ~12 h in the absence of aphid pressure until the trifoliates regained turgidity, thereby assuring proper insecticide uptake.

Fifteen wingless aphids were transferred to the trifoliates using a fine paintbrush (model 00 Connoisseur 367 W-Talklon Round, Beaverton, Oregon). The petioles of the trifoliates containing the insects were kept immersed in the treatment solutions during the experiment.

Each cell of the tray was then sealed with a transparent and porous plastic lid to avoid aphid escape. The numbers of dead and live aphids were recorded after 5 days.

The experiment included eight treatments (ethanolic seed extracts of *A. mucosa* and *A. muricata*, each at 100, 500, 1,000, 10,000 mg.L^{-1}), as well as the negative [deionized water and solvent solution water:acetone 1:1 (v/v)] and positive controls

(Warrior II®). Each cell containing one trifoliate with 15 aphids was considered a replication per treatment (4 in total, n=60) in a completely randomized design.

3.2.9 Repellence bioassay

The bioassay was carried out using transparent plastic petri dishes (15 cm of diameter). A disk of absorbent paper with the same diameter was used to cover the bottom of the petri dish. The paper area was symmetrically divided in two half circles.

The paper disks were treated prior to insertion into the petri dishes. The disks were cut in half, and one half of the circle received the treatment and the other half received only distilled water. Each half circle received 2.5 mL of its respective solution (treatment or water). After complete drying, they were connected with a tape, forming a complete circle again and placed inside the petri dish.

Twenty wingless aphids were then released in the middle of the petri dish, on the taped area (2 cm width, considering a neutral zone with no treatment). The petri dishes were kept closed during the evaluation.

The design was a completely randomized design with ten replications and four treatments (ethanolic seed extracts of *A. mucosa* at LC_{50} and LC_{90}; *A. muricata* at LC_{50} and LC_{90}) and positive controls (Anosom® 1 EC and Warrior II®). Each petri dish containing treated and untreated sections was considered a replication. The evaluations were performed by counting the number of insects attracted in each section at 15 min, 30 min and 60 min after aphid release.

3.2.10 Greenhouse bioassay

For this bioassay, soybeans plants were obtained as described for colony maintenance. When plants were at the V3-V4 stage, leaflets (one per plant) were infested with 10 wingless aphids.

The treatments evaluated were ethanolic seed extracts of *A. mucosa* and *A. muricata* at LC_{90}, in addition to negative (distilled water) and positive controls (Anosom® 1EC and Warrior II®). Each treatment was sprayed on aphids with a hand sprayer until the treatments began to run off (~1 mL). After spraying, the aphids were confined using clip-cages constructed with double sided 2.54 × 2.54 cm foam mounting squares (3M Scotch, Saint Paul, Minnesota), with an inner circular area of 1.2 cm^2 and covered with organdy fabric.

Each clip-cage with 10 wingless aphids on a leaflet was considered a replication per treatment (6 in total, n=60) in a completely randomized design. Mortality was evaluated at 1 and 3 days after application.

3.2.11 Statistical Analysis

For the analysis of all variables, normality of residuals was verified with the Shapiro-Wilk test (Shapiro and Wilk, 1965), and homogeneity of variances with the Bartlett test (Bartlett, 1937). When the assumptions were satisfied, the data were submitted to analysis of variance, and the means were compared by the Tukey or Scott-Knott test ($p < 0.05$) using the glht function of the multicomp package with adjustment of *p* values. All analyses were performed using the statistical software “R”, version 2.15.1 (R Development Core Team, 2012).

The data of the concentration-response bioassay were submitted to Probit analysis using Polo-Plus program (LeOra Software, 2003). The significance of the concentration-response curves was checked by the goodness-of-fit between observed and expected concentration-response curves using the chi-square ($\chi 2$) test. The mortality data were corrected for control mortality according to Schneider-Orelli (1947): (CM (%) = [(Mortal. (%) in T - Mortal. (%) in C) / (100 - Mortal. (%) in C)] * 100, where: CM (%) = corrected mortality in the control, T = mortality in the treatment and C = mortality in the control).

To examine the effects of the ethanolic seed extracts on the behavior of *A. glycines,* an infestation index (II) was calculated (adapted from Lin *et al.*, 1990). The formula was II = 2G/(G + P), where G = the number of insects counted on the test side on the petri dish and P = the number of insects counted on the control side of petri dish. Based on the indexes and standard deviations obtained, the classification intervals (CI) for the treatment averages were determined using the formula CI = [(1 ± t (n-1; ∞ = 0.05)) x (SD/n1/2)], where t = the value of Student’s t test at 5% probability; SD = the standard deviation; and n = the number of replications. The treatments were considered neutral when the value of their indices was included within the calculated CI, repellent when the values remained lower than the calculated CI, and attractive when the values were above the calculated CI (Silva *et al.*, 2012; Soares *et al.*, 2021b).

The survival curves and lethal times were obtained through Survival analysis. The analysis was performed using Kaplan–Meier estimators by the Log-Rank test

(SigmaPlot, version 12.5) and the survival curves were compared using the Holm-Sidak test (SigmaPlot, version 12.5).

3.3 Results

3.3.1 Vial Bioassay and concentration-response curves

The yield of ethanolic seed extracts was 21.1% for *A. muricata* and 18.8% for *A. mucosa.* All extracts showed insect mortality rates above 60% after 24 h of exposure, with a corrected mortality of 98.92% for *A. mucosa* (5,000 $mg.L^{-1}$) and *A. muricata* (5,000 $mg.L^{-1}$) (Table 2) and they did not differ from *A. mucosa* at 1,000 $mg.L^{-1}$ that reached a corrected mortality of 96.77%.

The seed extracts of *A. mucosa* at 500 $mg.L^{-1}$ (73.12%), *A. muricata* 1,000 and 500 $mg.L^{-1}$ (61.29 and 60.22% corrected mortality, respectively), positive controls Warrior II® (70.97% corrected mortality) and Anosom® 1 EC (46.24% corrected mortality) were not as effective as the other three treatments, but all of them differed from the second negative control water:acetone 1:1 (v/v) (2.15% corrected mortality).

The LC_{50} and LC_{90} values from ethanolic seed extract of *A. mucosa* on *A. glycines* control were 134.229 $mg.L^{-1}$ (95% CI: 77.225 – 215.584) and 487.890 $mg.L^{-1}$ (95% CI: 288.144 – 1435.243), respectively (h=0.41, X^2: 0.776, p=0.678, slope ± SE: 2.287 ± 4.40). For *A. muricata,* the LC_{50} and LC_{90} values were 305.721 $mg.L^{-1}$ (147.075 – 662.093) and 1290.3 $mg.L^{-1}$ (609.58 – 8140.10), respectively (h= 0.064, X^2: 0.201, p= 0.818, slope ± SE: 2.049 ± 3.79).

3.3.2 Lethal Time bioassay

All treatments differed from the negative controls water and water: acetone (1:1, v/v) (Figure 1) (Log-rank test: $\chi 2 = 241.802$; $P<0.001$). The ethanolic seed extracts of *A. muricata* and *A. mucosa* at LC_{90} caused 50% of mortality of *A. glycines* population at 20.12 (CI_{95}:18.266-21.974) and 20.08 h, (CI_{95}:18.203-21.957) respectively, and did not differ from the positive controls Warrior II® (LT_{50}: 19.14 h; CI_{95}: 17.170-21.124;) and Anosom® 1 EC (LT_{50}:22.24 h; CI_{95}:20.985-23.495).

Table 2. Mortality (± SE) of *Aphis glycines* in vial glasses containing ethanolic seed extracts of *Annona* extracts after 24 hours.

Treatments	% Mortality[1]	CM[2] (%)
A. mucosa 5,000 mg.L^{-1}	99.00 ±1.00 d	98.92
A. muricata 5,000 mg.L^{-1}	99.00 ±1.00 d	98.92
A. mucosa 1,000 mg.L^{-1}	97.00 ±2.00 d	96.77
A. mucosa 500 mg.L^{-1}	75.00 ±4.18 c	73.12
Warrior II®	73.00 ±4.64 c	70.97
A. muricata 1,000 mg.L^{-1}	64.00 ±7.48 bc	61.29
A. muricata 500 mg.L^{-1}	63.00 ±4.90 bc	60.22
Anosom® 1 EC	50.00 ±2.74 bc	46.24
Water:Acetone (1:1, v/v)	9.00 ± 3.67 a	2.15
Water	7.00 ± 3.74 a	--
F	66.65	--
Df	9, 40	--
p	<0.0001	--

[1] Means followed by distinct letters in the columns indicate significant differences between treatments by Tukey's ($p < 0.05$). [2]CM = corrected mortality, using the formula proposed by Schneider-Orelli (1947): CM (%) = [(Mortal. (%) in T - Mortal. (%) in C) / (100 - Mortal. (%) in C)] * 100, where: CM (%) = corrected mortality in the control, T = mortality in the treatment and C = mortality in the control.

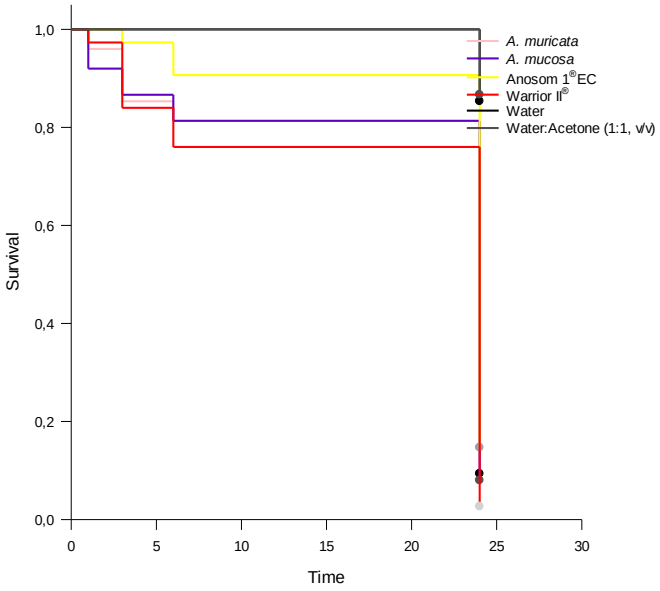

Figure 1. LT_{50} (hours) values of six treatments against *Aphis glycines* nymphs.

3.3.3 Detached leaf bioassay

Ethanolic seed extract of *A. mucosa* at 100 mg.L^{-1} had the highest number of insects among the treatments (32.75) and did not differ from the negative controls water (46.5) and water:acetone (1:1, v/v) (46.0) (Table 3). All other extract concentrations showed a significantly lower number of insects after five days– *A. mucosa* at 500, 1,000 and 10,000 mg.L^{-1} (22.75, 24.5 and 8.5, respectively); *A. muricata* at 100, 500, 1,000 and 10,000 mg.L^{-1} (9.5, 11, 7.5 and 11.25, respectively) – and did not differ from the positive control Warrior II®, with the lowest mean number of aphids (6.0).

Table 3. Number of *Aphis glycines* nymphs (± SE) after 5 days on soybean leaflets that had their petioles immersed in the different treatment concentrations.

Treatments	**Nymphs[1]**
A. mucosa 100 mg.L^{-1}	32.75 ± 11.45 a
A. mucosa 500 mg.L^{-1}	22.75 ± 11.45 b
A. mucosa 1,000 mg.L^{-1}	24.5 ± 16.07 b
A. mucosa 10,000 mg.L^{-1}	8.5 ± 5.01 b
A. muricata 100 mg.L^{-1}	9.5 ± 7.63 b
A. muricata 500 mg.L^{-1}	11.0 ± 2.52 b
A. muricata 1,000 mg.L^{-1}	7.5 ± 1.04 b
A. muricata 10,000 mg.L^{-1}	11.25 ± 3.68 b
Warrior II®	6.0 ± 2.27 b
Water	46.5 ± 19.52 a
Water:Acetone (1:1, v/v)	46.0 ± 21.12 a
F	2.21
Df	10, 33
p	0.0427

[1] Means followed by distinct letters in the columns indicate significant differences between treatments by Scott Knott's ($p < 0.05$).

3.3.4 Repellence bioassay

All *Annona* treatments (LC_{50} and LC_{90} of ethanolic seed extracts of *A. muricata* and *A. mucosa*) repelled *A. glycines* nymphs compared to the control for all evaluation

periods (Table 4). Anosom® 1 EC had similar results to *Anonna* extracts. In contrast, the insecticide Warrior II® was considered neutral after 30 min, but attractive after 15 and 60 min from insect release in the Petri dishes.

Table 4. Means (± SE) of aphids/section and infestation index of *Aphis glycines* in soybean subjected to different treatments at three evaluation periods.

15 min	aphids/section	II (M ± SE)[a]	CI[b]	Classification[c]
Anosom® 1 EC	0.6	0.22 ± 0.1	(0.82 ; 1.18)	repellent
Control	3.8			
Warrior II®	4.1	1.23 ± 0.1	(0.81 ; 1.19)	attractive
Control	2.7			
A. muricata LC_{50}	0.3	0.2 ± 0.11	(0.80 ; 1.20)	repellent
Control	2.5			
A. muricata LC_{90}	1.1	0.45 ± 0.11	(0.79 ; 1.21)	repellent
Control	3.0			
A. mucosa LC_{50}	0.4	0.18 ± 0.09	(0.83 ; 1.17)	repellent
Control	1.4			
A. mucosa LC_{90}	0.9	0,38 ± 0.17	(0.69 ; 1.31)	repellent
Control	2.8			
30 min				
Anosom® 1 EC	1.3	0.43 ± 0.2	(0.63 ; 1.37)	repellent
Control	3.9			
Warrior II®	3.8	1.15 ± 0.14	(0.74 ; 1.26)	neutral
Control	3.0			
A. muricata LC_{50}	0.7	0.37 ± 0.14	(0.75 ; 1.25)	repellent
Control	2.5			
A. muricata LC_{90}	0.9	0.43 ± 0.13	(0.76 ; 1.24)	repellent
Control	2.1			
A. mucosa LC_{50}	0.9	0.38 ± 0.16	(0.71 ; 1.29)	repellent
Control	2.6			
A. mucosa LC_{90}	1.2	0,58 ± 0.14	(0.74 ; 1.26)	repellent
Control	2.2			
60 min				
Anosom® 1 EC	1.1	0.38 ± 0.15	(0.73 ; 1.27)	repellent
Control	3.2			

Warrior II®	5.0	1.36 ± 0.09	(0.84 ; 1.16)	attractive
Control	2.4			
A. muricata LC_{50}	0.9	0.47 ± 0.14	(0.74 ; 1.26)	repellent
Control	2.2			
A. muricata LC_{90}	0.6	0.33 ± 0.12	(0.78 ; 1.22)	repellent
Control	2.1			
A. mucosa LC_{50}	0.8	0.56 ± 0.21	(0.62 ; 1.38)	repellent
Control	2.7			
A. mucosa LC_{90}	1.0	0.44 ± 0.15	(0.72 ; 1.28)	repellent
Control	2.1			

[a] Infestation index; [b] Classification interval; [c] Classification = neutral (within the classification range, CIi < II < CIs); repellent (II<Cli), or attractive (II > Cls); CIi = lower limit of the rating range and CIs = upper limit of the rating range.

3.3.5 Greenhouse bioassay

Under greenhouse conditions, *A. glycines* mortality was high for Warrior II®, with almost 100% of mortality (96.67%) one day after application (1 DAA). The ethanolic seed extract of *A. mucosa* at LC_{90} did not significantly differ from Warrior II®, although it presented a lower level of control (78.33%). Anosom® 1 EC and *A. muricata* did not differ significantly, with 66.67 and 45% mortality, respectively. All treatments differed significantly from water at 1 DAA (Table 5).

For the second evaluation (3 DAA), treatments did not significantly differ from each other with nymphal mortality rates above 65%, with 100% mortality for Warrior II®, followed by *A. mucosa* CL_{90} (92.1%), Anosom® 1 EC (68.4%) and *A. muricata* CL_{90} (65.7%).

Table 5. Mortality (± SE) of *A. glycines* nymphs on soybean 1 and 3 days after application (DAA) of *Annona* extracts.

	Mortality (%)[1]		
	1 DAA	3 DAA	CM[2]
A. muricata	45.00 ± 8.85 b	78.33 ± 7,03 b	65.79
A. mucosa	78.33 ± 4.77 cd	95.00 ± 5.00 b	92.11
Anoson® 1 EC	66.67 ± 8.43 bc	80.00 ± 10.00 b	68.42
Warrior II®	96.67 ± 2.10 d	100.00 ± 0.00 b	100.00
Water	0.00 ± 0.00 a	36.67 ± 11.15 a	0
F	39.02	10.39	--
Df	4, 25	4, 25	--
p	<0.001	<0.001	--

[1] Means followed by distinct letters in the columns indicate significant differences between treatments by Tukey's ($p < 0.05$). ²CM = Corrected mortality calculated in the last evaluation period, using the formula proposed by Schneider-Orelli, 1947.

3.4 Discussion

The present study demonstrated that the application of ethanolic seed extracts from *A. mucosa* and *A. muricata* has potential to manage *A. glycines* on soybean. Other studies have demonstrated the insecticidal potential of Annonaceae species derivatives to control important pest insects, such as lepidopterans, other hemipterans, coleopterans, and dipterans (Krinski, 2014; Ribeiro *et al.*, 2015, 2016; Souza *et al.*, 2017, 2019; Soares *et al.*, 2021 a, b), but the activity of these extracts on *A. glycines* was previously unknown.

The ethanolic seed extracts achieved nearly 100% mortality at the highest concentrations evaluated in vial bioassays (1,000 $mg.L^{-1}$ for *A. mucosa* and 5,000 $mg.L^{-1}$ for *A. muricata*), confirming the bioactivity of these extracts documented for other pest insects. For example, in bioassays conducted with *B. tabaci* MEAM1, ethanolic seed extracts of *A. muricata* and *A. mucosa* at 5,000 $mg.L^{-1}$ sprayed on second nymphal instars caused 93.5 and 100% of mortality, respectively (Soares *et al.*, 2021 a). *Annona mucosa* extracts also caused high mortality (95%) in *Helicoverpa armigera* neonates in tomato (Souza *et al.*, 2017) and in Asian citrus psyllid *D. citri* nymphs and adults (Ribeiro *et al.*, 2015).

In our study, *Annona* spp. seed extracts demonstrated potential as botanical insecticides, although additional studies at a larger scale are needed to further investigate their potential as novel insecticides for soybean aphid control. New insecticides are needed given that *A. glycines* populations have developed resistance to pyrethroids (Xi *et al.*, 2015; Menger *et al.*, 2022; Valmorbida *et al.*, 2022 a, b). Similar results were observed for *B. tabaci* MEAM1 with these extracts, which presented higher rates of nymphal mortality for *B. tabaci* MEAM1 under laboratory conditions when compared to thiamethoxam (Soares *et al.*, 2021 a).

The estimated values of LC_{50} and LC_{90} of *A. mucosa* (134.229 and 487.89 $mg.L^{-1}$, respectively) and *A. muricata* (305.721 and 1290.3 $mg.L^{-1}$, respectively) for *A. glycines* were higher than those reported for *B. tabaci* MEAM1 (LC_{50} 10.83 $mg.L^{-1}$ and LC_{90} 200.24 $mg.L^{-1}$ for *A. mucosa*), suggesting that the whitefly is more susceptible to this extract (Soares *et al.*, 2021 a). However, according to other studies that estimated LC_{50} of ethanolic seed extract of *A. mucosa* for lepidopteran species, the values obtained for *S. frugiperda* and *H. armigera* were at least six (842.9 $mg.L^{-}1$) and three (411.55 $mg.L^{-}1$) times higher (Ansante *et al.*, 2015; Souza *et al.*, 2019), respectively, suggesting that these insects are less susceptible to the extracts.

In this study, the ethanolic seed extracts also presented efficient translocation through the sap, showing similar effect as the synthetic insecticide Warrior II® on aphid control. This contrasts with results obtained for *B. tabaci* MEAM1 nymphs, where no systemic effect was reported for *A. mucosa* at the same concentration used in this study (10,000 $mg.L^{-1}$) (Soares *et al.*, 2021 a). One possible explanation is that in the present study a leaflet was used, and its petiole was immersed in a small vial (5 mL) with the treatment in solution. In the study with *B. tabaci* MEAM1, the extracts were added to the soil (2 L), and the whole plant needed to absorb and distribute the compounds present in the extracts. In addition, these compounds could have interacted with soil particles, reducing the amount absorbed, and possibly required higher concentration to have a measurable effect. Similar bioassays with soybean aphid should be conducted with *Annona* seed extracts.

Furthermore, a common problem with systemic uptake bioassays has been the uncertainty of knowing the concentration of an insecticide to which the insect has been exposed to. This has led some researchers to choose foliar bioassays instead of systemic uptake bioassays, even though the ingestion of the insecticide could be a more toxic mode of exposure relative to contact (Castle *et al.*, 2014).

The extracts were effective in less than 24 h. These results could vary according to the insect species and to the extract used. In a similar study, it was observed that the LT_{50} of 10 different species of Annonaceae on *S. frugiperda* varied from 61 to 264 h, and the extract of stem bark of *Duguetia lanceolata* A. St.-Hil. showed the fastest activity (61.4h) (Alves *et al.*, 2016).

Regarding the repellent effect, both ethanolic seed extracts demonstrated potential, presenting lower numbers of insects compared to negative (water) and positive (Warrior II®) controls. The same was observed for the ethanolic seed extract of *A. muricata* on *B. tabaci* MEAM1 adults, reducing insect infestation at all periods of evaluation (Soares *et al.*, 2021 b). Other plant species from several botanical families have also been shown to reduce infestations of aphids in treated plants, such as *Cymbopogon citratus*, *Salvia officinalis* and *Origanum majorana* that repel *Myzus persicae*, *Aphis gossypii*, *Aphis spiraecola* and *Aphis fabae* (Khaled-Gasmi *et al.*, 2021). Surprisingly, the synthetic insecticide Warrior II® was considered attractive for nymphs. Similar findings have been observed with thiamethoxam and *B. tabaci* adults on tomato leaflets after 12 h of evaluation (Soares *et al.*, 2021 b). The colonization inhibition for an extract could be due to the inactivation or stimulation of specific receptors in insects as demonstrated for Meliaceae plants (Tan and Luo, 2011). Attractiveness is expected to follow the same mechanism.

For the contact bioassay in the greenhouse experiment, the most promising among the ethanolic seed extracts was *A. mucosa* at LC_{90}. Similar findings were observed with *B. tabaci* nymphs under field conditions (Soares *et al.*, 2021a) and with *Myzus persicae* under laboratory and greenhouse trials. Although all *Annona* extracts tested caused reductions in aphid populations, *A. mucosa* showed the greatest aphicidal activity (Ribeiro et. al, 2014 a).

The bioactivity of extracts based on *A. mucosa* may be related to several compounds in its composition, which can act separately or synergistically in causing insect mortality. Alkaloids, triglycerides and, notably, ACGs are the most common classes of compounds found on *A. mucosa* extracts (Ribeiro *et al.*, 2014 b; Ansante *et al.*, 2015). The insecticidal properties of ACGs are related to the inhibition of complex I (NADH: ubiquinone oxiredutase) of the mitochondrial electron transport system of insects (Lewis *et al.*, 1993), inducing programmed cell death (apoptosis) due to ATP deprivation (Tormo *et al.*, 1999). In addition to the promising insecticidal action (Alali *et al.*, 1999; Ribeiro *et al.*, 2013), ACGs have attracted much interest due to their

effects on feeding and oviposition behavior (repellence/deterrence) (Blessing *et al.*, 2010). Studies on the structure-activity relationship have proven that ACGs with adjacent bis-tetrahydrofuranic rings and 3 hydroxyl groups (eg., Rolliniastatin-1) have more pronounced insecticidal activity compared to ACGs containing other distribution of functional groups in their structure (He *et al.*, 1997).

The results of this study demonstrated the potential of ethanolic seed extracts of *A. mucosa* and *A. muricata* to be used as an insecticide to manage *A. glycines* nymphs on soybean. In addition, these products could be a valuable tool to be used in organic soybean, a market that has been increasing considerably (USDA, 2022). To manage pests in an organic production system, growers often rely on IPM, but with a limited number of certified insecticides (Hartman *et al.*, 2016). In the future, new eco-friendly approaches must be implemented for sustainable aphid control, and botanical derivatives, such as ethanolic extracts of *Annona* species, should be developed and commercialized. Further trials are still necessary to better understand the selectivity of these extracts on non-target organisms, their persistence, as well as their biodegradability under field conditions (Soares *et al.*, 2021 a).

3.5 Conclusion

The ethanolic seed extracts of *A. mucosa* and *A. muricata* caused high mortality of *A. glycines* nymphs at low concentrations in laboratory and greenhouse assays. The repellence caused by the extracts was also an important finding, since preventing insects from colonizing plants also leads to reduced direct and indirect damage. The significant bioactivity demonstrated with derivatives of *Annona* spp. on *A. glycines* can serve as a stimulus for future research, aiming at the discovery and development of new products to be available for IPM programs and as additional tools to improve pest management strategies in organic farming systems.

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CONSIDERAÇÕES FINAIS

O uso do TS é uma valiosa ferramenta a ser considerada no manejo das populações de diferentes pragas de importância agrícola, incluindo a mosca-branca *B. tabaci* MEAM1, porém, para seu sucesso, deve ser utilizada com outras práticas de controle.

Os inseticidas tiametoxam e ciantraniliprole apresentaram resultados positivos no manejo da mosca-branca, diminuindo a infestação dos insetos nas plantas de soja e interferindo na viabilidade das ninfas. O tiametoxam apresentou maior residual nas plantas ao longo do ciclo da soja, apresentando efeito mais prolongado sobre os insetos.

A técnica do EPG permite destacar que os inseticidas sintéticos promoveram menor ingestão de seiva do floema pela mosca-branca, o que pode justificar a interferência na duração do período ninfal. Além do mais, esse resultado é interessante para os manejos envolvendo o controle de insetos vetores de viroses, uma vez que diminuem o processo de aquisição dos vírus pelos insetos, devido ao menor tempo de contato com o floema. Por outro lado, o inseticida botânico à base de anonina não apresentou efeitos positivos quando utilizado via TS.

Os extratos etanólicos de sementes de *A. mucosa* e *A. muricata* usados no controle de *Aphys glycines* ocasionaram elevada mortalidade das ninfas, tanto em ensaios de laboratório quanto em semi-campo. Resultados quanto à repelência dos extratos ao pulgão-da-soja também foram observados, sendo esta uma outra ferramenta para diminuir a infestação e alimentação dos insetos, diminuindo possíveis infecções por viroses.

Os resultados obtidos neste estudo são inéditos para o controle de *A. glycines* e, no geral, se mostraram promissores, evidenciando o grande potencial de uso desses extratos botânicos.

As espécies de *Annona* estudadas são nativas das Américas e ainda são pouco exploradas nas regiões Neotropicais, podendo servir de base para investigações futuras, inclusive servindo de modelo para a síntese de novos inseticidas sintéticos.

De forma geral, o residual obtido com os extratos é baixo, sugerindo maior aplicabilidade em pulverizações próximas à colheita, sendo este um nicho de mercado ainda bastante deficiente, com poucas alternativas aos produtores. Em adição, a matéria-prima para obtenção dos extratos pode ser obtida em indústrias, onde muitas

vezes, é considerada como resíduo, como o caso das sementes de *A. muricata*, extraídas e descartadas no processo de fabricação da polpa de graviola.

A significativa bioatividade demonstrada com derivados de *Annona* spp. sobre *A. glycines* pode servir como estímulo para futuras pesquisas, visando a descoberta e desenvolvimento de novos produtos a serem disponibilizados para os programas de manejo integrado de pragas.

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