CAMILA OLIVEIRA SANTOS

Potential of integrating mating disruption with resistance management and stable isotope to track dispersion of lepidopteran pests

Thesis submitted to the Graduate Program in Entomology of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Adviser: Eliseu José Guedes Pereira

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I dedicate this thesis to God Almighty, my creator and endless source of
inspiration, wisdom, knowledge, and understanding.
To my husband, Maikon
To my parents, Anisete and Janio

I dedicate.

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My utmost regard also goes to my husband Maikon, my greatest motivator to keep flying high.

Remain in me, as I remain in you. Just as a branch cannot bear fruit on its own unless it remains on the vine, so neither can you unless you remain in me.

I am the vine, you are the branches. Whoever remains in me and I in him will bear much fruit, because without me you can do nothing.

Anyone who does not remain in me will be thrown out like a branch and wither; people will gather them and throw them into a fire and they will be burned.

If you remain in me and my words remain in you, ask for whatever you want and it will be done for you.

By this is my Father glorified, that you bear much fruit and become my disciples.

As the Father loves me, so I also love you. Remain in my love.

John 15:4-9

ABSTRACT

SANTOS, Camila Oliveira, D.Sc., Universidade Federal de Viçosa, February, 2024. **Potential of integrating mating disruption with resistance management and stable isotope to track dispersion of lepidopteran pests.** Adviser: Eliseu José Guedes Pereira.

Recent advances in organic synthesis have enabled the production of more cost-effective insect pheromone formulations for mating disruption over extensive agricultural areas. Mating disruption can interrupt the chemical communication system between males and females, safeguarding crops in treated regions. The technique can suppress *Spodoptera frugiperda* populations, a polyphagous lepidopteran pest causing significant global economic losses. However, validating mating disruption remains an ongoing effort. Results revealed a high percentage of mating disruption in four out of the five locations tested and a reduction in plant damage in three locations due to the pheromone application. While the crop yield was not significantly impacted, a significant decrease in ear damage was observed in treated areas. These findings highlight the potential of synthetic pheromones to disrupt mating and mitigate plant damage in maize fields infested by this pest. Escalating issues of evolution of resistance to conventional control methods in *S. frugiperda* increases the need for alternative approaches. Mating disruption, which affects the success of pest mating, is a potential tool to benefit resistance management. In that matter, we aimed to monitor the susceptibility of *S. frugiperda* populations to insecticides and Bt traits while exploring the feasibility of mating disruption as a resistance management tool. Neurotoxic insecticides demonstrated continued efficacy against the larvae despite concerns regarding potential resistance development within the benzoylurea insecticide group. Two populations exhibited incomplete resistance to the dual-gene Bt maize, which impacted various life-history population traits. We discussed the potential benefits and challenges of implementing mating disruption within insect resistance management strategies. Additionally, understanding the insect movement patterns between host plants is essential to effectively implementing these biorational pest management tools. Nitrogen-15 (^{15}N) is a stable isotope and offers a promising tool for tracking insect dispersal among agricultural fields. Applying ^{15}N-enriched fertilizers to specific crop areas allows plants to be labeled with a distinct isotopic signature, which can be transferred between host plants and their herbivores. The ^{15}N levels in plant tissues were significantly higher in treatments sprayed with the isotope than in the control. The moths originating from isotope-treated plants absorbed the labeled nitrogen isotope into their tissues, although a non-significant difference was found among insects from treated and control plants. Our findings highlight the potential of integrating mating disruption with resistance management and ^{15}N isotope tracing as feasible tools for improving our capacity to manage agricultural pests effectively. Further field trial replications should help refine and integrate these techniques into sustainable pest management programs, ensuring effective and resilient crop protection strategies.

Keywords: pheromone, *Spodoptera frugiperda*, behavioral manipulation, insecticides and Bt crops, tracing tool, nitrogen.

RESUMO

SANTOS, Camila Oliveira, D.Sc., Universidade Federal de Viçosa, fevereiro de 2024. **Potencial de integrar confusão sexual com manejo de resistência e isótopo estável para rastrear dispersão de lepidópteros pragas.** Orientador: Eliseu José Guedes Pereira.

Avanços recentes na síntese orgânica permitiram a produção de formulações de feromônios de insetos mais econômicas para a confusão sexual em extensas áreas agrícolas. A confusão sexual pode interromper o sistema de comunicação química entre machos e fêmeas, protegendo as plantações nas regiões tratadas. A técnica pode causar a supressão das populações de *Spodoptera frugiperda*, um lepidóptero-praga altamente polífago que causa perdas econômicas globais significativas. No entanto, a validação dessa técnica continua sendo um esforço contínuo. Os resultados mostraram uma alta porcentagem de confusão sexual em quatro dos cinco locais testados e a redução nos danos às plantas em três locais devido à aplicação do feromônio. Embora o rendimento da colheita não tenha sofrido impacto significativo, foi observada uma redução significativa nos danos às espigas nas áreas tratadas. Essas descobertas destacam o potencial dos feromônios sintéticos para interromper o acasalamento e reduzir os danos às plantas em campos de milho infestados por essa praga. Os problemas crescentes de evolução da resistência aos métodos de controle convencionais em *S. frugiperda* aumentam a necessidade de abordagens alternativas. A confusão sexual, que manipula o comportamento reprodutivo da praga, é uma ferramenta potencial que pode beneficiar o manejo da resistência. Nesse sentido, nosso objetivo foi monitorar a suscetibilidade das populações de *S. frugiperda* aos inseticidas e às culturas Bt e, ao mesmo tempo, explorar a viabilidade da confusão sexual como uma ferramenta que beneficie o gerenciamento de resistência. Os inseticidas neurotóxicos demonstraram eficácia contínua contra as larvas, apesar das preocupações com o possível desenvolvimento de resistência dentro do grupo da benzoiluréia. Duas populações apresentaram resistência incompleta ao milho Bt piramidado, o que afetou várias características populacionais da história de vida. Discutimos os possíveis benefícios e desafios da implementação da confusão sexual nas estratégias de gerenciamento da resistência de insetos. Além disso, a compreensão dos padrões de movimento dos insetos entre as plantas hospedeiras é essencial para a implementação eficaz dessas ferramentas bioracionais de controle de pragas. O nitrogênio-15 (^{15}N), um isótopo estável, oferece uma ferramenta promissora para rastrear a dispersão de insetos em campos agrícolas. A aplicação de fertilizantes enriquecidos com ^{15}N em áreas específicas de cultivo permite que as plantas sejam marcadas com uma assinatura isotópica distinta, que pode ser transferida entre as plantas hospedeiras e seus herbívoros. Os níveis de ^{15}N nos tecidos das plantas foram significativamente mais altos nos tratamentos pulverizados com o isótopo do que no controle. As mariposas originárias de plantas tratadas com isótopo absorveram o isótopo de nitrogênio marcado em seus tecidos, embora não tenha sido encontrada uma diferença significativa entre os insetos de plantas tratadas e de controle. As nossas descobertas destacam o potencial de integração da confusão sexual com o manejo de resistência e o rastreio com isótopo ^{15}N como ferramentas viáveis para melhorar a nossa capacidade de gerir as pragas agrícolas de forma eficaz. Futuras repetições com ensaios de campo deverão ajudar a refinar e integrar estas técnicas em programas sustentáveis de gestão de pragas, garantindo estratégias de proteção de culturas eficazes e resilientes.

Palavras-chave: feromônio, *Spodoptera frugiperda*, manipulação comportamental, inseticidas e culturas Bt, ferramenta de rastreio, nitrogênio.

SUMMARY

GENERAL INTRODUCTION

By 2050, the world's population is expected to grow close to 10 billion, leading to a 40-50% increase in food demand compared to today (FAO, 2018). Additionally, climate change brings new challenges to food production, including higher temperatures and more frequent occurrences of pests, diseases, droughts, and floods. Insect pests are a significant concern among these challenges, causing global losses ranging from 6% to 19% (Oerke, 2006; Oliveira et al., 2014).

The fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), is a notorious agricultural pest with a widespread presence in the Americas. Its high polyphagia and dispersal among locations have posed significant economic losses and jeopardized food security worldwide (Day et al., 2017). Over the years, pesticides (insecticides, Bt crops) have been the primary means of controlling fall armyworm populations. However, selection pressure on these methods has led to various ecological and economic issues, including developing pesticide resistance in pest populations (Zhang et al., 2020). In response to this challenge, researchers and agricultural practitioners have been exploring innovative approaches to control and mitigate the impact of this destructive pest.

Semiochemicals, especially sex pheromones, have high potential as an environmentally friendly approach to suppressing insect pest populations (Bento et al., 2016). Pheromones were defined in 1959 by identifying the first sex pheromone of the silkworm, *Bombyx mori* (Lepidoptera: Bombycidae). It is defined as substances secreted to the outside by one individual and received by a second individual of the same species, in which they release a specific reaction, such as a defined behavior or a development process (Butenandt et al., 1959; Karlson and Lüscher, 1959).

The strategy of using sex pheromones is known as mating disruption, which promotes the disruption of communication among moths to hinder their ability to locate and mate with one another (Witzgall et al., 2010). This communication has developed through millions of years of natural selection, rendering mating disruption control far more resilient to the accumulated resistance observed in traditional chemical pesticides and Bt proteins in genetically modified crops (Harari & Sharon, 2022). Mating disruption has shown promise as an environmentally friendly and sustainable approach to managing resistant fall armyworm populations. However, its effectiveness and implications for resistance management remain as active research and debate areas.

In chapter one, we explore the potential of mating disruption to suppress the fall armyworm population, reduce plant damage, and increase maize yield. By deploying synthetic pheromones to interfere with fall armyworm mating patterns, we aimed to assess the efficacy of this approach in mitigating crop damage and enhancing yield in maize fields.

Managing pest resistance is crucial in sustaining the effectiveness of control strategies over time. Chapter two aims to explore the fall armyworm susceptibility status to pesticides and discuss mating disruption benefits in resistance management. We focused on phenotypic monitoring of fall armyworm populations to understand their susceptibility to insecticides and genetically modified crops (Bt traits). By monitoring population dynamics and changes in life-history traits, we aimed to increase insights into the potential of mating disruption as a tool in resistance management strategies.

Understanding insect movement patterns between crops is essential for effective pest management and crop protection. The third chapter explores using nitrogen-15 (^{15}N), a stable isotope, as a potential marker for tracking insect dispersal within agricultural fields. By applying ^{15}N-enriched fertilizers to specific crop areas, we aimed to label plants with a distinct isotopic signature, allowing for the tracking of insect movement and population dynamics among host plants. This approach can potentially advance our understanding of pest dynamics and develop more targeted and sustainable pest management strategies.

Collectively, these chapters represent a wide-ranging effort to explore the potential of integrating mating disruption with resistance management and stable isotope to track the dispersion of lepidopteran pests. Ultimately, we hope this thesis can inform and guide policymakers, farmers, and researchers in making informed decisions about fall armyworm (and other applicable pests) control strategies, which are both effective and environmentally responsible.

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CHAPTER 1

Mating disruption of fall armyworm: Can it reduce plant damage and increase maize yield?

ABSTRACT

Recent advances in organic synthesis processes have made it possible to produce more affordable insect pheromones for mating disruption in large areas. Mating disruption interferes with the chemical communication system between males and females, which can ultimately protect the crop in treated areas. This technique can suppress *Spodoptera frugiperda* populations, whose larvae are believed to cause worldwide economic damage. Mating disruption in maize still needs to be validated, hoping that it can protect against plant damage and yield loss. We aimed to evaluate the effectiveness of mating disruption in maize fields through the controlled release of a synthetic pheromone formulation and determine its impact on the damage caused by *S. frugiperda* larvae and the final crop yield. Maize fields of at least two hectares were selected in five locations. One plot received 40 pheromone dispensers per hectare; the other was a control. Delta traps with pheromone bait were used in both fields to monitor the male capture weekly and the percentage of mating disruption. The maize foliar damage and their final yield were also assessed. The synthetic pheromone caused a high percentage of mating disruption in four of the five locations tested and reduced plant damage in three locations. Although crop yield was not affected by the presence of the pheromone dispensers, there was a reduction in the damage to the ears in the treated areas. These results show that the synthetic pheromone of *S. frugiperda* caused mating disruption and reduced plant damage in the maize fields tested. More trials are needed to validate this technique in different infestation scenarios and combine it with other control techniques for sustainable and effective integrated management.

Keywords: behavioral control, *Spodoptera frugiperda*, *Zea mays*, sex pheromone, foliar injury.

1 Introduction

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae) is a notorious pest of maize (*Zea mays*) that has attracted significant attention in recent years due to its rapid dispersion and devastating impact on global maize production (Cock et al., 2017; Goergen et al., 2016; Guo et al., 2018; Ma et al., 2019; Sharanabasappa et al., 2018). Fall armyworm is a polyphagous pest, feeding on 300 plant species from 76 families, including some of economic importance, such as maize, soybeans, and cotton (Montezano et al., 2018).

Managing fall armyworm populations in maize fields has historically relied on chemical pesticides and Bt traits. Concerns over environmental pollution, ecological disruption, and ultimately, the development of insecticides and Bt traits resistance (Bernardi et al., 2015; Carvalho et al., 2013; Gutiérrez-Moreno et al., 2018; Nascimento et al., 2016; Okuma et al., 2018; Ríos-Díez & Saldamando-Benjumea, 2011; Santos-Amaya et al., 2015; Yu & Elzie McCord, 2007; Zhu et al., 2015) have pushed the investigation of alternative, more sustainable control strategies.

Mating disruption (MD), a pheromone-based technique, has emerged as a promising tool in the integrated pest management arsenal against fall armyworm infestations (Schirmer et al., 2023). Mating disruption aims to control population growth by targeting sexually active adults instead of the destructive larval stage (Sarfraz et al., 2006). By disrupting the communication system between the moths by releasing synthetic pheromones, this strategy aims to confuse and disorient the males, making it challenging for them to locate and mate with females (Cardé, 2007a; Miller & Gut, 2015). Therefore, the overall reproductive capacity of the pest population decreases, resulting in reduced larval infestations in maize fields (Mori & Evenden, 2013).

The disruption of insect mating is an attractive strategy because most pheromones used commercially are species-specific, so they do not adversely affect non-target organisms and are considered safe for the environment, making them ideal components of integrated pest management (Bento et al., 2016; Witzgall et al., 2010). The pheromones are effective even at minimal concentrations, and the insects are less likely to develop resistance to this method than to pesticides (Klassen et al., 2023). There are different ways to use it in agriculture, such as monitoring, mating disruption, and assessing insecticide resistance in pest populations (McNeil, 1991).

Nowadays, agricultural pests are estimated to be managed with mating disruption in more than 800,000 hectares (Benelli et al., 2019), mainly in orchards, vineyards, and annual vegetable crops (Ballesteros et al., 2021; Onufrieva et al., 2019; Ricciardi et al., 2019; Wu et al., 2012). The field applications of pheromones depend on the availability of efficient dispensing materials and the economical synthesis of pheromone chemicals. Recent innovations in organic synthesis technology have led to increased pheromone production, making the release in larger areas possible, unlike a few years ago (Arnold, 2019; Miller and Gut, 2015).

Besides the Americas, several other places, such as Africa, have suffered substantial economic losses from *S. frugiperda*. Fast and easy-to-adopt control methods, like chemicals and Bt traits, can have long regulatory processes and impact on natural enemies (Day et al., 2017; Feldmann et al., 2019). The mating disruption approach aims to minimize the reliance on traditional control methods and mitigate the economic losses (Rizvi et al., 2021) associated with fall armyworm infestations, contributing to the sustainability and resilience of maize production worldwide. We aimed to evaluate the effectiveness of mating disruption in maize fields through the controlled release of a synthetic pheromone formulation and determine its impact on the damage caused by *S. frugiperda* larvae and the final crop yield.

2 Materials and Methods

2.1 Locations and growing seasons

Field trials were carried out in two growing seasons during the 2020/2021 years. In the first season, the trials were conducted at Cajuri (20°47'01.3"S 42°48'29.2"W) and Viçosa locations (20°44'39.7"S 42°50'36.2"W). In the second season, the trials were in Canaã (20°39'13.5"S 42°41'17.2"W), Coimbra (20°49'22.6"S 42°47'12.9"W), and Rio Doce (20°14'47.7"S 42°56'25.0"W), all located in the state of Minas Gerais, Brazil. The maize fields belonged to partner growers and represented an area of mixed landscape with Atlantic Forest and pastures. The maize hybrids planted and their technologies, as well as the total size of the area, varied depending on the location (Table 1): Cajuri (RB9006 RR, non-Bt); Viçosa (DKB390PRO, Bt with Cry1A.105+Cry2Ab); Canaã and Coimbra (RB9006 RR, non-Bt and BM709PRO2, Bt with Cry1A.105+Cry2Ab); Rio Doce (BM3069PRO2, Bt with Cry1A.105+Cry2Ab).

Table 1. Information about the pheromone field trials to test mating disruption of fall armyworm.

Location	**Season**	**Application type**	**Purpose**	**Total plot size (ha)**
Cajuri	1st season	Dispenser	Silage	4.00
Viçosa	1st season	Dispenser	Grain	8.35
Canaã	2nd season	Dispenser	Silage	8.00
Coimbra	2nd season	Dispenser	Silage	4.00
Rio Doce	2nd season	Spray	Silage	10.50

2.2 Mating disruption in field conditions

Two main treatments were tested in the fields: commercial formulation of *S. frugiperda* sex pheromone ((Z)-9-Tetradecenyl Acetate + (Z)-11-Hexadecenyl Acetate)) (Provivi™, Santa Monica, United States), with sachet-type dispensers at 40 units/hectare (Figure 1). The other area was considered control, without the pheromone release and the management following the grower practices. In the Viçosa location, additional treatments were performed to test the integration of the pheromone with other control methods. The treatments were: 1) control - with non-Bt maize and no *S. frugiperda* control method applied; 2) grower standard (GS) - with Bt maize (Cry1A.105 +Cry2Ab) + seed treatment and control decision by the grower; 3) pheromone + action threshold for insecticide application - pheromone application and insecticide intervention at 20% of plants attacked by larvae; 4) pheromone + GS - pheromone + grower practices; 5) pheromone + seed treatment - pheromone and seed treatment only. The experimental plots were at least 2 ha in size (Table 1), separated by a distance large enough not to confuse the response of the moths (around 100 m).

Delta traps containing bait with sex pheromones of *S. frugiperda* (Z)-11-hexadecenyl acetate; (Z)-7-dodecenyl acetate and (Z)-9-tetradecenyl acetate) were set up 1.3 m from the ground in both treatments, with 40-70 m between traps, where the moths were counted. The pheromone lures in each trap were changed every 30 days. We standardized at least 200 meters between the traps of the different treatments. One week before planting and during the first three weeks after plant emergence, catches were evaluated twice a week, and after that, they were assessed weekly. The results of mating disruption were calculated by the percentage (%) of inhibition of the males capture in traps, calculated as: [1 - (average capture per trap in the pheromone-treated plot / average capture per trap in the control plot)] × 100%(Miller et al., 2006; Roelofs et al., 1970).

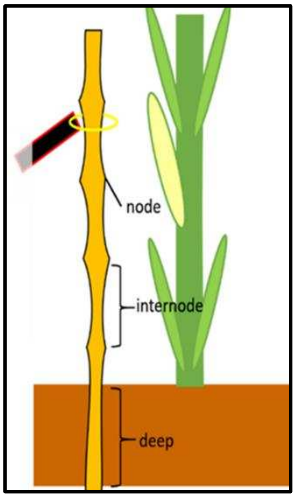

Figure 1. Guidance for installing the dispenser containing pheromone for mating disruption *of Spodoptera frugiperda* using bamboo or wooden stakes. Source: Provivi®.

2.3 Maize foliar damage and yield traits

Damage caused by *S. frugiperda* larvae was assessed weekly from emergence using the Davis scale (Table 2) (Davis et al., 1992). We set up four traps per plot/treatment, and 40 plants were damage-assessed per trap, totaling 160 plants per plot. We also assessed damage to the ear at the R4 stage of maize growth. Grain and silage yield data were sampled at harvest with a sample of plants in four rows (each row next to a trap) of 10 m in the experimental plots. The same experimental procedures were adopted for the sprayable pheromone, applied in a ground sprayer (90 to 130 mL/ha) along with other products during the maize cycle. The pheromone was sprayed three times during the crop cycle, starting from plant emergence to 7-10 days apart.

Table 2. Davis foliar damage scores caused by *Spodoptera frugiperda* on maize leaves.

Score	Definition
1	Leaves without any damage or with small dots on the leaves.
2	Small circular perforations on some leaves.
3	Few perforations, small circular lesions, and some small, elongated lesions (rectangular) up to 1.3 cm long are present on expanded and new leaves.
4	Several elongated lesions 1.3 to 2.5 cm long are present on some leaves.
5	Several large, elongated lesions over 2.5 cm long are present on some leaves.
6	Several large, elongated lesions are present on several leaves with large, uniform holes of irregular shapes.
7	Many elongated lesions of all sizes are present on several leaves and several large uniform to irregular holes.
8	Many elongated lesions of all sizes are present on most curled leaves and many medium to large, uniform to irregular holes.
9	Perforated leaves are almost destroyed.

2.4 Statistical analysis

The data on moth catches and foliar damage were subjected to descriptive statistical analysis with the dates of the evaluations and the treatments as factors. The moth's catches were analyzed using the Kruskal-Wallis test with treatment as a factor. When significant, Dunn’s method was used for multiple comparisons ($P < 0.05$). The percentage of foliar damage with a Davis scale score ≥ 3 was subjected to analysis of variance. The average proportions of mating disruption at the different sites were compared using the chi-squared test. The ear weight data, as well as the silage yield, were subjected to analysis of variance. When necessary, the means were compared using the Holm-Sidak test ($P < 0.05$).

3 Results

3.1 Mating disruption and foliar damage

Figure 2 shows the data on moth capture, foliar damage, and infestation in Cajuri during the first season. The number of moths trapped was higher in control plots during the maize cycle, with a peak in late October (Fig.2A). The median of the foliar damage in the Davis scale reached 3 in the control plot, while in the pheromone plot, the median did not exceed score 1 (leaves with no damage or only small punctures). Regarding the percentage of plants with foliar damage >3 on the Davis scale, the rates were similar in both treatments until late October. Afterward, the significant damage was higher in the control plot than in the pheromone.

The results in the Viçosa site during the first season are shown in Figure 3. Different treatments were adopted at that site, such as the association of pheromones with the grower standard, action threshold, and seed treatment. The response was homogeneous for all treatments covered by the traps, as indicated by the non-significant treatment × trap interaction for yield ($F = 1.16$, $df = 12, 60$, $P = 0.33$). The number of moths trapped in the control plot was higher, with a low population density in that field (maximum of 4 moths trapped). The foliar damage median was not different among all the treatments (score 1). The percentage of infestation in all treatments was below 20%, corroborating the low number of insects trapped (Fig. 3C).

During the second season in Canaã, the moths trapped in the control plot were higher than in the pheromone during all the crop cycle, as well as the median of foliar damage (Fig. 4A, B). The foliar damage caused by fall armyworm larvae above score 3 reached close to 50% in both treatments, then dropped as the maize developed. Figure 5A shows the number of moths trapped during the maize cycle in Coimbra. The control plot had the highest number of moths (above 100), while less than 5 were trapped in the pheromone plot. The foliar damage in Coimbra reached a score of 5 in both treatments, with no difference between the areas with and without the presence of pheromone. Accordingly, the plants with significant damage (> 3 on the Davis scale) reached more than 60% in control and pheromone plots.

In the Rio Doce location, two plots with pheromone were set up and one plot as control (Fig.6). The number of moths trapped in control was higher than in the pheromone plot between February to March months after that period one of the areas with pheromone was practically equal to control plot, reaching more than 40 moths in the peak. The median of foliar damage reached the score of 3 in the control and 2 in the pheromone plot (Fig. 6B). The percentage of plants with significant damage reached close to 80% in the control while in pheromone plots, the maximum was around 40% in one area and 22% in the other.

Figure 7 summarizes the moth capture, significant foliar damage between treatments, and overall mating disruption in the locations. The number of male captures in control was significantly higher than in the pheromone plot (H = 9.67, *df* = 1, *P* = 0.002) considering the catch in all the five locations tested (Fig. 7A). The percentage of significant foliar damage varied among the locations, ranging from less than 10% to almost 60% (Fig. 7B). In Cajuri, Coimbra, and Rio Doce the foliar damage was significantly higher in control than in the pheromone plots, while in Viçosa there was no difference between treatments. Only in the Canaã location the foliar damage was higher in the pheromone plot than in control. Figure 7C summarizes the means of mating disruption in all sites tested. All the locations reached a mating disruption above 80%, except Rio Doce, which averaged 69%. The highest value was recorded in Coimbra, with 98% of mating disruption. Despite that, no differences were found in the rates of mating disruption among the locations ($X^2 = 5.46$, $df = 3$, $P = 0.14$).

3.2 Ear quality and silage yield

Figure 8 shows the ear quality data and silage yield in three sites submitted to mating disruption. Regarding the ear damage, there was a difference among the locations ($F = 16.78$, $df = 2, 355$, $P < 0.001$) and between the treatments ($F = 14.13$, $df = 1, 355$, $P < 0.001$). In Cajuri, the ear damage was significantly higher in the control plot (1.8 cm^2) than in the pheromone plot (0.36 cm^2). In Canaã, there was no difference between the plots with and without pheromone, while in Coimbra, the damage in ears in the control was higher (3.5 cm^2) than in the pheromone plot (1.9 cm^2).

The variable ear weight was different among the locations ($F = 100.80$, $df = 2, 355$, $P < 0.001$) and between the treatments ($F = 5.92$, $df = 1, 355$, $P = 0.015$). In Cajuri and Coimbra, there was no difference between control and pheromone-treated plots, while in Canaã, the ear weight mean was higher in the area submitted to mating disruption (Fig. 9B).

There was a difference in silage yield among the sites ($F = 13.52$, $df = 2, 18$, $P < 0.001$) but not between the control or pheromone treatments ($F = 0.69$, $df = 1, 18$, $P = 0.417$). The Cajuri location had the highest silage yield (60-64 tons/ha) and Canaã the lowest (43-46 tons/ha) among the sites assessed. Despite the non-significant yield among the sites, the difference between the silage yield in the control and the pheromone areas is 2.286 tons/ha, saving at least US$135.77 per hectare (Fig. 8D).

4 Discussion

Mating disruption, a technique first adopted in the 1970s, found its roots primarily in orchards. However, its application in row crops and larger agricultural areas remains largely unexplored. The fall armyworm sex pheromone, discovered in 1967 (Sekul & Sparks, 1967; Tumlinson et al., 1986), initially served as a monitoring tool. Over the decades, as we have faced challenges like fall armyworm resistance to Bt traits and insecticides, sustainable practices using pheromone-based control are increasing for this pest (Schirmer et al., 2023). Recent advancements in synthesizing pheromones more affordably (Arnold, 2019) have opened new possibilities for deploying this technique on a larger scale.

Adapting mating disruption to crops such as maize and cotton, where fall armyworm can be a predominant threat, comes with numerous challenges. The success of mating disruption lies in its validation through laboratory and field trials. Along with safety regulations, specificity, and the limited availability of effective insecticides due to resistance in fall armyworm populations, mating disruption has proven worthy of further investigation (Rizvi et al., 2021). To the best of our knowledge, we describe the first field trials using synthetic pheromone for fall armyworm in maize.

Our recent field studies demonstrate a notable success rate in mating disruption for fall armyworm. The percentage of mating disruption was high in all tested areas, indicating the effectiveness of the pheromone-based strategy in interfering with fall armyworm reproductive behavior. This is a crucial step toward validating mating disruption in the field, where pheromone dispensers release the pheromone blend, leading to significantly higher moth captures in control plots compared to pheromone-treated areas. Other studies reported rates of mating disruption in field trials ranging from 89.5 to 93.8% for *Cydia* spp., and 85% for fall armyworm, corroborating with the high efficiency that we found in our study (Ferracini et al., 2021; Schirmer et al., 2023). This result can also be interpreted as >85% of male moths could not find mates in the treated fields. Tests with mating disruption of *Tuta absoluta* showed that this technique can be as effective as an insecticide in reducing male trapping, foliage, and fruit damage (Jallow et al., 2020). In cotton, the yield in the plots using pheromonal control for pink bollworms was higher than in control plots (Sreenivas et al., 2021).

The efficacy of mating disruption also depends on the species; in cases like pink bollworm and codling moth, the population levels within the treatment and in its periphery probably will determine if a pheromone maintains the foliar damage at a tolerable level (Carde & Minks, 1995), and this pattern likely match with fall armyworm populations. However, mating disruption will succeed if the foliar damage remains below the economic threshold (Baker & Heath, 2005). The foliar damage in locations with a minor moth density (Viçosa) was not different between control and pheromone areas (Fig. 7B). Trials conducted in low population densities may have to be run over several seasons to expose treatment differences (Eppo, 2019). In contrast, in higher densities, such as in Coimbra, the pheromone probably contributed to reduced foliar damage in the pheromone-treated plot.

Even though mating disruption can reduce the number of fall armyworm moths in the field, it may not eliminate them, and some damage will probably occur in the field (Cardé, 2007b). Accordingly, there was still noticeable damage to the plants, even in areas with high mating disruption rates. A reduction in trap capture does not always mean a reduction in mating success, so the catches cannot always be considered the only indicator of disruption success (Ferracini et al., 2021).

Mating disruption might not entirely prevent mating but can delay it, impacting the fitness and population dynamics of the insect (Baker & Heath, 2005). Delayed mating is believed to be a one-way mating disruption that regulates population growth (Jones et al., 2008). Theoretical modeling of populations under mating disruption points that regardless of a specified fertility curve, the net reproductive rate decreases due to delayed mating (Barclay & Judd, 1995). Delayed mating of females likely improves population suppression and may constitute one mechanism for mating disruption. Females who experienced delayed mating had a 32% reduction in fecundity and fertility compared with females who were not delayed (Mori & Evenden, 2013). A mechanism where mating disruption acts on the female and not only in males was found in *Lobesia botrana*, which was able to detect its synthetic pheromone and, because of that, reduced the calling and decreased the number of oviposited eggs (Harari et al., 2015).

The dispensers were kept on the field until close to the harvest, and the captures of moths were assessed for at least 50 days in Coimbra up to 99 days in Viçosa. The dispenser probably released satisfactory pheromone levels since mating disruption was detected late in the crop cycle (Fig. S1). Dispensers releasing pheromone until 80 days or more in the field have already been reported in orchards (Ferracini et al., 2021; Klassen et al., 2023). For persistent and more effective performance, the mating disruption should be practiced over extended periods and ideally in several hectares (>100 km^2) (Cardé, 2007b; Eppo, 2019). In addition, several factors can also affect the efficiency of pheromone coverage in the field, with the temperature being considered the most important and, ultimately, the airspeed, especially if the formulation is highly volatile (Nielsen et al., 2019; Tabata, 2018).

We used the membrane dispenser type (Klassen et al., 2023), which is considered passive, continuously releasing the pheromone. There are some limitations in that type of dispenser, such as the labor cost for the higher density per hectare (Vacas et al., 2016). Several studies with aerosol formulations (i.e., programmed to release pheromone in specific intervals) have been conducted in orchards primarily, being faster and cheaper to apply (Benelli et al., 2019). However, whether aerosol is as effective as passive dispensers is not well established.

The sprayable formulation seems to fit better in landscapes like the row crops, but more studies should focus on comparing both deployment methods (Klassen et al., 2023). A microencapsulated sprayable pheromone formulation was tested for *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) and was as effective as the hand-applied dispensers, reducing the labor cost and facilitating the application (Il'ichev et al., 2006). Pheromones deployed from the ground up to the crop canopy just before the start of moth emergence would be the best approach for insects that pupae on the soil to reduce the incidence of mating, such as fall armyworms (Ferracini et al., 2021). Since we have partially overcome the limiting barrier of high synthesizing cost (Miller & Gut, 2015), new technologies should now improve the deployment and formulation techniques for maintaining satisfactory pheromone levels in the field.

While the primary goal of pheromone-based mating disruption is not to prevent ear damage in the field, there were observed differences in damage between treated and untreated plots in Cajuri and Coimbra locations (Fig. 8A). Maize kernel quality, a crucial component of crop yield, can be adversely affected by kernel-destroying insects (Silva et al., 2018). Therefore, using pheromones may improve ear quality by suppressing insect populations, potentially resulting in significant economic benefits for maize growers. Even though there was no difference in silage yield between the treatments, considering the economics between both areas, the savings can average US$135.77 per hectare, which can be significant for maize growers. Farmers and policymakers must carefully consider the cost-effectiveness of implementing mating disruption on a commercial scale. Factors like the purchase and deployment costs of pheromone dispensers should be weighed against the potential benefits, including increased crop yield and reduced pesticide usage (Klassen et al., 2023). Reducing insecticide use can provide a marketing advantage for growers, potentially boosting sales, particularly for environmentally conscious customers.

In conclusion, our study highlights the potential of mating disruption as a valuable tool in fall armyworm control, achieving high rates of mating interference (Schirmer et al., 2023). Integrating tactics that combine mating disruption with other pest control methods may be necessary to mitigate crop damage fully. Future studies should explore variations in deployment techniques, timing, and dosages to refine this promising approach further. More in-depth mating/oviposition behavior and dispersal experiments are still needed and should be encouraged.

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Figures

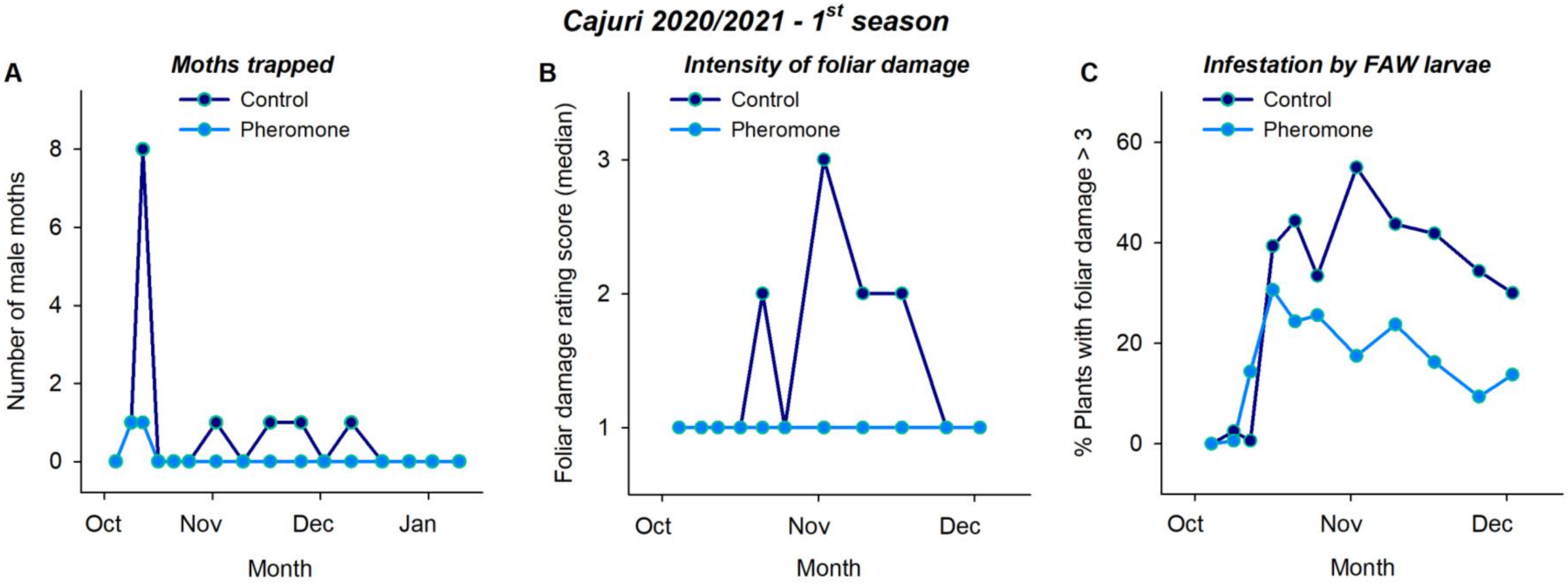


Figure 2. Mating disruption of *Spodoptera frugiperda* and its relationship with foliar damage by larvae on plants: 1st harvest 2020/2021, Cajuri, MG. Data were obtained from plants in four quadrants around each of the four traps installed in the area with each treatment (control or pheromone). A) Number of males caught in the traps. B) Median intensity of foliar damage by larvae to plants. C) Average infestation of plants with damage equal to or greater than a score of 3 on the Davis scale.

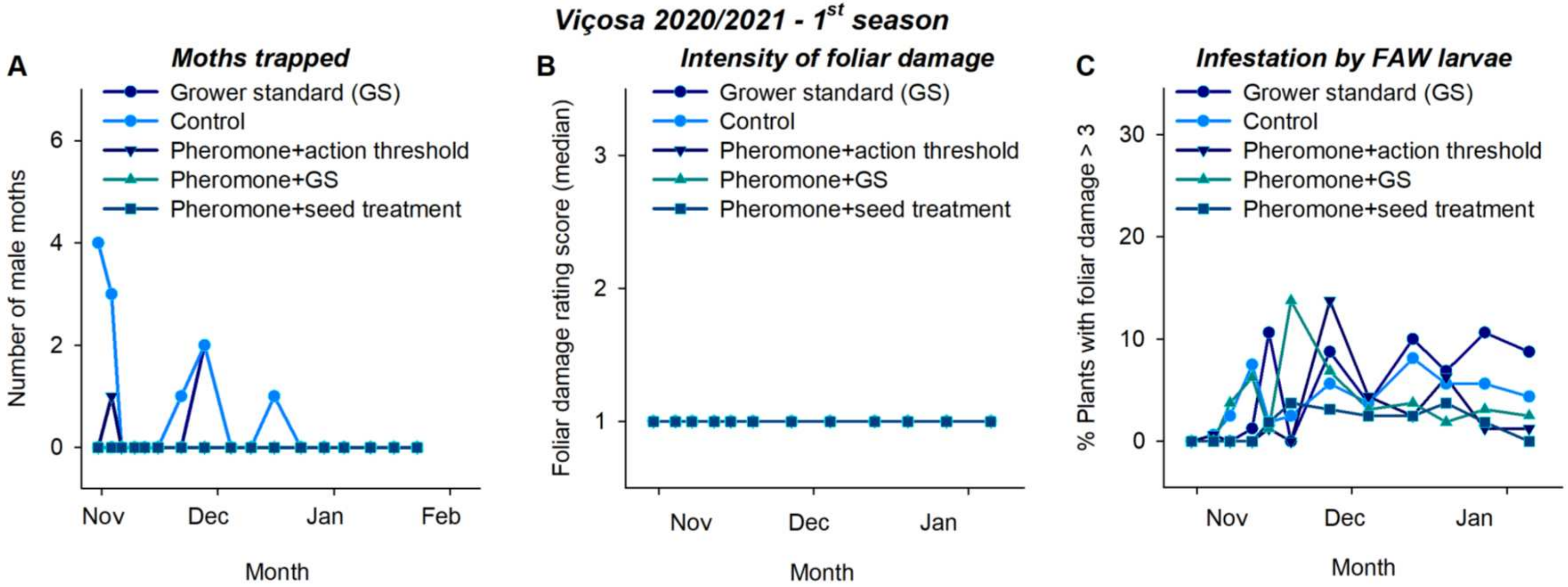


Figure 3. Mating disruption of *Spodoptera frugiperda* and its relationship with foliar damage by larvae on plants: 1st harvest 2020/2021, Viçosa, MG. Data were obtained from plants in four quadrants around each of the four traps installed in the areas with each of the treatments: Grower standard (GS), practices commonly adopted by growers - Bt Cry1A.105+Cry2Ab maize + seed treatment; Control - non-Bt corn without application of *S. frugiperda* control method; Pheromone + action threshold for insecticide application - pheromone application and insecticide intervention at 20% of plants with larvae damage; Pheromone+ grower standard, pheromone application e practices usually adopted by growers; Pheromone + seed treatment, pheromone and seed treatment only. A) Number of males caught in the traps. B) Median intensity of larvae damage to plants. C) Average infestation of plants with damage equal to or greater than grade 3 on the Davis scale.

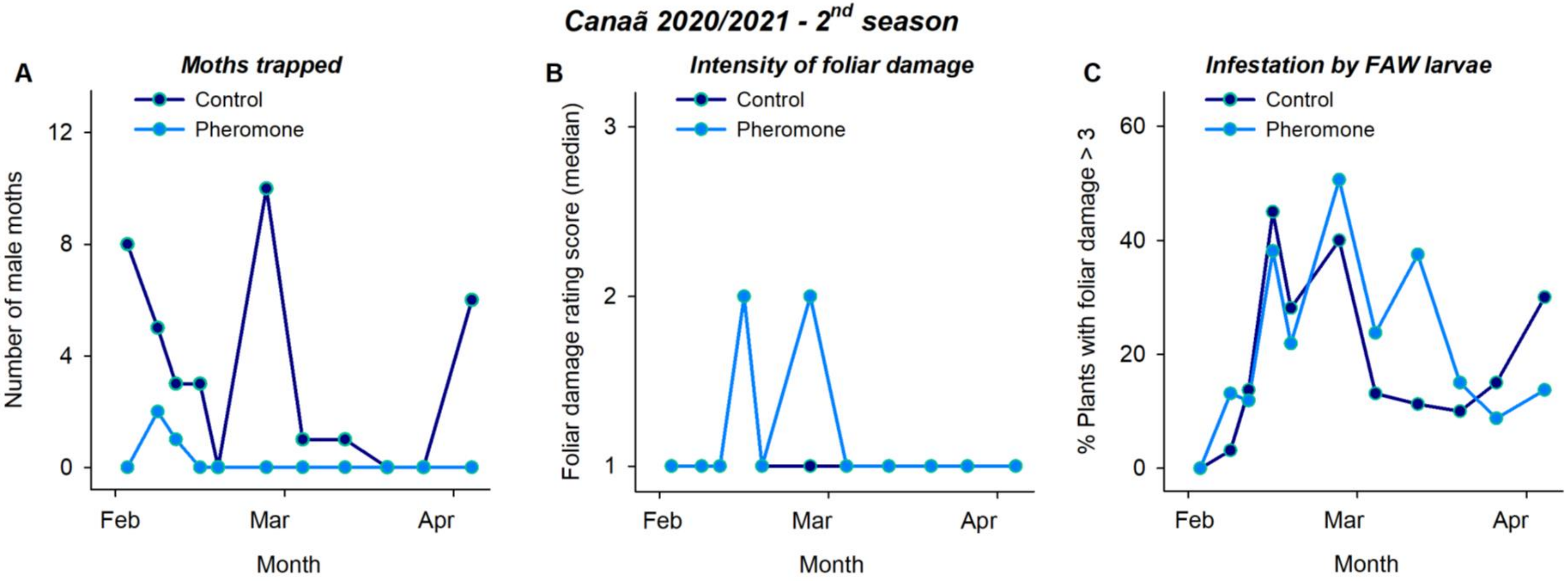


Figure 4. Mating disruption of *Spodoptera frugiperda* and its relationship with foliar damage by larvae on plants: 2nd harvest 2020/2021, Canaã, MG. Data were obtained from plants in four quadrants around each of the four traps installed in the area with each treatment (control or pheromone). A) Number of males caught in the traps. B) Median intensity of foliar damage by larvae to plants. C) Average infestation of plants with damage equal to or greater than a score of 3 on the Davis scale.

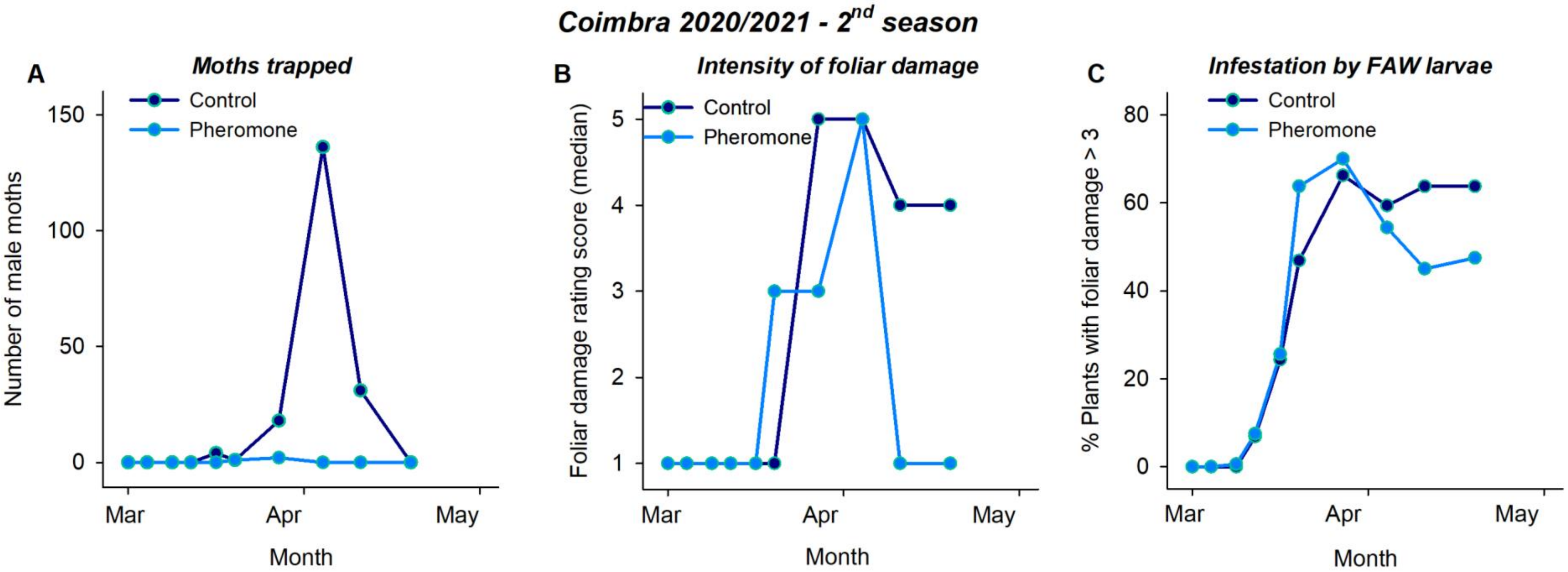


Figure 5. Mating disruption of *Spodoptera frugiperda* and its relationship with foliar damage by larvae on plants: 2nd harvest 2020/2021, Coimbra, MG. Data were obtained from plants in four quadrants around each of the four traps installed in the area with each treatment (control or pheromone). A) Number of males caught in the traps. B) Median intensity of foliar damage by larvae to plants. C) Average infestation of plants with damage equal to or greater than a score of 3 on the Davis scale.

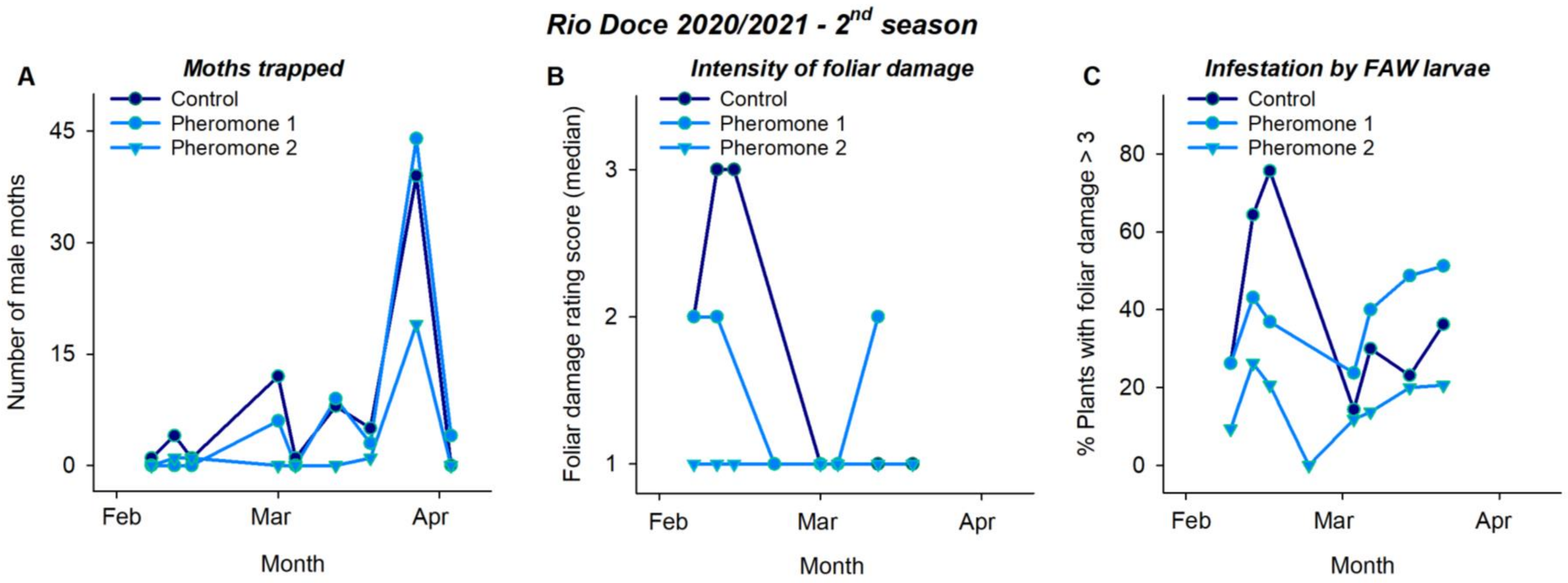


Figure 6. Mating disruption of *Spodoptera frugiperda* and its relationship with foliar damage by larvae on plants: 2nd harvest 2020/2021, Rio Doce, MG. Data were obtained from plants in four quadrants around each of the four traps installed in the areas with each treatment (control, pheromone 1, and pheromone 2). A) Number of males caught in the traps. B) Median intensity of foliar damage by larvae to plants. C) Average infestation of plants with damage equal to or greater than a score of 3 on the Davis scale.

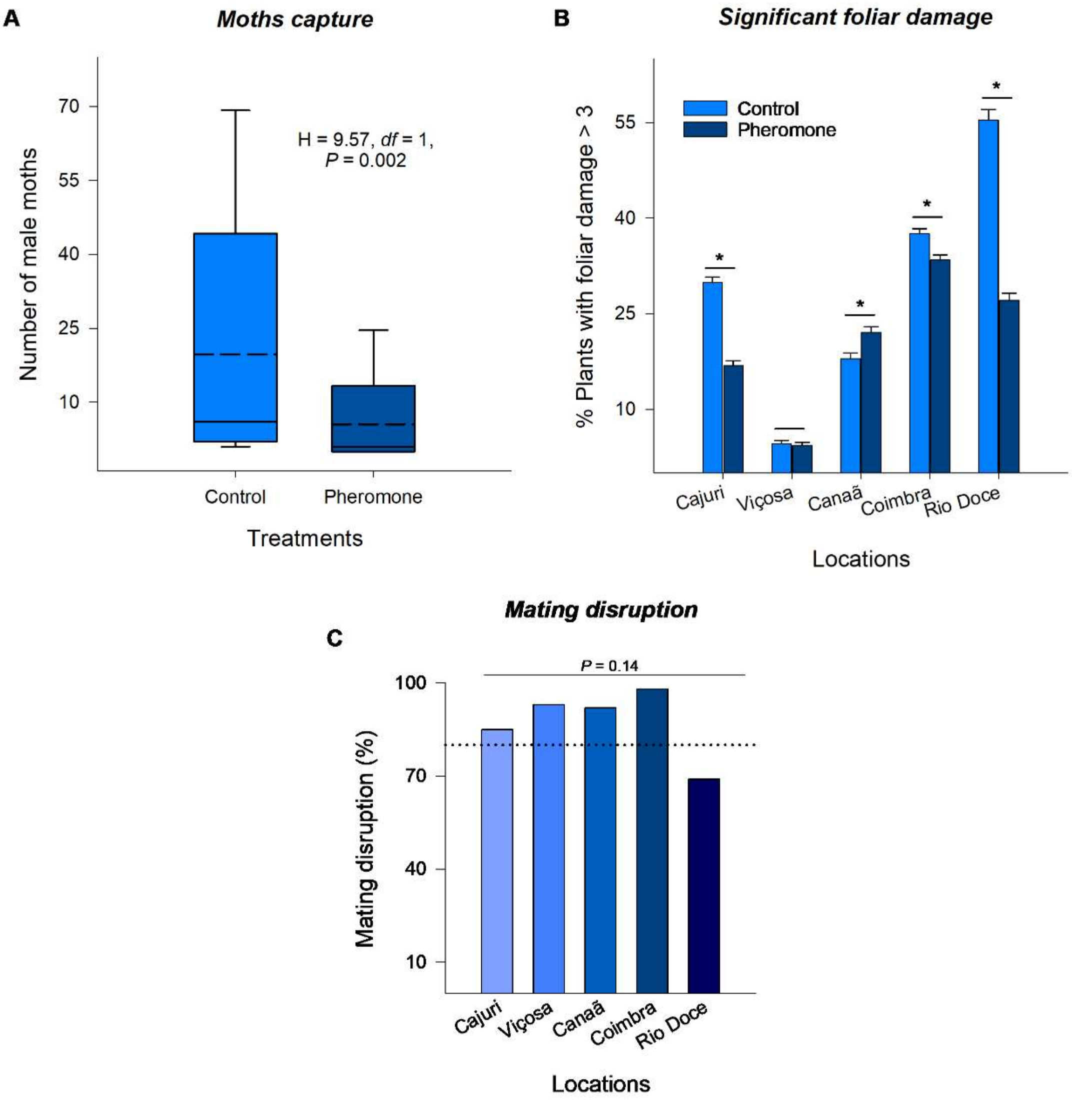
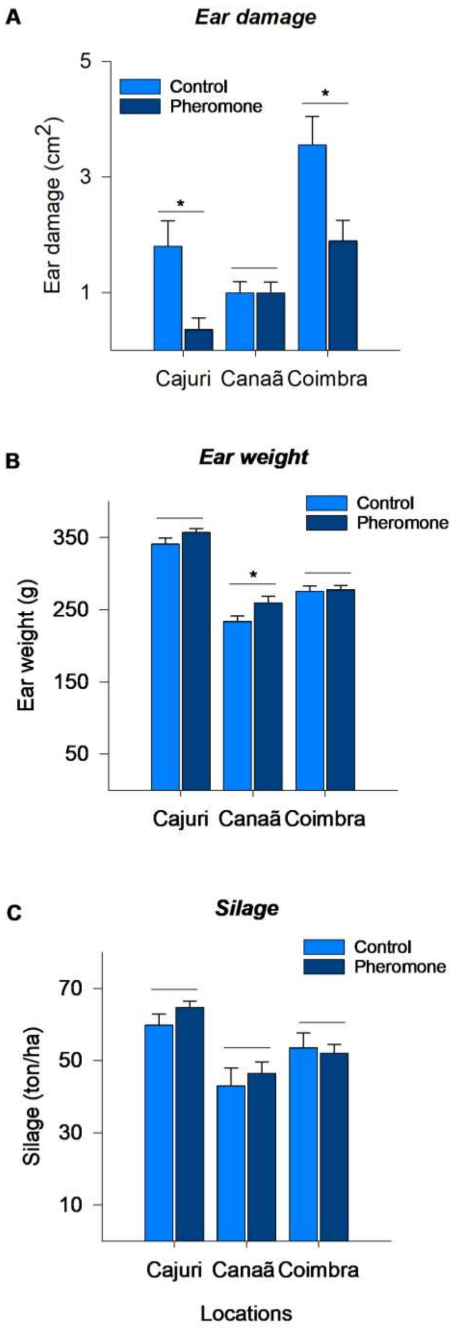


Figure 7. *Spodoptera frugiperda* male capture, foliar damage, and mating disruption. A) Males trapped in control and pheromone plots in all five locations tested. Dashed lines are means, and solid lines are median. B) Percentage of plants with foliar damage ≥ 3 score on Davis scale in control and pheromone plots. Groups with an asterisk (*) mean significantly different within the group ($P < 0.05$). C) Mating disruption average during the maize crop cycle in all locations tested. The dotted line refers to 80% of mating disruption.



D

Economics

Treatment	Silage (ton/ha)	Difference Pheromone - Control		
Control	52.134	ton/ha	R$	US$
Pheromone	54.420	2.286	685.65	135.77

Figure 8. Ear quality and silage yield in maize fields submitted to mating disruption. A) Ear damage, B) Ear weight, and C) Silage yield in different locations submitted or not to mating disruption. Data are means ± standard errors. Asterisk (*) indicates a significant difference between groups within each location ($P < 0.05$). D) Economic comparison adopting

values of R$300 per ton of silage in the local market. The data are the mean of silage yield in the three locations.

Supplementary information

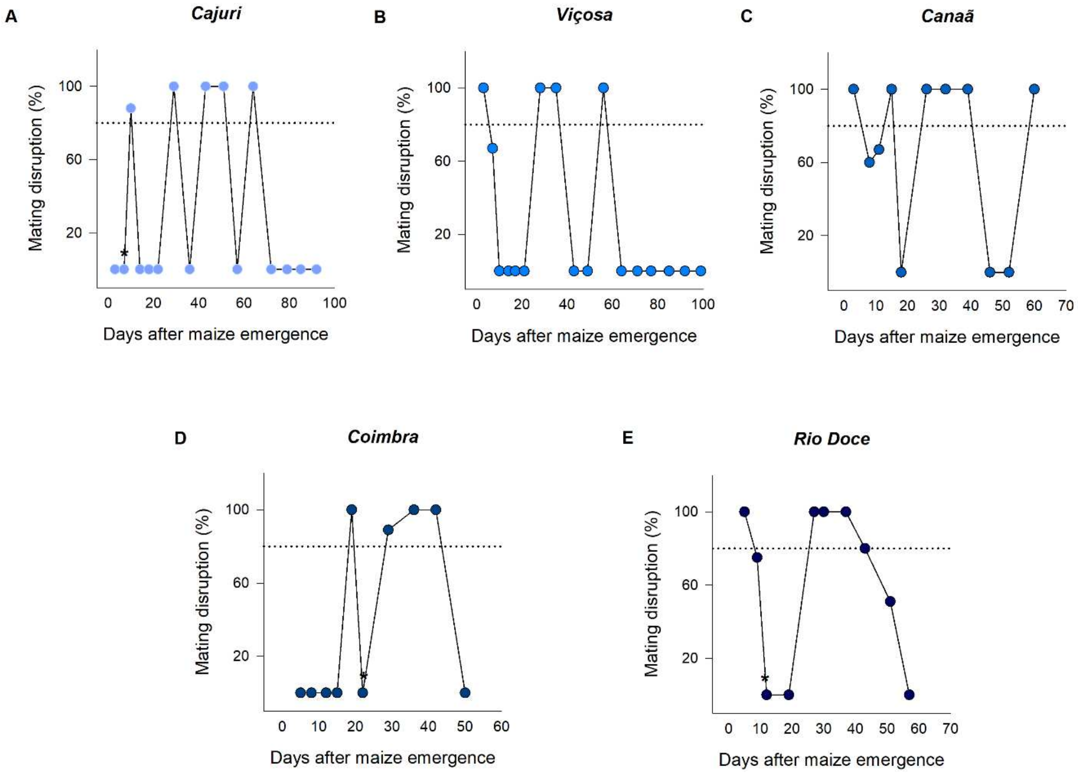


Figure S1. Mating disruption of *Spodoptera frugiperda* in maize fields. Percentage of mating disruption during maize development stage in A) Cajuri, B) Viçosa, C) Canaã, D) Coimbra, and E) Rio Doce. Data were obtained from plants in four quadrants around each of the four traps installed in the area with each treatment (control or pheromone). The dotted line refers to 80% of mating disruption. All the points located in zero on the Y axis from Figures A-E mean that there were no moths trapped, except where there is an asterisk (*), which means the catch in the control plot was equal to the pheromone plot (i.e., mating disruption = 0%).

CHAPTER 2

Phenotypic monitoring of fall armyworm populations: first steps to understand mating disruption in resistance management

ABSTRACT

The fall armyworm (*Spodoptera frugiperda*) is a significant threat to global agriculture, causing substantial economic losses and challenging pest management strategies. The escalating resistance to conventional methods emphasizes the need for innovative approaches to control fall armyworm populations. Mating disruption, a technique that interferes with insect reproductive behavior by releasing synthetic pheromones, is a promising opportunity for suppressing *S. frugiperda* populations. The objective was to monitor the susceptibility status of *S. frugiperda* populations to insecticides and Bt traits and explore the potential implementation of mating disruption as a tool in resistance management. We obtained data showing that neurotoxic insecticides continue to exhibit efficacy in most populations tested, although there was some evidence that benzoylurea compounds (growth regulators) may have lost efficacy due to potential resistance development. The Cry1A.105+Cry2Ab Bt maize caused reduced survival, while Vip3a20 maize effectively controls *S. frugiperda* populations. Analysis of life-table parameters indicated incomplete resistance to the dual-gene Bt maize in 2 out of 5 populations. There was no difference in fitness in the other three populations on non-Bt or Bt maize. The latter affected other life-history traits in the populations, with lower survival and larval weight and a higher developmental time. These findings are discussed in terms of the potential benefits and challenges in implementing mating disruption within pest management strategies. Further fundamental research and field trials should help to refine and integrate this tool into pest management programs, promoting a sustainable and resilient approach to *S. frugiperda* control.

Keywords: Bt-resistant, insecticide, life table, fitness cost, fertility.

1 Introduction

In recent years, the global agricultural landscape has faced an escalating threat by the fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae), an invasive pest that feeds on several crops (Montezano et al., 2018). The fall armyworm, native to the Americas, has rapidly spread across continents, causing substantial yield losses and economic damage (Wan et al., 2021). Conventional control methods, such as chemical pesticides and Bt crops, have been widely employed to combat the fall armyworm; however, their efficacy is increasingly compromised by resistance in pest populations (Gutiérrez-Moreno et al., 2018; Tavares et al., 2021).

The more effective and successful an arthropod pest management tactic is, the more likely it is that the pest will develop resistance to this management tactic (Onstad, 2014). Considering this, insect resistance management is a critical aspect of pest control, aiming to preserve pesticide effectiveness and prevent resistance development within insect populations (Sparks et al., 2021). The escalating concern about the emergence of resistance has driven a search for alternative and sustainable pest management strategies (Akutse et al., 2020).

No revolutionary tool can replace conventional control techniques; however, some methods can be integrated into pest management strategies. One promising is mating disruption (MD), a technique rooted in disrupting the reproductive behavior of pest species (Witzgall et al., 2010). It works by interfering with the reproductive behavior of insects by releasing synthetic pheromones that confuse male insects, making it difficult for them to locate and mate with the females (Miller & Gut, 2015). Researchers have interfered with these communication systems to decrease the number of successful matings, subsequently dropping the number of offspring (Rizvi et al., 2021). Besides preventing mating, pheromone treatment can also delay it, impacting the target pest's fitness and subsequent population dynamics (Mori & Evenden, 2013).

Mating disruption has been regarded as a potential tool to delay insect resistance (Caprio & Suckling, 1995; Suckling et al., 1990), although studies on its use in row crops and its consequences on resistance management are rare. There is no information on the potential impact of MD on the allele frequency of resistance to insecticides or Bt toxins used in transgenic cultivars. Since recessive alleles are usually found in resistant populations (Carrière et al., 2010), MD can suppress the population and reduce the chance of resistant individuals to mate.

Research suggests that applying MD with other pest management strategies, such as biologicals and Bt crops, can further enhance its effectiveness and potentially delay the onset of resistance (Karlsson Green et al., 2020; Tabashnik et al., 2013). Mating disruption can minimize dependence on conventional control methods (Witzgall et al., 2010), reducing the selective pressure that generates resistance. If resistant individuals carry a fitness cost, they may be less successful at finding partners (Zhang et al., 2015) in the presence of synthetic pheromones used to interrupt mating.

Besides the potential benefits in resistance management, the MD is also an environmentally friendly approach targeting the pest species, reducing the need for broad-spectrum pesticides that can harm non-target organisms and ecosystems (Pålsson et al., 2022). In addition, it has been shown that natural enemies can delay the evolution of insect resistance to Bt crops (Liu et al., 2014).

In previous studies on the MD effects in resistance management, the first step was to monitor resistance in potential application areas (Suckling et al., 1987). Considering this, our goal was to monitor the current susceptibility status of fall armyworm populations to insecticides and Bt traits in important producing regions of Brazil. We expected to find resistant populations of fall armyworm as there have been some complaints of control failure in fields planted with Bt maize hybrids. We discussed the potential benefits of mating disruption as a tool in resistance management. As we continue to face pest management challenges in intensive agricultural systems with a trend for reducing the reliance on conventional control methods, understanding the viability and limitations of MD becomes crucial to a future of resilient and sustainable agriculture.

2 Materials and Methods

2.1 Insect collection

Samples from field populations of *S. frugiperda* were collected in important maize or cotton-producing regions in Brazil (Table 1). The insects were placed in 50 mL plastic cups containing diet with a lid containing holes for air entry. Samples were collected from areas with Bt or non-Bt cultivars from pheromone-treated and control plots. Depending on the region, collections took place during different periods to represent the succession of intensive cultivation in one agricultural year in Brazil.

Table 1. Information on the *Spodoptera frugiperda* populations collected in the field and used for the bioassays and laboratory characterization.

Population	Collection site	Date	Crop
Bahia-CGP	São Desidério-BA	May/2021	Cotton
Bahia-PFP	São Desidério-BA	May/2021	Cotton
Ilha Solteira	Ilha Solteira- SP	July/2021	Maize
Inha-CGP	Inhaúma-MG	April/2022	Maize
Inha-PFP	Inhaúma-MG	April/2022	Maize
Maranhão	Balsas- MA	May/2022	Maize

Upon arrival at the laboratory, the plastic cups containing the larvae were incubated in an air-conditioned room (27 ± 2 °C temperature and 14 h of photophase) until the larvae reached the pupal stage. Samples of the pupae were sorted to separate the healthy individuals that started the first filial generation (F_1) in the laboratory. We established the field populations in the laboratory and characterized their susceptibility to relevant insecticides and Bt toxins used in maize cultivation.

2.2 Characterizing susceptibility to insecticides

The Insecticide Resistance Action Committee (IRAC) recommended the leaf-dip method for bioassays with some lepidopterans (IRAC, 2020). The discriminating concentration of the insecticide used was the upper limit of the label dose recommended in the field. The following insecticides were tested: flubendiamide, chlorantraniliprole and cyantraniliprole (diamides), spinetoram (spinosyn), thiodicarb (carbamate), lufenuron and novaluron (benzoylureas), as well as bifenthrin (pyrethroid) in the last populations tested (Inha-CGP, Inha-PFP e Maranhão). Non-Bt maize leaves were collected from plants at the V4-V9 growth stage and grown under favorable conditions in the field. Leaf sections of approximately 3 cm were immersed in the insecticide mixture for 5 seconds and left to dry for 60 minutes. Using tweezers, the treated leaf sections were carefully placed in 16-cell plastic trays (Advento do Brasil, Diadema, SP). A third-stage *S. frugiperda* larva (<1cm) was transferred to each cell. Four trays were used in each treatment, totaling 64 larvae. Mortality was recorded after 24, 48, 72, and 96 h, and larvae that did not move when touched with a fine brush were considered dead. All bioassays were conducted under the following laboratory conditions: 27 ± 2 °C, 70 ± 15% relative humidity, and a 14 h light: 10 h dark photoperiod.

2.3 Characterizing susceptibility to Bt toxins

The susceptibility of the fall armyworm populations to the proteins Cry1A.105+Cry2Ab, present in transgenic event MON89034, and Vip3a20, transgenic event MIR162, was determined. The BM709PRO2 hybrid, which expresses the Cry1A.105+Cry2Ab proteins and its isoline (which does not express the insecticidal proteins), and the 30F35V hybrid, which expresses the Vip3a20 protein and its isoline, were cultivated under optimal growth conditions. The maize hybrids expressing the Bt proteins commonly used and the isolines made it possible to assess the susceptibility of populations from different regions.

Maize leaves at the V4-V6 growth stage were used for the bioassays and placed in 16-cell plastic trays. Groups of 5 neonates (24 h after hatching) were placed in each cell, using 2-3 trays, totaling 160-240 larvae per treatment. Survival curves were generated from evaluations 24, 48, 72, and 96 hours after the larvae were exposed. Larvae that did not move when touched with a fine brush were considered dead. The mean survival time in each location sampled and for each Bt maize was estimated. All bioassays were conducted under the following laboratory conditions: 27 ± 2 °C, 70 ± 15% relative humidity, and a 14 h light: 10 h dark photoperiod.

2.4 Impact of Bt maize on reproductive and population growth parameters

Resistance-related costs were investigated by estimating life table parameters and other life history traits. To determine whether there is a cost associated with resistance to Bt proteins, larvae from different populations of *S. frugiperda* were exposed to maize plants expressing the Cry1A.105+Cry2Ab or Vip3a20 proteins, as well as to their respective hybrids that do not express these proteins. The exposure was through maize leaves taken from plants at the V6-V9 growth stage, cultivated as described above. Initially, five neonates were transferred to each cell of a plastic tray containing leaf sections of the maize under study. Three 16-cell trays were used, totaling 240 larvae per treatment. As the larvae developed, those that survived were individualized, and the leaves changed every two days until their larval period was complete. The pupae that survived this exposure were weighed, sexed, and used to form pairs to study the life history of the populations.

For the life table, adult couples freshly emerged from each population collected in the field (n = 15-25) were taken randomly and placed in PVC cages (10 cm high x 10 cm in diameter) and fed a solution containing 10% sugar and 5% ascorbic acid. The cages were lined with sheets of sulfite paper to provide a substrate for oviposition. Adult survival and the number of egg masses laid by each female were recorded daily until the insects died. The number of hatched neonates was counted to determine the fertility of *S. frugiperda* (Sousa et al., 2016).

The sex ratio and the number of times a female mates on average (spermatophore number) were also determined. The number of spermatophores was counted by dissecting the females after mortality and ending the experiment. Survival, longevity, and fertility data were used to estimate the demographic parameters of the populations tested. Based on the data, the net reproductive rate (R_0; female offspring produced per female), the intrinsic population growth rate (r_m; daily production of female offspring per female), and the generation time (T) were estimated. All experiments were conducted under the following laboratory conditions: 27 ± 2 °C, 70 ± 15% relative humidity, and a 14 h light: 10 h dark photoperiod.

2.3 Statistical analysis

Survival data for the experiments with insecticides and Bt toxins were analyzed using the non-parametric Kaplan-Meier procedure (Kaplan & Meier, 1958) and log-rank $\chi 2$ tests ($P < 0,05$) for the hypothesis of equality between the survival curves. For multiple comparisons, the Holm-Sidak method was used ($\alpha = 0.05$). The lethal time (LT_{50}), the mean survival time, and the confidence limits provided in the survival analysis were used to compare the insecticides among the populations.

The life-table parameters were estimated using a SAS protocol previously developed (Maia et al., 2000). This procedure obtained the net reproductive rate (R_0), the intrinsic rate of increase (r_m), and the mean generation time (T). The jackknife method estimated the variances associated with the population growth parameters (Meyer et al., 1986) using a SAS protocol, providing confidence intervals, t values, and their respective *P* values for comparison among groups. The data on larva/pupa weight and development time were subjected to analysis of variance. The means were compared using the Holm-Sidak test ($P<0.05$) when necessary.

3 Results

3.1 Fall armyworm susceptibility to insecticides

Figure 1 shows the lethal time (TL_{50}) data of insecticides tested in fall armyworm populations. For the flubendiamide, 72h was the lethal time for both Bahia and Ilha Solteira populations, while in Inha-PFP and Maranhão, it was 48h (Fig. 1A). Only in the Inha-CGP population, the lethal time was 96h, which means that it took 96h to flubendiamide kill 50% of the population (Fig. 2A). Chlorantraniliprole pattern was similar with flubendiamide, differing only in Inha-PFP population, where the lethal time was 72h (Fig. 1B). Cyantraniliprole also caused similar results, differing in Ilha Solteira lethal time (96h).

Spinetoram lethal time was 48h in most populations (Bahia CGP/PFP and Inha-CGP), followed by 24h in Ilha Solteira and Maranhão and 72h in Inha-PFP (Fig. 1D). Thiodicarb lethal time was 24h for all the populations tested (Fig. 1E). For lufenuron, the lethal time in Bahia populations was 96h, differing from the 72h in Ilha Solteira and Inha populations, and 48h in Maranhão (Fig. 1F). Novaluron only caused 50% of the mortality in Maranhão population, with a lethal time of 48h (Fig. 1G). Bifenthrin, only tested in Inha-CGP, Inha-PFP, and Maranhão, had a lethal time of 24h in all these populations (Fig. 1H).

Figure 2 summarizes the 96-h survival of fall armyworm populations in all insecticides tested. The 20% survival threshold reference line shows that the Inha-CGP population crossed the line on the three diamides tested (flubendiamide, chlorantraniliprole, and cyantraniliprole) and in novaluron. Regarding lufenuron, Bahia-CGP/PFP, Ilha Solteira, and Inha-PFP survived above 20%. All the populations, except Maranhão, survived above 50% when exposed to novaluron. For all other insecticides not mentioned, the survival rate of fall armyworm populations was below 20%.

3.2 Fall armyworm susceptibility to Bt-traits

Figure 3 shows the survival curves and mean survival time of fall armyworm populations exposed to Bt maize. The maize expressing the proteins Cry1A.105+Cry2Ab caused different curve patterns among the populations ($X^2 = 174.1$, $P<0.001$). Bahia and Inha-CGP had the highest survival rates, 0.6 and 0.43, respectively (Fig. 3A). Maranhão and Ilha Solteira did not differ between their curves, with survival of 0.30 and 0.26, respectively. Inha-PFP was the population with the lower survival rate, 0.18. The Cry1A.105+Cry2Ab maize did not cause extinction probability in any of the populations tested.

The maize expressing the Vip3a20 protein caused different curves over time ($X^2 = 149.7$, $P<0.001$), with low survival probability among the populations. Only the Ilha Solteira and Inha-CGP curves were significantly equal. However, until the end of the evaluations, all the populations were expected to be extinct when exposed to the Vip3a20 protein.

Regarding the mean survival time when exposed to the Cry1A.105+Cry2Ab and the non-Bt isoline maize, all the populations differed between these two groups, except for Bahia (Fig. 3C). For the Vip3a20 and its isoline maize, all the populations were significantly different when comparing the survival time in the transgenic or conventional Bt (Fig. 3D).

3.3 Insect life-history traits

Figure 4 and Table 2 show the life table parameters of fall armyworm populations raised in the Cry1A.105+Cry2Ab maize and its isoline. The net reproductive rate (R_0) differed among the populations, with the highest values in non-Bt maize in Bahia (87) and Maranhão (64) (Fig. 4A). Bahia and Maranhão had significant differences in R_0 when raised in Bt and non-Bt maize. In the other populations (Ilha Solteira, Inha CGP/PFP), there was no difference in that parameter between the maize genotypes. A similar pattern was observed in the intrinsic rate of increase (r_m), with the highest values in non-Bt maize in Bahia (0.18) and Maranhão (0.17) populations (Fig. 4B). Significant differences in r_m parameters were detected between the Bt and non-Bt maize in Bahia and Maranhão populations. The generation time (T) differed slightly among maize genotypes and populations, ranging from 24 to 28 days. Bahia was the only population where generation time differed between Bt and non-Bt genotypes (Fig. 4C).

Figure 5 shows some life-history traits in fall armyworm populations raised in the Bt and non-Bt maize. The larvae weight of populations varied from 240 to 380 mg, and the larvae raised on non-Bt maize had significantly lowest weight in all populations, except Inha-PFP, where no differences were found (Fig. 5A). The pupa weight ranged from 170 to 200 mg and differed between the maize genotypes in all populations. However, in Inha-CGP and Inha-PFP, the pupae from the Bt maize had significantly higher weight values (Fig. 5B). The development time to pupa varied from 15 to 20 days, with the lowest values observed when the insects were raised in non-Bt maize. Bahia, Ilha Solteira, and Inha-CGP had differences in pupal time within the maize genotypes (Fig. 5C). The development time to adulthood ranged from 25 to 30 days, and all populations excepting Inha-PFP had differences in the time to reach adult within the Bt and non-Bt maize (Fig. 5D).

The overall survival until adults varied across populations. In Bahia, the survival percentage differed when raised in non-Bt (50.41±3.23) and Bt maize (30.83±2.30). In Ilha Solteira, the survival in non-Bt was 53.75±3.95, while in Bt maize was 29.73±3.37. Inha-CGP survival in non-Bt was 40.00±3.17 and 23.43±2.37 in Bt maize. Regarding Inha-PFP, survival in non-Bt was 27.50±2.89 and 12.50±1.85 in Bt maize. Maranhão survival in non-Bt maize was 34.58±3.07 and 16.56±2.08 in Bt maize. We did not find a difference in the number of egg masses between Bt or non-Bt maize genotypes (F = 1.23 *df* = 1, 902, *P* = 0.27), nor in the number of spermatophores (F = 0.03 *df* = 1, 230, *P* = 0.85), or weight of spermatophores (F = 0.48 *df* = 1, 225, *P* = 0.49) in any of the populations tested.

4 Discussion

Since the last significant reports on the association between mating disruption (MD) and resistance management (Suckling et al., 1990), papers discussing this topic are rare or nonexistent. Back in the 1990s, the effects of mating disruption in resistant populations of *Epiphyas postvittana* (Lepidoptera: Tortricidae) were investigated. In that case, the first step was to monitor resistance in potential application areas (Suckling et al., 1987). The MD complemented an ineffective insecticide program and effectively controlled the resistant population, leading to a complete cessation of trap catch in the treated area. Pheromones have been used as part of resistance-monitoring programs for a long time (Suckling et al., 1985).

The adoption of MD can be driven by a strong laboratory and field research foundation allied to a limited insecticide/Bt crop availability due to resistance (Miller & Gut, 2015). Monitoring the susceptibility status of representative insect pest populations is imperative to ensure a successful program. A systematic monitoring approach over several harvests is a must to generate reliable empirical results. Our study characterizes fall armyworm (FAW) populations' susceptibility and population growth parameters concerning insecticides and Bt traits.

The observed lethal times (LT_{50}) among populations highlight the diverse responses of FAW to insecticides. The neurotoxic insecticides tested are still effective in most populations assessed (Fig. 2). The survival thresholds at 96 hours reveal population-specific insecticide responses, with Inha-CGP exhibiting reduced susceptibility to diamides and novaluron. The availability of several insecticide molecules can reduce the reliance on a single mode of action, minimizing the risk of rapid resistance development (Sparks & Nauen, 2015). This is an optimistic result because MD does not show promise as a stand-alone strategy (Welter et al., 2005), and combining it with other control methods, such as the rational use of insecticides and biological control agents, may enhance its overall effectiveness and sustainability (Higbee & Burks, 2021).

Benzoylurea was the only insecticide group that did not cause satisfactory mortality in most populations (<80%). One possible explanation is that the evaluation time was insufficient to detect the lethal effect due to its slower mode of action (chitin synthesis inhibitors). Besides that, a resistance case was already detected for this group in FAW (Nascimento et al., 2016). Notably, the lack of LT_{50} for novaluron in most populations (Fig. 1G) suggests a potential resistance issue, emphasizing the need to keep monitoring when using this active ingredient. We compared the insecticides in populations from areas with (PFP) and without pheromone application (CGP) to detect possible differences, but the results between these two populations were similar (Fig. 1 and 2).

Understanding the variation across populations is crucial for tailoring insecticide applications based on regional differences (Guedes, 2017). A sustainable integrated IRM strategy requires using insecticides with multiple modes of action applied in space and time (rotations and mosaics) and using insecticide mixtures in concert with as many other approaches as possible (Zhu et al., 2016).

The variable survival rates across populations suggest a potential resistance to specific Bt traits (Fig. 3). The Cry1A.105+Cry2Ab maize caused reduced survival across populations ($\leq 20\%$), with some showing higher rates (60% in Bahia and 43% in the Inha-CGP population). This indicates the existence of distinct genetic differences in FAW populations that influence their response to Bt traits. The Vip3a20 maize showed the potential to cause extinction in all populations, remaining effective in controlling FAW (Fig. 3B). Mating disruption can be integrated into Bt resistance management by introducing non-lethal stress that complements Bt traits, slowing down the reproductive capacity of resistant individuals (Mori & Evenden, 2013). In cotton, the sprayed pheromone reduced FAW damage and brought benefits along with using Bt cotton, indicating that MD is an excellent matching tool to other existing control techniques (Schirmer et al., 2023). As Bt crop varieties are widely planted, the risk of resistance increases, and MD, with its non-lethal nature, can contribute to preserving the efficacy of Bt traits over time (Sreenivas et al., 2021).

Mating disruption can influence key life table parameters by reducing the fitness of resistant individuals as an additional stressor, complementing Bt traits. By disrupting mating signals, this approach can decrease the net reproductive rate (R_0) and intrinsic rate of increase (r_m), potentially slowing population growth. The life table parameters showed that only 2 out of 5 populations (Bahia and Maranhão, located in the Matopiba region) have evidence for incomplete resistance to the dual-gene Bt maize. Incomplete resistance is the resistance in which fitness is lower for resistant individuals exposed to a pesticide relative to resistant individuals not exposed to the pesticide (Tabashnik et al., 2014). Bahia and Maranhão had differences in R0 and r_m between the maize genotypes, which are important parameters in determining population growth (Fig. 4A, B). In the other three populations, insect fitness was not different when exposed to Bt or non-Bt maize. We could not carry out the life table for Bahia-CGP and Bahia-PFP due to colony maintenance problems, so the life table was only set for one joint population (Bahia).

In some cases, resistance brings disadvantages such as altered mating behavior or diminished reproductive capacity (Zhang et al., 2015; Zhao et al., 2009). If adaptative costs are present in the population, the insects carry disadvantages when not exposed to the stressor (Bt maize). Strong fitness costs to the dual-gene Cry1A.105+Cry2Ab2 Bt maize were reported in fall armyworm (Santos-Amaya et al., 2020). In the same way that these costs reported were magnified on less-suitable host plants, the pheromone would act as an additional stressor, reducing the chance of successful matings. The cost can also have phenotypic plasticity, appearing in some environments but not others (Onstad, 2014). In scenarios with cost, natural selection may help maintain or restore the insect susceptibility to the Bt proteins (Paddock et al., 2021; Santos-Amaya et al., 2020). There is a discussion about the return to susceptibility in resistant populations under mating disruption. The susceptibility is a finite and valuable resource that needs to be conserved to maintain the success of control strategies over time (Carrière et al., 2020). The susceptibility of pests to Bt is considered a common property resource, where the political goal is to prevent the depletion of this resource. In countries such as Australia, India, and the USA, their respective governments have declared pest susceptibility in Bt crops a public good (Carrière et al., 2020).

Regarding the other FAW life-history traits, the larva and pupa weight tended to be lower in Cry1A.105+Cry2Ab2 Bt than in non-Bt maize in most populations, strengthening the dual-Bt protein effect (Fig. 5A, B). The overall survival until adulthood was higher in non-Bt maize than in Bt maize in all populations. The differences in time development between insects originating from Bt and non-Bt maize (Fig. 5C, D) may result in developmental asynchrony, which may lead to assortative mating in the next generations, likely accelerating the rate of resistance evolution (Anilkumar et al., 2008; Blachford & Agrawal, 2006). However, assortative mating fitness differences will favor restoration of susceptibility in the absence of the stressor treatment. Besides that, the delayed mate that MD can cause has a high impact on female fitness.

Females have a limited window for mating and detecting suitable oviposition hosts, and any delay in the mating process can significantly impact female fitness (Jones & Aihara-Sasaki, 2001). Such delays in mating can lead to diminished fecundity and/or fertility as older females experience arrested oocyte production, potential egg resorption before oviposition, and a reduced ability to store sperm (Torres-Vila et al., 2002). The timing of a female's initial oviposition period is also crucial in shaping the population growth rate (Jones et al., 2008). Moreover, natural mortality before mating may decrease the overall number of females, further improving the efficacy of MD (Barclay & Judd, 1995). Mating disruption may also exacerbate the population density of multivoltine species (i.e., fall armyworm) as the number of individuals able to mate in each subsequent generation is reduced (Mori & Evenden, 2013).

In summary, insect mating disruption could help manage pesticide resistance problems, which are reported here. However, taking a proactive stance by incorporating the technique into pest management programs (Tabata, 2018) before control issues arise would be a more forward-thinking approach to resistance management (Suckling & Karg, 1997). By interfering with their mating behavior, MD limits the reproduction of resistant individuals, reducing the likelihood of passing on resistance genes to the next generation. Additional studies addressing pest spatial dynamics and resistance genetics are still needed to refine the integration of mating disruption and resistance management.

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Figures and tables

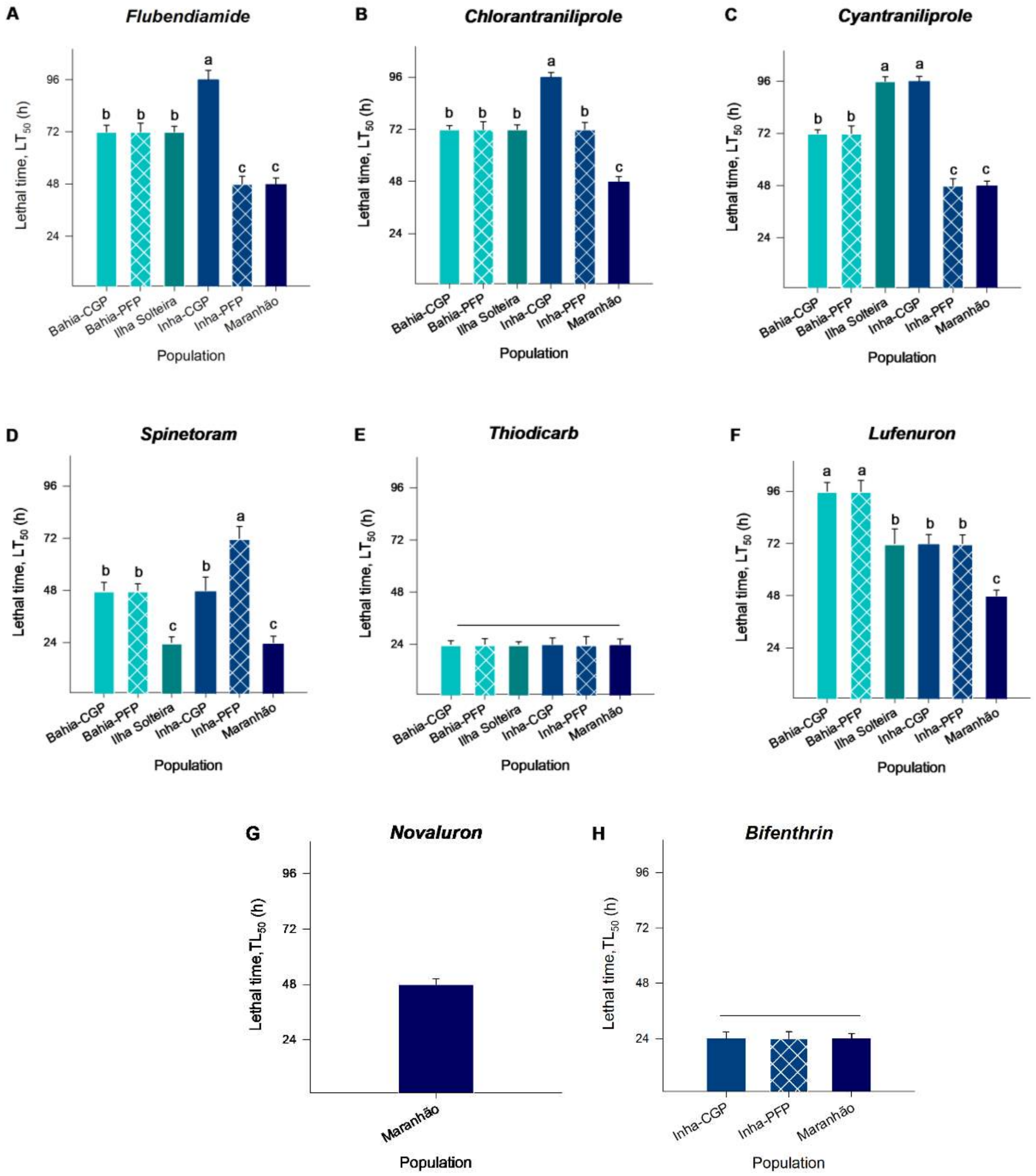

Figure 1. Lethal time in hours (LT_{50}) of insecticides in fall armyworm (*Spodoptera frugiperda*) populations. A) Flubendiamide, B) Chlorantraniliprole, C) Cyantraniliprole, D) Spinetoram, E) Thiodicarb, F) Lufenuron, G) Novaluron, H) Bifenthrin. Data are means ± standard errors. Groups with different letters indicate a significant difference ($P < 0.05$). Groups with straight bars are not significantly different ($P > 0.05$).

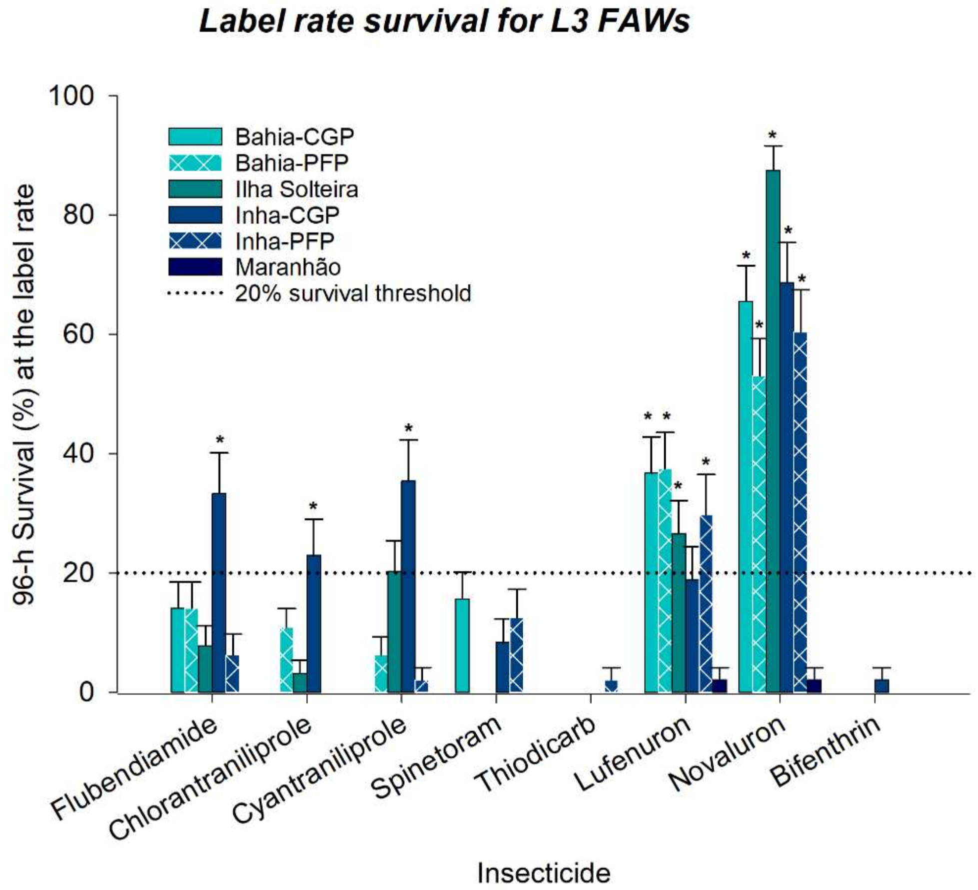

Figure 2. Summary of fall armyworm (*Spodoptera frugiperda*) populations survival to different insecticides. Asterisk (*) means insecticides that caused less than 80% mortality (or 20% survival).

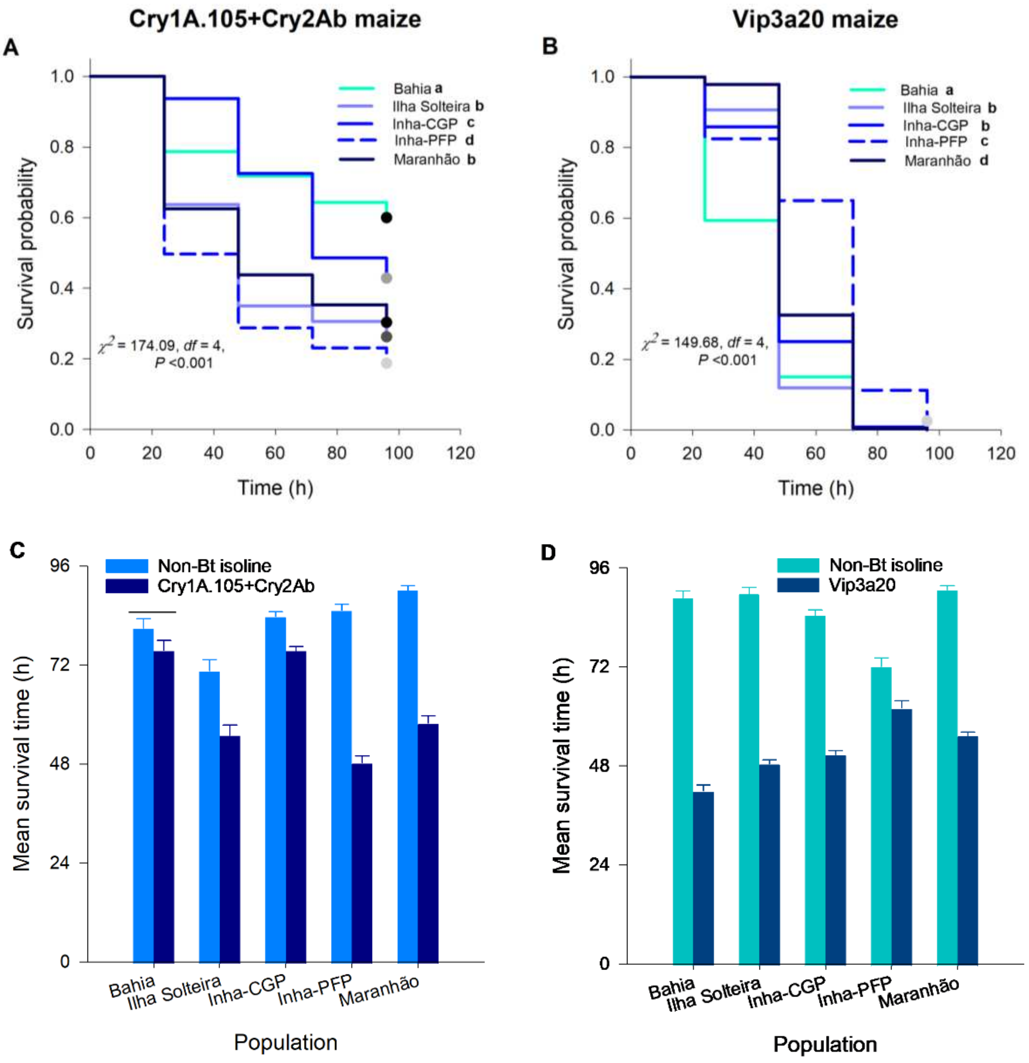

Figure 3. Survival curves and mean survival time (h) of fall armyworm (*Spodoptera frugiperda*) populations exposed to maize A, C) Cry1A.105+Cry2Ab and B, D) Vip3a20. Curves with different letters indicate a significant difference ($P < 0.05$). Data are means ± standard errors. Groups with straight bars are not significantly different ($P > 0.05$).

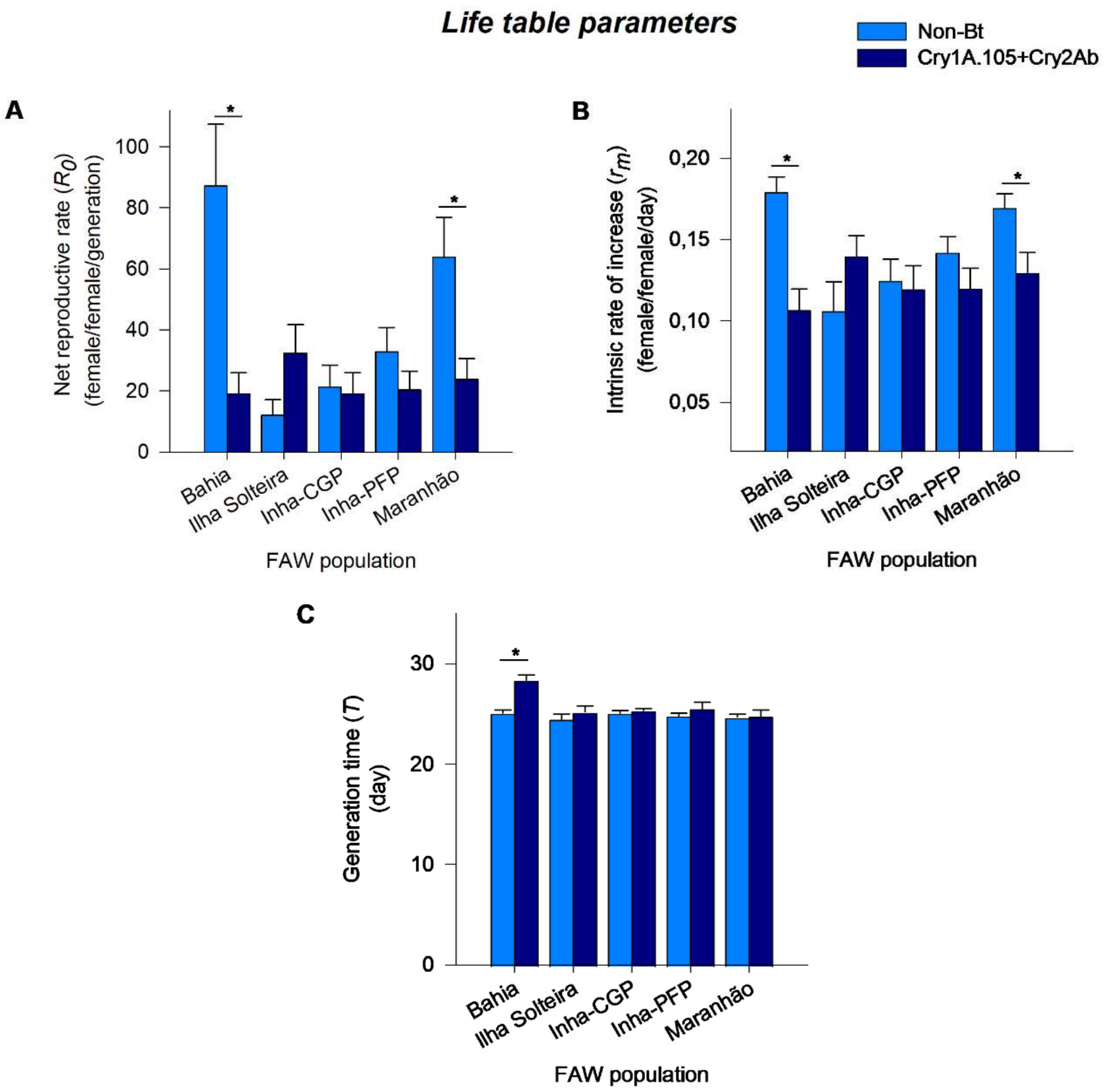

Figure 4. Life table parameters of fall armyworm (*Spodoptera frugiperda*) populations raised on Bt (Cry1A.105+Cry2Ab) and non-Bt maize. A) Net reproductive rate (R_0), B) Intrinsic rate of increase (r_m), C) Generation time (T). Data are means ± standard errors. Groups with an asterisk (*) mean significantly different ($P < 0.05$).

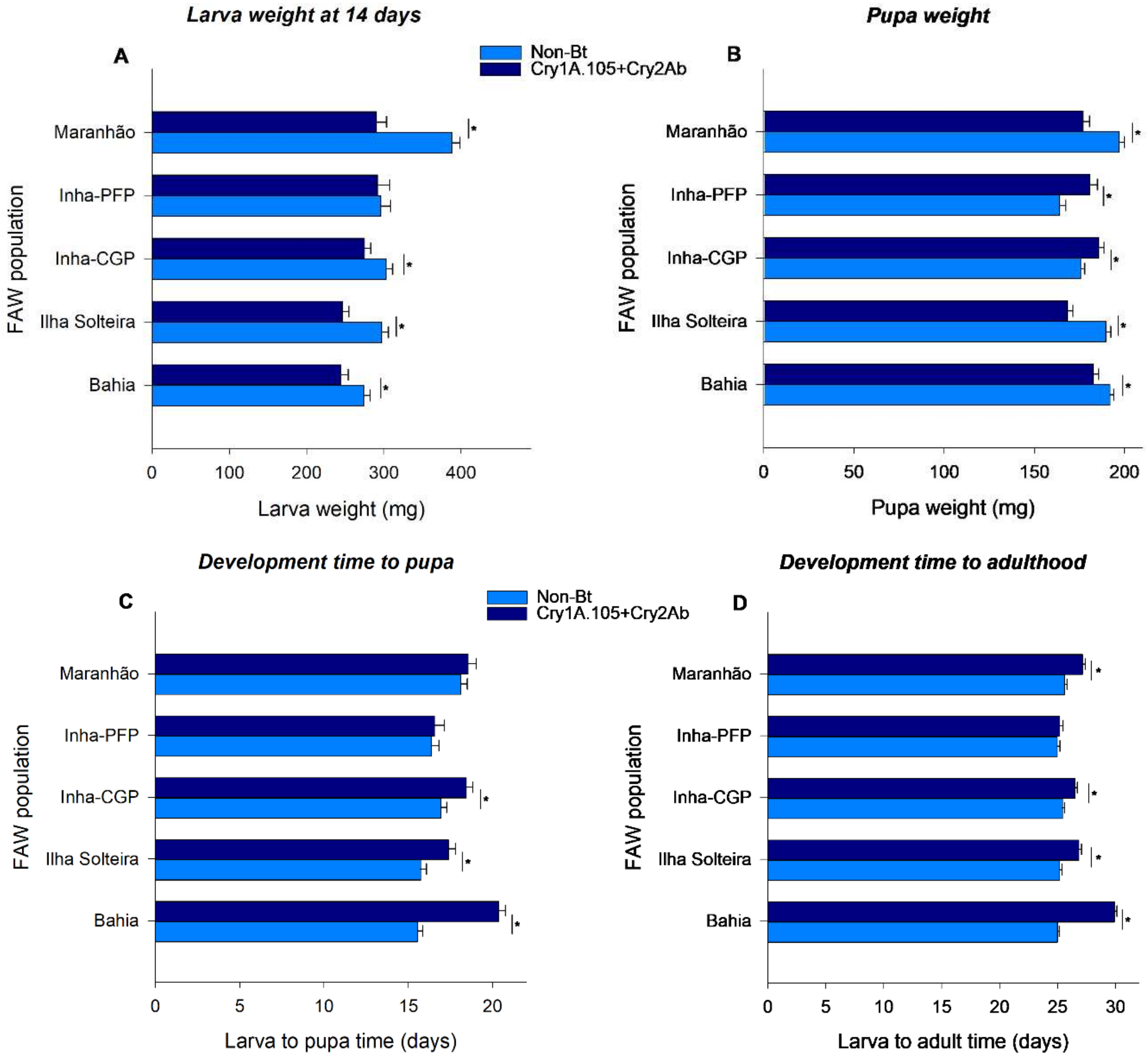

Figure 5. Life-history traits of fall armyworm (*Spodoptera frugiperda*) populations raised on Bt (Cry1A.105+Cry2Ab) and non-Bt maize. A) Larva weight at 14 days, B) Pupa weight, C) Time development until pupa, D) Time development until adult. Data are means ± standard errors. Groups with an asterisk (*) mean significantly different ($P < 0.05$).

Table 2. Life-table parameters of fall armyworm (*Spodoptera frugiperda*) populations raised in dual-gene Bt maize (Cry1A.105+Cry2Ab2) and its non-Bt isoline. Population growth statistics for each population in each maize genotype are shown.

Fall armyworm population	Population growth parameter*	Maize genotype		*P*
		non-Bt isoline	Cry1A.105+Cry2Ab	
Bahia	R_0	87.28 ± 20.13	19.34 ± 6.58	<0.01
	r_m	0.179 ± 0.010	0.107 ± 0.013	<0.01
	T	25.10 ± 0.313	28.37 ± 0.506	<0.01
Ilha Solteira	R_0	12.30 ± 4.92	32.56 ± 9.11	0.9706
	r_m	0.106 ± 0.018	0.140 ± 0.013	0.9339
	T	24.50 ± 0.486	25.21 ± 0.608	0.1828
Inha-CGP	R_0	21.53 ± 6.96	19.34 ± 6.63	0.4106
	r_m	0.124 ± 0.013	0.119 ± 0.015	0.3985
	T	25.10 ± 0.240	25.35 ± 0.168	0.1971
Inha-PFP	R_0	33.08 ± 7.68	20.56 ± 5.94	0.1030
	r_m	0.142 ± 0.010	0.120 ± 0.013	0.0883
	T	24.83 ± 0.223	25.56 ± 0.616	0.1396
Maranhão	R_0	64.03 ± 12.90	24.06 ± 6.51	<0.01
	r_m	0.169 ± 0.009	0.130 ± 0.013	<0.01
	T	24.69 ± 0.265	24.81 ± 0.566	0.4252

***** Net reproductive rate (R_0), intrinsic rate of increase (r_m), and generation time (T). Data are means ± SE and *p* values for one-tailed t-tests on the reduced fitness hypothesis (i.e., reduced R_0 and r_m; increased T) in Bt-maize raised insects.

CHAPTER 3

Potential of nitrogen-15 isotope as a marker to track insect movement between crops

ABSTRACT

Understanding insect movement patterns between host plants is crucial for effective pest management and crop protection strategies. Nitrogen-15 (^{15}N) is a stable isotope that plants can uptake through fertilization. The ^{15}N can be used as a marker to track insect movement between agricultural fields. By applying ^{15}N-enriched fertilizers to specific crop areas, we can label plants with a distinct isotopic signature, allowing their transfer between the host plant and its herbivore. ^{15}N-enriched ammonium-nitrate doses (0.1 and 1 grams/liter) were applied in Almond and Pistachios trees as a liquid solution, and unsprayed plants were used as a control. The trees received three applications every two weeks, starting 2-4 weeks after the fruit set. Leaf and nut tissue samples from almonds and pistachios were collected 1, 5, 10, and 20 weeks after the first spray. At hull split (almond) and hull slip (pistachio), cages with nut clusters were inoculated with five mated *Amyelois transitella* females. Moths were allowed to oviposit onto the nuts, which were collected four weeks later to collect emerging *A. transitella* adults to test for the ^{15}N marker. Stable isotope analysis with mass spectrometry was performed to quantify ^{15}N levels in plant tissues and insects. The percentage of ^{15}N atoms varied depending on the dose of isotope solution applied, with higher doses resulting in increased atom percentages. The ^{15}N levels in almond and pistachio tissues were significantly higher in treatments sprayed with the isotope than in the control. The overall ^{15}N levels in both crops peaked after the third spray, tending to decrease after that. The moths originating from isotope-treated plants absorbed the labeled nitrogen isotope into their tissues, although a non-significant difference was found among insects from treated and control plants. Exploring ^{15}N as a marker for tracking insect dispersal among hosts is a promising tool for advancing our understanding of pest dynamics in agricultural ecosystems.

Keywords: stable isotope, ecology behavior, orchards, navel orangeworm.

1 Introduction

Dispersal is defined as an intergenerational movement, displacement from a natal habitat, or movement between breeding habitats (Asplen, 2018). The diverse agricultural landscapes provide a temporal and spatial concentration of resources, making them attractive to insects from various distances (Dingle & Drake, 2007). Noctuids are generally considered strong fliers, capable of dispersing at nighttime and in the direction of winds (Lewis & Taylor, 1964). These insects exhibit variable movement patterns in response to different weather fronts, enhancing their dispersal capabilities. Although the migration of lepidopterans species has been well-documented (Westbrook et al., 2016; Zhan et al., 2014), less is understood about the dispersal behavior of insect pests within agricultural landscapes (Tavares et al., 2019). Tracing methods are required to comprehend insect pest dispersion, and the stable isotope technique stands out as one of the most promising.

Nitrogen-15 (^{15}N) isotope is a heavy form of the element that is taken up and translocated in plants as the common nitrogen (^{14}N). It can be naturally present at low concentrations (0.36%), and it can be applied as a fertilizer solution and then distributed throughout plants (Fry, 2006b). The isotope signature is often allocated to reproductive plant structures (Ledgard & Smith, 1992). The natural abundance of ^{15}N may be used as a natural signature of populations. However, using ^{15}N to enrich host plants generates a robust isotopic signature that is easily traceable in laboratory and field studies (Hood-Nowotny and Knols 2007). Nitrogen isotopes can be supplied to plants through various methods, including foliar spraying, soil irrigation, or trunk injections (Porras et al., 2020). When foliar-spraying isotope solutions, nitrogen enters the plant through the epidermis, incorporating ^{15}N into metabolic processes and distributing it throughout the plant tissues (Gauthier et al., 2013). Consequently, the herbivores consuming the tissues can uptake the isotope signature, making it possible to track them.

Navel orangeworm (NOW), *Amyelois transitella* (Lepidoptera: Pyralidae), is a polyphagous and major pest in almond and pistachio crops, leading to substantial economic losses. Due to the staggered phenology of the tree crops, almond and pistachio orchards may act as reservoirs for adult navel orangeworm, potentially leading to spillover into other hosts (walnuts, figs) (Burks & Brandl, 2004). Understanding the movement patterns of this pest between orchards is critical for effective pest management strategies. The isotope technique has been successfully used to mark many different types of insects (Caudill, 2003; Hamer et al., 2014), including Lepidoptera (Hobson et al., 2017), and application of markers to trees and vines (Han et al., 2017; Neilsen et al., 1997; Porras et al., 2020). Moreover, a study has already demonstrated the uptake of ^{15}N by navel orangeworm (Steffan et al., 2001).

Through feeding activities, the insect pest incorporates the ^{15}N-enriched plant material into their tissues, allowing for detecting and quantifying ^{15}N levels in insect samples collected from different treatments (Porras et al., 2020). When ^{15}N-enriched fertilizers are applied to selected crop patches, the following step is monitoring insect populations in adjacent untreated areas (Quinby et al., 2020). Studies investigating isotope tracing techniques in navel orangeworm have not been established. Characterizing the pest spillover dynamics is critical to improving our understanding of navel orangeworm dispersal into adjacent orchards.

Here, we examine the potential of ^{15}N as a tracer for studying navel orangeworm movement dispersal between crops. By applying different application rates of ^{15}N in almond and pistachio trees, we measured the transfer of this marker to navel orangeworm adults that emerge from the nuts of labeled trees. In the last instance, we want to use the ^{15}N to mark multiple orchard blocks and measure navel orangeworm dispersal outward into surrounding orchards. Future directions and applications in other crops and insect pests are discussed.

2 Materials and Methods

2.1 Insects and study areas

The navel orangeworm (NOW) mated moths used for this experiment were provided by the U.S. Department of Agriculture (USDA), San Joaquin Valley Agricultural Sciences Center in Parlier, CA. The local "Phoenix" strain was established in 2018 from NOW larvae from the USDA APHIS mass-rearing facility in Phoenix, AZ. The NOW colony was raised on an artificial wheat bran diet (Tebbets et al., 1978) at 26°C in a 14:10 (L:D) photoperiod. Newly emerged female moths were set up to mate one night before being used on the inoculations. The almond and pistachio orchard areas were at the UC Kearney Agricultural Research and Extension Center, Parlier, CA.

2.2 Isotope application

Two ^{15}N doses (0.1 and 1 grams/liter) were evaluated, and unsprayed plants were used as a control (Table 1). ^{15}N was applied in the spring as a liquid solution of ^{15}N-enriched ammonium-nitrate (Cambridge Isotope Laboratories, Inc.). Three treatments in total were tested, with a high and low rate of ^{15}N isotope plus the control. The experiment was arranged in a randomized block design in each orchard with four replications (blocks). Three trees were used per treatment, and the spray focused on the middle tree for each block. The middle trees received three total applications of 2 liters each per tree every two weeks starting 2-4 weeks after fruit set (i.e., beginning April 1 in almonds and May 15/2022 in pistachio). A backpack fogger sprayer (Tomahawk, 4-gallon tank, 220 mph air speed) was used for applications. A parallel set of control trees received a water-only application. The treated trees from different treatments were spaced at least 10 feet apart to avoid cross-contamination.

Table 1. Information on the crops, treatments, and rates for the ^{15}N isotope experiments.

Crop	**Label**	**Treatment**	**Rate**
Almond Pistachio	Control	Water	--
	Low	^{15}N enriched ammonium nitrate	0.1 g/L
	High	^{15}N enriched ammonium nitrate	1 g/L

2.3 Sampling and isotope analysis

Following the first application of the ^{15}N marker, leaf, and nut tissue samples were collected from each tree 1, 5, 10, and 20 weeks later (the final collection from the almond was in late August, and the pistachio in late September/2022). Almond and pistachio clusters were caged early in the season using window screen material. At hull split (almond) and hull slip/tatter (pistachio), cages were inoculated with five mated (gravid) females NOW. Moths were allowed to oviposit onto the nuts, which were collected four weeks later to collect emerging NOW adults to test for the ^{15}N marker. All plant and insect materials were dried at 60°C for 72 hours and then stored at -20°C. Samples of each material were weighted in tin capsules, placed in a 96-cell plate, and shipped to the isotope analysis facility. Single isotope (^{15}N) analyses were carried out by the UC Davis Stable Isotope Facility. Data were analyzed to determine differences in the concentration of ^{15}N between different plant parts and dilutions over time and, most importantly, between NOW adults reared from nuts on the marked and unmarked trees.

2.4 Statistical analysis

We tested normality (Shapiro–Wilks test) and homogeneity of variance (Levene's test); however, the data sets did not meet the assumptions for parametric analysis, and alternative non-parametric methods were employed. The data on the percentage of ^{15}N atoms in each crop tissue and the moths were analyzed with the nonparametric Kruskal-Wallis chi-squared test, followed by post hoc pairwise comparisons with the Wilcoxon test. The ^{15}N atom levels (disregarding material type) over time were plotted for each crop. The stable isotope ratios are expressed in atom percent (absolute number of atoms of an isotope in 100 atoms) because this notation is generally recommended when dealing with enriched samples (Fry, 2006a). The delta notation (δ ^{15}N (‰)) is also commonly found in literature. All statistical analyses were performed in R (v. 4.2.2, CRAN project).

3 Results

3.1 ^{15}N uptake in almond tissues

Figure 1 shows the ^{15}N atom percentage in almond tissues. The percentage of ^{15}N atoms in nuts (Fig. 1A) and leaves (Fig. 1B) of almonds sprayed with the isotope solution was significantly higher than in the unsprayed plant tissues (nut: $H = 32.07$, $df = 2$, $P < 0.0001$; leaf $H = 38.78$, $df = 2$, $P < 0.0001$) (Table 2). The natural ^{15}N content (control) ranged around 0.36% in nut and leaf materials. The ^{15}N levels in the low dose varied from 0.37 to 0.38%, and the high dose varied from 0.37 to 0.67%. All the treatments (control, low, and high) differed from each other both in nuts and leaves tissues ($P<0.05$) (Fig. 1A, B). The ^{15}N overall levels in almond tissues over time are shown in Figure 4. In the first collection (one week after the first spray), the ^{15}N levels started to rise, reaching the peak in the high treatment after the three isotope applications (Fig. 4A). The atom percentage then decreased and stabilized in the third and last collection (10 and 20 weeks after the first spray, respectively).

3.2 ^{15}N uptake in pistachio tissues

The ^{15}N atom percentage in pistachio tissues is shown in Figure 2. ^{15}N atom level in nuts (Fig. 2A) was significantly higher from control and low treatments (H = 30.96, *df* = 2, $P < 0.0001$) (Table 2). The atom percentage in pistachio leaves (Fig. 2B) was also different from control and low treatments (H = 33.70, *df* = 2, $P < 0.0001$) (Table 2). The natural ^{15}N content in unsprayed tissues was similar to almond levels (0.36%). The ^{15}N levels in the low dose varied from 0.37 to 0.63%, and the high dose varied from 0.37 to 0.91%. The treatments (control, low, and high) differed from each other in pistachio nuts and leaves ($P<0.05$). In general, the range of the ^{15}N atom percentage was higher in pistachio than in almonds, especially on the leaves (Fig. 2). The overall ^{15}N levels in pistachio tissues over time are shown in Figure 4. The isotope levels started to rise in low and high treatments after the first spray, increasing it until the third spray. In both low and high treatments, the ^{15}N levels reached a peak during the second collection (5 weeks after the first spray) and started to decrease during the third collection (10 weeks after the first spray) (Fig. 4B).

3.3 ^{15}N isotope transfer to moths

Figure 3 shows the percentage of ^{15}N atoms in moths that developed on the almond and pistachio trees. The ^{15}N atom levels were higher in moths collected in plants sprayed with the isotope than in control plants (Fig. 3). However, the atom percentage was not different among the treatments (H = 3.06, *df* = 2, $P = 0.21$) (Table 2). The natural ^{15}N content in moths from the control treatment was similar to the plant tissue results (~0.36%). The ^{15}N levels in moths from the low dose ranged from 0.36 to 0.60% and varied from 0.36 to 0.68% in the high dose.

4 Discussion

Understanding how pests disperse is essential for developing effective pest management strategies. Information on pest movement patterns, including seasonal migration, crop dispersal, and colonization of new habitats, helps implement timely interventions to prevent or minimize crop damage (Mazzi & Dorn, 2012). The isotope tracing technique is one of the most promising tools to help understand insect pest dispersion (Quinby et al., 2020). This study demonstrated that ^{15}N-enriched ammonium nitrate caused a significant increase in the isotope atoms in sprayed trees. The ^{15}N levels varied depending on the dose of isotope solution, with higher doses resulting in increased atom percentages (>0.36%) since the relative atom abundances in nature are approximately 0.36% (Ostrom & Ostrom, 1998). The percentage of ^{15}N atoms in almond nuts and leaves showed a dose-dependent response, with low and high treatments significantly higher than unsprayed plants (Fig.1). The high treatment was approximately 1.31 times higher in the atom percentage than the control. These results indicate successful uptake and incorporation of the labeled nitrogen isotope into almond tissues.

Similarly, studies in grapes showed an increase of the ^{15}N levels in the trunk and leaves for four weeks (Porras et al., 2020). The target invasive pest (spotted lanternfly) raised on grapes also assimilated the isotope in all the body parts, confirming the method's feasibility. This latter study showed higher ^{15}N atom levels than in the present work (around 4% in leaves and 10% in trunks). The reason is probably due to the increased spray number and the collections performed four weeks in a row. In our study, the collections were until 20 weeks, which means that the ^{15}N isotope remained traceable up to 140 days after the first application (Fig. 4). Since nitrogen fertilizers are the most important in tree crops (Sperling et al., 2023), long-term isotope application can be achieved through fertilization.

Similar to almonds, pistachio tissues also exhibited a significant increase in the percentage of ^{15}N atoms in both nuts and leaves following isotope spraying compared to control tissues (Fig.2). This suggests that pistachio trees also efficiently take up and assimilate the labeled nitrogen isotope. The high treatment was approximately 1.45 times higher in the atom percentage than the control treatment. Interestingly, the range of ^{15}N atom percentages in pistachio tissues was higher than in almond tissues, particularly in leaves, indicating differences in the uptake and metabolism of nitrogen in pistachio trees.

All the navel orangeworm insects fed on ^{15}N-enriched plants assimilated the stable isotope, which persisted throughout the insect development (Fig. 3). The navel orangeworm larvae consumed the nuts from sprayed plants and incorporated the labeled nitrogen isotope into their tissues. However, there was no significant difference in the atom percentage in moth from different treatments, which might be associated with the reduced moth sample size (Table 2). Previous studies indicated that navel orangeworm absorbed ^{15}N isotope in a lab setting (Steffan et al., 2001). Higher ^{15}N rates should be tested for an increased transfer in adults. More trials are also needed to increase the number of cages inoculated and consequently increase the number of emerging adults. Other isotopes like Rubidium (Rb) are reported to be effective in being transferred to insects, an alternative option besides C and N isotopes (Zhengxuan et al., 2023).

The navel orangeworm has the propensity to disperse across orchards in search of suitable hosts and oviposition sites. Traditional tracking methods, such as pheromone traps and field surveys (Burks et al., 2008; Koffi et al., 2021), offer valuable insights into population dynamics but often lack spatial resolution and accuracy in identifying movement patterns. For those species included in a sterile insect technique (SIT), such as navel orangeworm, the stable isotope signatures can be used as markers to distinguish mass-reared from wild moth species (Hood-Nowotny et al., 2016).

Another species in evidence of its high dispersal capacity is the fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae). This species, the primary maize pest, is also well known for its resistance to several control methods (Tavares et al., 2021; Zhang et al., 2021; Zhu et al., 2015). Alternative methods like mating disruption are being tested in the field (Schirmer et al., 2023), and information on the dispersal among hosts will be beneficial since it is a highly polyphagous pest. Studies relating to mating disruption and its contribution to resistance management can benefit from isotope studies on many levels. Variations in the isotopic compositions of spermatophores in European corn borers (*Ostrinia nubilalis*) raised on C3 and C4 plants were analyzed to evaluate assortative mating strategies (Ponsard et al., 2004). Employing stable isotopes in studies on mating and sperm competition can provide valuable insights into hostplant and insect interactions, preferences in oviposition, and assortative mating strategies (Quinby et al., 2020). These studies would be highly beneficial for understanding fall armyworm dispersal across several hosts.

The incorporation of ^{15}N-labeled was already described in maize plants, so it can be used for incorporation into herbivore pests (Below et al., 1985). Incorporating the ^{15}N isotope into plant tissues allows us to track fall armyworm dispersal patterns by analyzing the atom percentage in insects collected from different locations. The fall armyworm larvae exhibit preferences for certain host plants over others, so by analyzing the ^{15}N levels in insects collected from different hosts, we can determine the crop origin to which the larvae fed (Dourado et al., 2021; Jia et al., 2021). Monitoring the isotope percentage in the pest over time allows us to identify host plant utilization shifts and adapt management strategies accordingly (Holder et al., 2014). Studies on resistance management (Gould et al., 2002) and determining fall armyworm strains are also potential applications of isotope tracing (Nagoshi et al., 2007). Long-term monitoring studies using isotope labeling can help predict future trends in fall armyworm dynamics and develop management strategies for this invasive species.

In summary, applying the nitrogen-15 tracing technique offers a promising approach to enhance our understanding of pest dispersal patterns, host plant utilization, and movement dynamics across different crop environments. More field trials are needed to improve the isotope uptake in hosts and its assimilation by the insect pest. By elucidating pest movement, we can improve and integrate sustainable methods such as mating disruption and biological control into pest management. Ultimately, these practices will mitigate pest damage and optimize the control efficacy while minimizing environmental impacts.

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Figures and tables

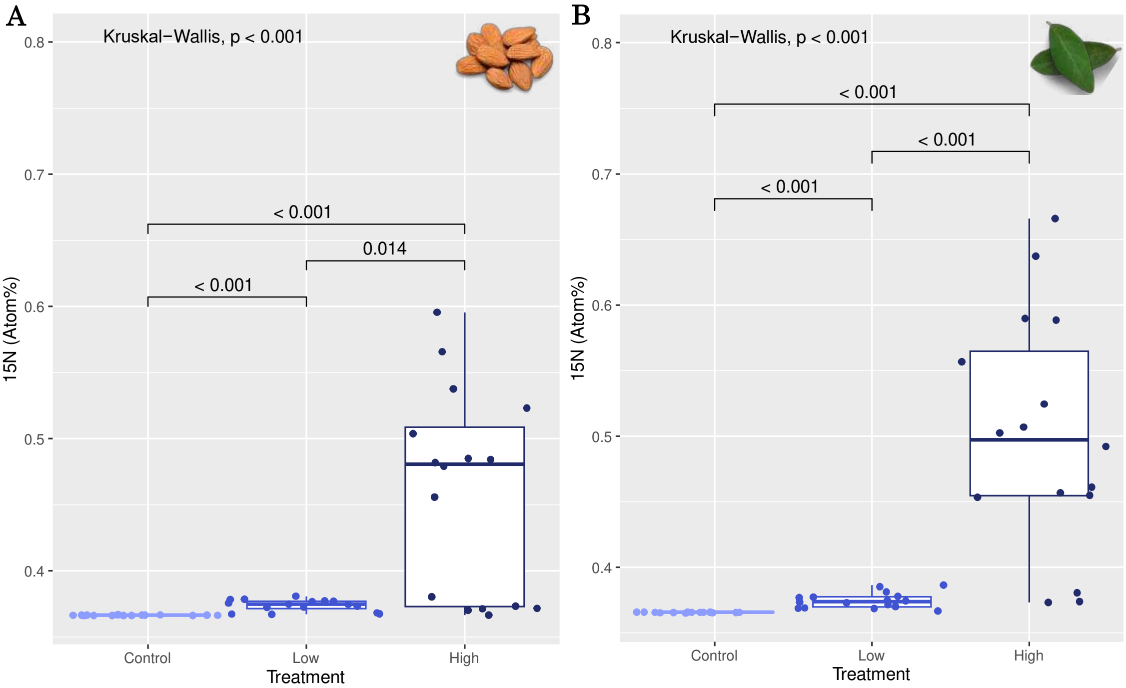

Figure 1. Percentage of ^{15}N atoms in A) Almond nuts and B) Almond leaves in different ^{15}N enriched ammonium nitrate doses (0.1 and 1g/L). The boxplots display the interquartile range (IQR) with the median indicated by the horizontal line inside. The whiskers extend to the minimum and maximum values within 1.5 times the IQR from the first and third quartiles. The p-values from the Wilcoxon comparison tests are plotted in the graph.

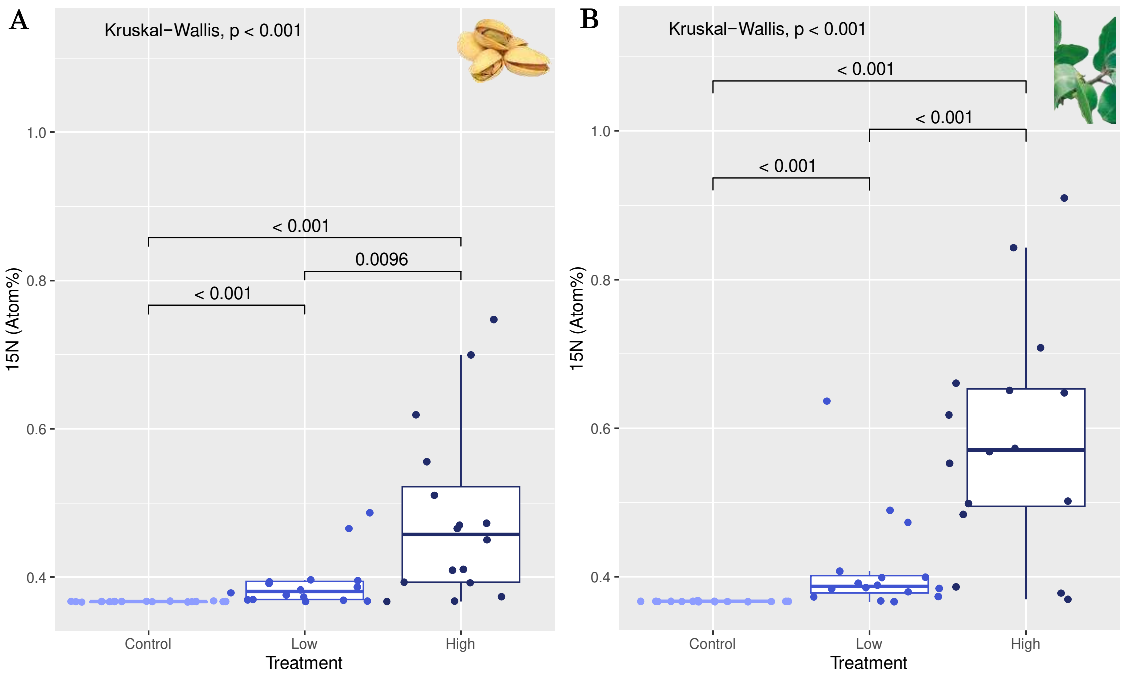

Figure 2. Percentage of ^{15}N atoms in A) Pistachio nuts and B) Pistachio leaves in different ^{15}N enriched ammonium nitrate doses (0.1 and 1g/L). The boxplots display the interquartile range (IQR) with the median indicated by the horizontal line inside. The whiskers extend to the minimum and maximum values within 1.5 times the IQR from the first and third quartiles. The p-values from the Wilcoxon comparison tests are plotted in the graph.

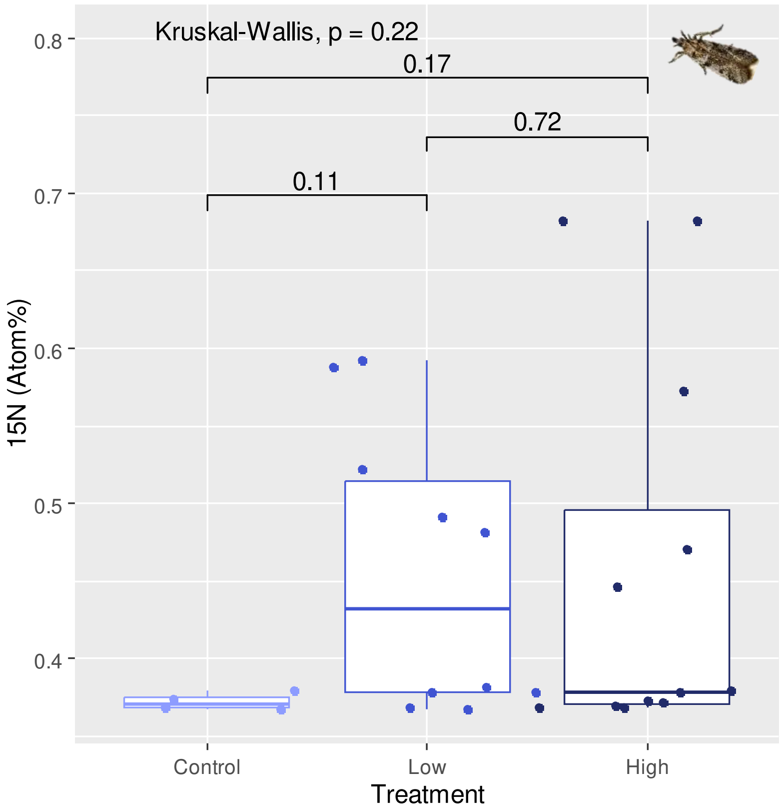

Figure 3. Percentage of ^{15}N atoms in moths originated from the plants submitted to different ^{15}N-enriched ammonium nitrate doses (0.1 and 1g/L). The boxplots display the interquartile range (IQR) with the median indicated by the horizontal line inside. The whiskers extend to the minimum and maximum values within 1.5 times the IQR from the first and third quartiles. The p-values from the Wilcoxon comparison tests are plotted in the graph.

Table 2. Summary of Kruskal-Wallis test results and its p-values in each crop and material type.

Crop	**Material type**	**n**	***df***	**H (χ^2)***	***p***
Almond	Nut	48	2	32.068	1.088e-07
	Leaf	48	2	38.786	3.783e-09
Pistachio	Nut	48	2	30.961	1.892e-07
	Leaf	48	2	33.701	4.808e-08
--	Moth[1]	26	2	3.0667	0.2158

* Kruskal-Wallis chi-squared test.

[1] Number of moth samples in each treatment: control (4), low (10), and high (12).

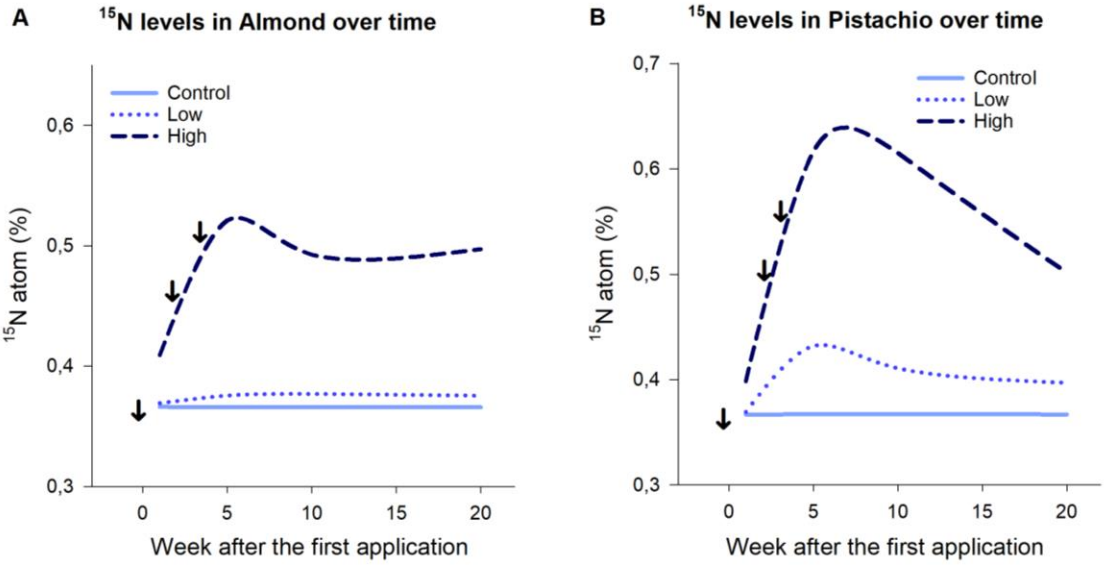

Figure 4. ^{15}N atom percentage over time in A) Almond and B) Pistachio. Arrows in (A) and (B) indicate the time of the isotope solution application (each two weeks). The plant materials were collected 1, 5, 10, and 20 weeks after the first application (first arrow).

GENERAL CONCLUSIONS

The results indicate that *Spodoptera frugiperda* sex pheromone caused a high percentage of mating disruption and reduced plant damage in most of the locations tested. Although crop yield was not affected by the presence of the pheromone dispensers, there was a reduction in the damage to the ears in the treated areas. These results reinforce the high rates of *S. frugiperda* mating disruption, validating the method's efficacy in maize fields.

The neurotoxic insecticides still exhibit efficacy in most populations tested, although there was evidence that growth regulators have lost efficacy due to potential resistance development. The Cry1A.105+Cry2Ab Bt maize has a high risk of control failure of *S. frugiperda* populations, in contrast to Vip3a20 maize, for which the risk was low. The life-table parameters indicated incomplete resistance to the dual-gene Bt maize in 2 out of 5 populations. There was no difference in fitness in the other three populations on non-Bt or Bt maize. The latter affected other life-history traits in the populations, with lower survival rates, larval weights, and a higher developmental time. These findings show that resistance to pesticides, especially Bt proteins, is likely, and integrating the mating disruption and its potential benefits may help minimize the spread of resistance. We suggest constant monitoring in pheromone-treated areas and the best practices of insect resistance management programs.

We explored the ^{15}N isotope as a marker for tracking insect dispersal, and we found high uptake rates in plant tissues. The percentage of ^{15}N atoms was dose-dependent, with higher doses resulting in increased atom percentages. The overall ^{15}N levels in both crops peaked after the third spray, tending to decrease after that. The isotope signature was found until 140 days after the first spray, validating the long-term tracing. The moths originating from isotope-treated plants absorbed the labeled nitrogen isotope into their tissues, although a non-significant difference was found among insects from treated and control plants. The ^{15}N tracing method might be particularly important for tracing moth movement in the environment when using pheromone-based techniques. More field trials are needed to improve the isotope uptake in hosts and its assimilation by the insect pest. In the last instance, we can apply the ^{15}N to mark multiple fields and measure insect pest dispersal outward into surrounding fields. These practices will potentially mitigate pest damage and enhance sustainability in integrated management programs.