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POPULATION-DEPENDENT PHEROMONE BLEND CHANGES IN BT CORN RESISTANT SPODOPTERA FRUGIPERDA FROM BRAZIL

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Doctor Scientiae

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

Adviser: Eraldo Rodrigues de Lima

Co-adviser: Marcelo Coutinho Picanco

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“Quem acredita sempre alcança”.
(Autor desconhecido)

ABSTRACT

TSCHOEKE, Lucas Felipe Prohmann, D.Sc., Universidade Federal de Viçosa, August, 2024. **Population-dependent pheromone blend changes in Bt corn resistant Spodoptera frugiperda from Brazil**. Adviser: Eraldo Rodrigues de Lima. Co-adviser: Marcelo Coutinho Picanco.

Sex pheromones play a crucial role in agriculture to monitor and control pests such as the fall armyworm (FAW), Spodoptera frugiperda. It is important to investigate how resistance to Bt (Bacillus thuringiensis) affects sexual communication and reproductive behavior in resistant and susceptible FAW populations, as well as in their hybrids. Variations can affect the efficiency of pheromone traps in capturing males in corn crops in different regions. Our objective was to evaluate the effect of Bt resistance on the ratio of the critical component Z7-12:Ac in FAW females and how this influences the oriented flight response of males in wind tunnel. We also investigated how hybridization between Bt-resistant and susceptible FAW populations affects the ratio against wich components of the FAW pheromone and the flight of hybrid males. Furthermore, we analyzed how different ratios of the critical component Z7-12:Ac impact the capture of FAW males in corn fields in Brazil. Our results show that the geographic location of FAW populations had a greater impact on decreasing the female Z7-12:Ac component ratio than Bt toxin resistance. Males from Minas Gerias state population (rr-MG) showed a wider window of response to the proportions of the Z7-12:Ac component in the wind tunnel than males from the Bahia state populations (rr-BA and SS-BA). Hybrid males from the rr-MG population also showed the best responses in the wind tunnel. Field results showed that traps with 2% and 4% Z7-12 captured more FAW males than those with 10% and 12%. This capture was greater in the vegetative than in the reproductive phase, indicating a preference of FAW adults for corn at an early stage. Furthermore, there was annual variation in the number of males captured, possibly influenced by climatic conditions and changes in the pest population dynamics. These findings are important for integrated pest management and the use of pheromones in FAW manegement.

Keywords: bt resistance, male response, pheromone traps, sexual communication

RESUMO

TSCHOEKE, Lucas Felipe Prohmann, D.Sc., Universidade Federal de Viçosa, agosto de 2024. **Mudanças na mistura de feromônios dependentes da população em Spodoptera frugiperda resistente ao milho Bt do Brasil**. Orientador: Eraldo Rodrigues de Lima. Coorientador: Marcelo Coutinho Picanco.

Os feromônios sexuais desempenham um papel crucial na agricultura para monitorar e controlar pragas como a lagarta-do-cartucho do milho (FAW), Spodoptera frugiperda. É fundamental investigar como à resistência ao Bt (Bacillus thuringiensis) pode afetar a comunicação sexual e o comportamento reprodutivo em populações de FAW resistentes e suscetíveis, assim como em seus híbridos. Essas variações podem afetar a eficiência de armadilha de feromônio no monitoramento de machos em lavouras de milho em diferentes regiões. Nosso objetivo foi avaliar o efeito da resistência ao Bt na proporção de Z7-12:Ac em fêmeas de FAW e definir como isso influencia a resposta dos machos em túneis de vento. Também investigamos como a hibridização entre populações de FAW resistentes e suscetíveis ao Bt afeta a proporção do componente crítico Z7-12:Ac em fêmeas híbridas e o voo dos machos híbridos. Além disso, analisamos como diferentes proporções do componente crítico Z7-12:Ac impactam a captura de machos de FAW em campos de milho no Brasil. Nossos resultados mostram que a localização geográfica das populações de FAW teve um impacto maior na proporção do componente Z7-12:Ac na glândula das fêmeas do que a resistência ao Bt. Os machos da população de Minas Gerias (rr-MG) apresentaram uma janela mais ampla de resposta à variação de proporções do componente Z7-12:Ac em túnel de vento do que os machos das populações resistentes e suscetíveis da Bahia (rr-BA e SS-BA). Os machos híbridos provenientes da população rr-MG também apresentaram as melhores respostas no túnel de vento. Os resultados do campo mostraram que armadilhas com 2% e 4% de Z7-12 capturaram mais machos de FAW independente da área, que as de 10% e 12%. Capturas foram maiores na fase vegetativa do milho do que na fase reprodutiva, indicando uma preferência dos adultos de FAW pelo milho em estágio inicial. Além disso, houve variação anual no número de machos capturados, possivelmente influenciada por condições climáticas e mudanças na dinâmica populacional da praga. Essas descobertas são importantes para o manejo integrado de pragas e o uso de feromônios no

manejo de FAW.

Palavras-chave: resistencia ao bt; resposta dos machos; armadilhas de feromônio; comunicação sexual.

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SUMMARY

GENERAL INTRODUCTION

Integrated Pest Management (IPM) is essential for a sustainable and efficient agriculture, particularly as cultivated areas expand and farming practices intensify (Stern et al., 1959; FAO, 2024). IPM integrates various pest management strategies, either individually or in combination to mitigate crop damage (Elliott et al., 1995). These strategies include chemical, genetic, biological, cultural, and behavioral methods (Day et al., 2017). About the environmental impact, there is a rising demand for sustainable technologies that minimize pollution and ecosystem disruption (Busch et al., 2020). Consequently, farmers are increasingly adopting integrated approaches that reduce dependency on chemical pesticides while enhancing overall pest control efficacy.

The use of genetically modified plants expressing *Bacillus thuringiensis* (Bt) toxins is a crucial tool in IPM as it plays a significant role in reducing economic damage and minimizing the use of chemical control (Shelton et al., 2002; Romeis et al., 2006; Téllez-Rodríguez et al., 2014). Bt technology has been widely adopted for decades to control lepidopterans in crops such as corn, soybeans, and cotton (Van den Berg et al., 2013; Omoto et al., 2016; Yang et al., 2022). However, the large-scale adoption of Bt crops and the constant presence of Bt plants in the field have increased the risk of resistance development due to strong selective pressure on insect populations (Gassmann et al., 2009; Santos-Amaya et al., 2017; Orozco-Restrepo et al., 2024). This Bt resistance evolves due to continuous exposure of insect pests to toxins present in transgenic plants (Tabashnik et al., 2013). Which process can be accelerated when there is high selective pressure over multiple insect generations per year, extensive presence of plants with Bt technologies in the field and lack of refuge adoption (Santos-Amaya et al., 2017).

To slow the evolution of resistance to Bt crops, high-dose and refuge strategies have been adopted (Carrière et al., 2015; Tabashnik et al., 2023). These strategies assume that transgenic plants produce a high amount of insecticidal protein capable of killing the target population. In refuges, where there are non-Bt host plants, susceptible insects could mate with resistant insects from areas with Bt plants and if the resistance is heterozygous recessive in nature the offspring of these crosses would die when feeding on the Bt plants (Gould, 1998; Tabashnik et al., 2013; Welch et al., 2015).

Spodoptera frugiperda, known as fall armyworm (FAW), is a pest of global importance due to its polyphagous capacity, causing significant damage to crops such as corn, sorghum, soybeans and cotton (Yang et al., 2022). Recently, this pest has become even more problematic due to the loss of effectiveness of transgenic technologies, which are currently the main control tool against this insect (Gonçalves et al., 2024; Orozco-Restrepo et al., 2024). FAW resistant to all currently available Bt technologies including VIP3 have already been reported (Bernardi et al., 2016; Amaral et al., 2020; Orozco-Restrepo et al., 2024).

Fitness costs associated with Bt resistance may play a role in delaying the development of resistance (Santos-Amaya et al., 2017; Ribas et al., 2022; Orozco-Restrepo et al., 2024). Fitness costs represent the compensation that the insect needs to support the acquired resistance gene. In scenarios where there is no exposure to Bt toxins, resistant insects should, in principle, have lower fitness compared to susceptible ones (Gassmann et al., 2009). Although several studies have been carried out reporting costs related to the development of resistance in insects, few specifically explore the costs of Bt resistance in Lepidoptera reproduction (Santos-Amaya et al., 2022; Ribas et al., 2022; Carrière et al., 2023).

Sexual communication in moths as in many species of the order Lepidoptera is mediated by sexual pheromones (Tamaki, 1985; Löfstedt & Kozlov, 1997). Sex pheromones are blends of specific chemical compounds produced by females and released to attract males of the same species over long distances (Tamaki, 1985; Allison & Cardé, 2016). This chemical mediated communication is essential for successful mating, as it allows males to locate females and coordinate their reproductive behaviors (Löfstedt, 1993; Cardé & Haynes, 2004). Changes in the composition of sex pheromones, or the response of males to these signals, can lead to reproductive isolation, potentially contributing to speciation over time (Cardé & Roelofs, 1973; Phelan, 1992; Baker, 2002; Smadja & Butlin, 2009; Groot, 2014).

Several factors can influence sexual communication in insects, including age (young and old) (Webster & Cardé, 1982; Gemeno & Haynes, 2000; Kawazu &

Tatsuki 2002; Mazor & Dunkelblum, 2005; Ming et al., 2007), mating status (virgin or mated) (Delisle et al., 2000; Foster & Roelofs 1994; Mazo-Cancino et al., 2004) and male size (small and large) (McNeil, 1991). Insecticide resistance can also impact sexual communication in several insects altering the production or response of sexual pheromones (Campanhola et al., 1991; Delisle & Vincent, 2002; Wei & Du, 2004; Xu et al., 2010; Navarro-Roldán et al., 2017). Furthermore, possible fitness costs associated with resistance to Bt toxins could harm the development and reproductive success of both male and female insects (Ribas et al., 2022; Santos-Amaya et al., 2022). However, the specific impacts of Bt resistance on Lepidoptera sexual communication are still little explored in the scientific literature.

The FAW sex pheromone consists of a mixture of three main components: (Z)-9-tetradecenyl acetate (Z9-14:Ac), (Z)-7-dodecenyl acetate (Z7-12:Ac) and (Z)-11-hexadecenyl acetate (Z11-16:Ac) (Groot et al., 2008; Lima & McNeil, 2009; Allison & Cardé, 2016). Z7-12:Ac is a critical component of the sex pheromone (Tumlinson et al., 1986; Groot et al., 2008; Lima & McNeil, 2009; Unbehend et al., 2013), and small changes in the proportion of this component have a significant impact on the attraction of males of these insects (Andrade et al., 2000; Batista-Pereira et al., 2006; Mozuraitis, 2000; Yang et al., 2009; Uehara et al., 2014). It is known that the proportion of these components can vary depending on the breed of the species, geographic distribution and resistance to insecticides (Lima & McNeil, 2009; Allison & Cardé, 2016). These variations are important for adapting pest control strategies that use sex pheromones highlighting the need for local studies to ensure the effectiveness of these management techniques.

An effective strategy to monitor insect pest population in crops is the use of sex pheromone traps (Stokstad, 2017; Hendrichs et al., 2021). These traps are designed to sexually attract or confuse males (El-Ghany, 2019). In the attract and kill technique, males are attracted by the synthetic pheromone and become trapped in a glue from which they can no longer escape and die (Phillips, 1997; El-Ghany, 2019). The mating disruption technique consists of flooding the system with sexual pheromone in such way that the male becomes confused and is unable to find the female in the area and mate to generate offspring (Barclay & Judd, 1995; Mafra-Neto et al., 2014).

Studies on possible changes in sexual communication of insects resistant to Bt toxins are essential to understand the impacts of resistance on reproduction and population dynamics. Variations could affect the effectiveness of synthetic pheromones used to monitor and control fall armyworm in the field. Furthermore, fitness costs in sexual communication of FAW populations resistant to multitoxin Bt maize have not yet been studied. If they exist, fitness costs may provide new insights for integrated pest management.

Our work consisted of three main parts: in the first chapter, we evaluated whether resistance to Bt influences the ratio of the critical component Z7-12:Ac in FAW female sex pheromone composition, and if so how this might change affects the response of males in the wind tunnel. Second, in the second chapter, we investigated whether hybrids from the cross between Bt resistant and susceptible strains affects the proportion of the Z7-12:Ac in hybrid FAW females and the pheromone mediated flight of hybrid males. Finally, in the third chapter, we analyze whether different proportions of Z7-12:Ac influence the capture of FAW males in corn fields in Brazil. This work aims to understand how resistance to Bt and variations in the composition of sexual pheromones might affect the interaction between FAW males and females, both in controlled environments and in field conditions, thus contributing to more effective integrated pest management strategies.

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CHAPTER 1

Sexual communications of Bt-corn-resistant FAW caries geographically

ABSTRACT

Developmental costs in Bt (*Bacillus thuringiensis*) resistant insects were reported for populations of *Spodoptera frugiperda,* an important maize pest. However, the effect of costs associated with Bt resistance on the sexual communication of these lepidopterans has not yet been investigated. Therefore, we aimed to evaluate the cost of resistance in the ratio of the critical component Z7-12:Ac of their sex pheromone in one susceptible (SS-BA) and two resistant (rr-BA and rr-MG) *S. frugiperda* populations to the Cry1A.105 + Cry2Ab Bt toxins. The rr-BA and SS-BA populations were selected from a lineage from Bahia, Brazil and the rr-MG population was from Minas Gerais, Brazil. We tested the response of *S. frugiperda* males in a wind tunnel to different ratios of Z7-12:Ac found in these resistant and susceptible female populations. Our results showed a reduced ratio of the Z7-12:Ac component in the sex pheromone of the rr-BA population when compared to the SS-BA and rr-MG on the pheromone emitted during the females first calling day. Males of the rr-MG population reached in the pheromone source faster than the others two populations. Therefore, we did not find any orientation performance cost related to the Bt resistance. However the differences appear to be carreted to geographic location of the FAW populations tested. These findings provide important information for the integrated pest management with the use of sex pheromones in corn crops to manage this pest.

Keywords: Sex pheromone; Semiochemicals; Reproductive behavior; *Spodoptera frugiperda*; Wind tunnel.

1. INTRODUCTION

The sexual communication of many insect species is mediated by sex pheromones produced and released by females that mediate the response of males; this chemical communication is fundamental for mating and reproduction (Tamaki, 1985; Löfstedt & Kozlov, 1997). In Lepidoptera, in most cases, sex communication occurs with females releasing species-specific compounds that attract males over long distances (Tamaki, 1985; Cardé, 2016). Therefore, to successfully mate, males must be able to efficiently recognize and respond to chemical signals emitted by conspecific females (Löfstedt, 1993; Cardé & Haynes, 2004). Changes in female sex pheromone composition together with the males' response to this new signal may result in reproductive isolation and, therefore, be the basis for speciation in lepidoptera (Roelofs & Cardé, 1974; Phelan, 1992; Baker, 2002; Smadja & Butlin, 2009; Groot, 2014).

Several factors can affect insect sexual communication (McNeil, 1991; Raina, 1993; Nosil et al., 2007; Robbins et al., 2008), including age (young and old) (Webster & Cardé, 1982; Gemeno & Haynes, 2000; Kawazu & Tatsuki 2002; Mazor & Dunkelblum, 2005; Ming et al., 2007), mating status (mated or virgin) (Delisle et al., 2000; Foster & Roelofs 1994; Mazo-Cancino et al., 2004) and male size (small and large) (McNeil, 1991). In addition, resistance to insecticides can be conclated to changes in the sexual communication of several insects (Campanhola et al., 1991; Delisle & Vincent, 2002; Wei & Du, 2004; Xu et al., 2010; Navarro-Roldán et al., 2017). Costs related to resistance to *Bacillus thuringiensis* (Bt) technology can impair the development and reproductive success of both sexes (Ribas et al., 2022; Santos-Amaya et al., 2022) and could help in the resistance decline (Gassmann et al., 2009). However, the costs of Bt resistance on lepidopteran reproduction has been little investigated.

The fall armyworm (FAW), *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae), is a highly polyphagous moth species and one of the most important pests of maize in the world (Yu et al., 1991; Baudron et al., 2019; Fan et al., 2020). The use of genetically modified crops that express insecticidal proteins from the bacteria *B. thuringiensis* is currently the main control method for Lepidoptera in maize, cotton, and soybeans plants (Gould et al., 1998; Okumura et al., 2013;

ISAAA, 2017; Kenis et al., 2022). However, resistance to Bt maize in this specie has been observed in Puerto Rico, the United States and Brazil (Farias et al., 2014; Huang et al., 2014; Rodriguez-Chalarca et al., 2024). To delay the evolution of resistance, crops should have plants with high Bt protein expression and adjacent refuge areas (Ferré et al., 2008; Huang et al., 2011; Li et al., 2017). A refuge consists of an area cultivated with a non-Bt crop, wich help to reduce selection pressure and maintain the occurrence of susceptible insects in the field. The proximity of refuge favors mating between resistant insects from the Bt area and susceptible ones from the non-Bt area, generating recessive heterozygous offspring that, when in contact with the toxin at high doses, will not be able to survive (Gassmann et al., 2009).

Bt crop cultivars expressing Cry and/or Vip insecticidal proteins are used in the field as technologies to control FAW larvae (Santos-Amaya et al., 2022). However, large-scale cultivation of Bt plants imposes high selection pressure for resistance development (Santos-Amaya et al., 2015; Tabashnik & Carrière, 2017). This resistance might have a fitness costthat is commonly assessed by comparing life cycle characteristics, such as survival, development, fecundity, and fertility parameters (Carriére et al., 2006; Dangal & Huang, 2015; Santos-Amaya et al., 2022; de Souza Ribas et al., 2022). However, few studies have addressed changes in sexual communication in lepidopterans associated with resistance to Bt-expressing transgenic plants.

The FAW sex pheromone consists of a blend of three components: (Z)-9-tetradecenyl acetate (Z9-14:Ac) the major component, (Z)-7-dodecenyl acetate (Z7-12 :Ac), and (Z)-11-hexadecenyl acetate (Z11-16:Ac) (Groote et al., 2008; Lima & McNeil, 2009; Allison & Cardé, 2016). Z7-12:Ac and Z9-14:Ac are the most important components for male attraction, whereas effect of the third component (Z11-16:Ac) on male behavior remains unclear (Tumlinson et al., 1986; Groot et al. al., 2016) and it’s presence varies depending on the region where the insect was collected (Andrade et al., 2000; Batista-Pereira et al., 2006; Jiang et al., 2022). Z7-12:Ac is a critical component (Tumlinson et al., 1986; Groot et al., 2008; Lima & McNeil, 2009; Unbehend et al., 2013), and change in it’s ratio is much more likely to produce variation in attractiveness than the change of the major component Z9-14:Ac. Small changes in the Z7-12:Ac ratios had a significant impact on the FAW male attraction (Andrade et al., 2000; Batista-Pereira et al., 2006; Mozuraitis, 2000; Yang et al., 2009; Uehara et al., 2014).

Studies on potential changes in pheromone communication in Bt-resistant insects, might provide helpful information about possible reproductive constraints as resistance selection would potentially affect the attraction of Bt-resistant and -susceptible individuals. Possible variations in this communication system could interfere in the efficiency of the synthetic sex pheromone used to monitor or control the FAW. Additionally, fitness costs in sexual communication of *S. frugiperda* populations resistant to multi-toxin Bt maize have not yet been studied. In the present study, we examined changes associated with Bt resistance in the ratio of the Z7-12:Ac component of the FAW sex pheromone. In addition, we tested the behavioral response of resistant and susceptible males in wind tunnel to different ratios of the Z7-12:Ac found in these populations.

2. MATERIALS AND METHODS

2.1 Insects

Three FAW populations were used in this study; two (rr-BA and rr-MG) are resistant to Cry1A.105 + Cry2Ab Bt maize while the third is susceptible (SS-BA). The rr-BA and SS-BA populations were collected in maize fields in Luís Eduardo Magalhães, Bahia state, Brazil, in 2013 (Santos-Amaya *et al*., 2015). The resistance in the rr-BA population is recessive and carries fitness costs to life history and demographic parameters (Santos-Amaya *et al*., 2022). The rr-MG population was collected in Cajuri, Minas Gerais state, Brazil, in 2016, and the resistance is complete and not associated with fitness costs to life-history traits (Orozco-Restrepo, 2019). Every seven generations, we reared the larvae of the resistant populations on Cry1A.105 + Cry2Ab *Bt* maize foliage, and the survivors were advanced to the next generation (Santos-Amaya *et al*., 2015).

Insects were reared in the laboratory under controlled conditions at 27 ± 3º C, 70 ± 15% RH, and 14:10 L/D photoperiod. The adults were maintained in cylindric cages (20 cm diameter x 30 cm height) covered with paper on the inner walls for oviposition, and a piece of cotton soaked in a 10% sugar and 1% ascorbic acid solution was offered every two days *ad libitum* as food source. Egg masses were collected every two days and distributed in 500-mL plastic pots coated with larval diet (Greene et al., 1976). Groups of neonates were maintained in the pots until they reached the 2nd instar. After that, the larvae were individually held in 16-well plastic trays (Advento do Brasil Ind. e Comércio de Plásticos Ltda, São Paulo, Brazil) until pupation.

We used the insects in two types of assays. First, the ratio of Z7-12:Ac of the female sex pheromone was determined for all populations, and then behavioral assays with males were conducted in a wind tunnel considering the aplitude of ratio of Z7-12:Ac found in the females.

2.2 Ratio of Z7-12:Ac in female moths

Each FAW populations was considered a treatment, we had three treatments. The sex pheromone glands, located between the VII and IX abdominal segments, from three females were excised (Sekul & Sparks, 1967) using micro scissors, placed in a 1.5 mL glass vial with 15 µL of hexane and 40 ng of n-heptyl acetate as internal standard (IS) for 30 min. Next, the glands were removed, and the extract obtained was kept at -20◦C until needed for analysis. The extraction was performed using virgin females within one and two days of first calling, in the third hour of scotophase (Lima & McNeil, 2009). Each treatment, had ten replicates, each consisting of the pooled extract of three females.

Pheromone extracts were subjected to chemical analysis using gas chromatography with a flame ionization detector (GC-FID) in a Shimadzu GC-17A equipped with an Rtx-5 cross bond 5% diphenyl-95% dimethylpolysiloxane capillary column (30 m, 0.25 mm i.d., and 0.25 µm film thickness, Thames Restek UK Ltd). One microliter of each extract replicate was injected in splitless mode with the injector at 250°C. The column oven was maintained at 80ºC for 2 min, the temperature increased to 180ºC at 30ºC/min, and to 250°C at 5 °C/min and held for 2 min. Helium was used as carrier gas at an 1 mL/min constant flow rate. We quantified the amount of the minor component Z7-12:Ac of the blend based on an analytical dose curve ran at 0.1, 0.5, 0.7, 1.0, 10, 30, and 50 ng/µL with an authentic standard of Z7-12:Ac (Wang et al., 2022). The ratio of the Z7-12:Ac component in each sample was obtained by dividing the amount of Z7-12:Ac by the sum of the amount of Z7-12:Ac and Z9-12:Acin the sample.

2.3 Male behavior in wind tunnel

The wind tunnel consisted of a Plexiglas 3 m long x 1 m wide x 1 m high, through which a fan pushes clean air, producing a laminar airflow of 0.3 m/s. The bioassays were carried out at 24 ± 2ºC, 40 ± 20% RH, and 1 lux of diffused red light on the top, following the method of Vickers and Baker (1997) and Jiang et al. (2019, 2020).

Sex pheromone lures for the wind tunnel bioassay were formulated with the six different ratios of Z7-12:Ac found in the pheromone glands of FAW females of the three populations (rr-BA, rr-MG, and SS-BA). The formulations contained Z9-14:Ac and Z7-12:Ac in the following percentages: 99:1 (1%), 98,5:1,5 (1,5%), 98:2 (2%), 97,5:2,5 (2,5%), 97:3 (3%) and 96:4 (4%),corresponding to the amplitude of ratios found in females glands of the three FAW populations, as determined in the analytical part of this work. Based on our GC-FID results the dose reference of Z9-14:Ac was fixed at 70 ng (equivalent to one female), uses were prepared by pipetting ten microliters of solution for each treatment. onto filter paper (2 cm x 1 cm). The test lure was placed upwind, on the landing platform, located in the center of the wind tunnel30 cm high and 200 cm from the males' release. Thirty three-day-old virgin males from each population were tested for each of the six ratios of Z7-12:Ac. Males were testedindividualy, and each constituted a replicate.

Wind tunnel assays were performed between the 2nd and 6th hour of scotophase, corresponding to the main period of calling. Males were separated at the pupa stage and left in a separate room to avoid exposure to female sex pheromones. The adult males were individually placed in small glass cylinders (15 cm high and 7 cm in diameter) and transferred to the wind tunnel room 1 h before testing for acclimatization. Each male was individually placed on the release platform intercepting the tested pheromone plume,and observed for three min. The filter paper lure was changed after testing each male moth. The male's behavioral responses were recorded and classified according to the following categories: (1) upwind flight: males flew upwind until reaching close (5 cm) to the pheromone source; (2) upwind: flight speed (m/s) of males until reaching close (5 cm) to the pheromone source; (3) landing: males reached and landed on the pheromone source; (4) upwind flight: speed (m/s) of males reaching the pheromone source; and (5) courtship and mating displays on with the landing platform.

2.4 Statistical analysis

All analyses were performed using the R statistical program (v. 4.0.0; R Development Core Team, 2020). Always considering the factors under study according to the assay conducted, we analyzed each variable recorded using generalized linear models (GLM) with the adequate error distribution. They were as follows: 1) Gaussian for the ratio of Z7-12:Ac produced by females; 2) binomial and Gaussian, respectively, for the percentages and speed of males that arrived close to the source and that landed on the pheromone source; 3) binomial for the percentage of males that displayed courtship. We used the least squares means (emmeans) to compare the significance of treatment differences. Prior to applying the statistical methods used here, we performed residual analyses to confirm the model's error distribution and suitability.

3. RESULTS

3.1 Ratios of Z7-12:Ac in the females

The ratios of the compound Z7-12:Ac found in the sex pheromone glands of females from each of the three populations during two calling days are displayed in figure 1. We found an interaction between genotype and age ($F = 3.42$; $df = 2, 54$; $P = 0.03$). On the first calling day, resistant rr-BA females produced lower ratios of Z7-12:Ac (rr-BA: 1.24 ± 0.07%) than females from the susceptible (SS-BA: 1.53 ± 0.12%) and resistant (rr-MG: 1.82 ± 0.12%) populations. On the second day of calling, however, there were no differences between populations on the ratio of Z7-12:Ac sex in the pheromone blend produced by the females ($P > 0.05$).

3.2 Male behavior in wind tunnel

The results of a the behavioral responses of males from the three FAW populations in the wind tunnel at ratios of 1; 1.5; 2; 2.5; 3 and 4% of the Z7-12:Ac component of the sex pheromone (figure 2 and 3).

3.2.1 Response of males approaching (5 cm) the landing platform

The results related to the percentage of males that arrived close to the pheromone source showed that there was no difference between males from the three FAW populations when testing the ratios of 1% (F = 0.13; df = 2.87; P = 0 .87), 1.5% (F = 0; df = 2.82 ; P = 1), 2.5% (F = 0.15; df = 2.87; P = 0.86), 3% (F = 1; df = 2, 87; P = 0.37) and 4% (F = 0.5; df = 2 , 87; P = 0.60) of Z7-12:Ac in the blend with Z9-14:Ac (figure 2). However, for the 2% source of the Z7-12:Ac component, the results related to the percentage of males that arrived close to the odor source showed that this percentage was higher for SS-BA males when compared to rr-MG males, and rr-BA males did not differ from rr-MG and SS-BA males (F = 3.95; df = 2.87; P > 0.02) (figure 2).

The flight speed (m/s) of FAW males that arrived close to the pheromone source was affected by the following Z7-12:Ac ratio 1% (F = 6.50; df = 2, 79; P < 0.01), 2 (F = 3.65; df = 2, 81; P = 0.03) and 2.5% (F = 3.76; df = 2, 80; P = 0.02) of the Z7-12:Ac (Figure 3). Males of the resistant rr-MG population flew upwind at a greater speed for the above treatments than the susceptive SS-BA and resistant insects rr-BA.

3.2.2 Male response to the odor source

The ratio of Z7-12:Ac influeced the upwind flight of males that landed on the odor source 1% (F = 14.96; df = 2.87; P > 0.01), 1.5% (F = 3.46; df = 2, 82; P = 0.03), 2% (F = 7.42; df = 2.87; P < 0.01), 2.5% (F = 19.05; df = 2, 87; P < 0.01), 3% (F = 4.27; df = 2.87; P = 0.01) and 4% (F = 12.72; df = 2.87; P < 0.01) of the Z7-12:Ac. Also, using these ratios of Z7-12:Ac, the percentage of rr-MGmales that landed on the odor source was higher than that for the rr-BA and SS-BA populations (Figure 2).

Only the ratio 3% (F = 3.29; df = 2, 65; P = 0.04) of the Z7-12:Ac (Figure 3) had an influence on the flight speed ($m.s^{-1}$) of males that landed on the odor source . With the use of this ratio of the Z7-12:Ac component, males from the susceptible (SS-BA: 0.1 ± 0.02 $m.s^{-1}$) and resistant (rr-BA: 0.09 ± 0.02 $m.s^{-1}$) populations flew faster upwind than resistant insects (rr-MG: 0.05 ± 0.006 $m.s^{-1}$).

3.2.3 Males courtship with the odor source

The behavior of FAW males curling their abdomen after landing on the pheromone source was classified as a mating courtship attempt. A effect ($p < 0.05$) was verified on the percentage of FAW males that did the courtship at the pheromone source as a function of insect's population when the ratios of 1% were used ($F = 31.41$; $df = 2.87$; $P < 0.01$), 1.5% ($F = 4.41$; $df = 2, 82$; $P = 0.01$), 2% ($F = 6.82$; $df = 2, 87$; $P < 0.01$), 2.5% ($F = 14.04$; $df = 2, 87$; $P < 0.01$), 3% ($F = 4.09$; $df = 2, 87$; $P = 0.01$) and 4% ($F = 8.2$; ; $df = 2, 87$; $P < 0.01$) of the Z7-12:Ac component. With these ratios of Z7-12:Ac, the percentage of males performing courtship behavior at the pheromone source was higher for the rr-MG population than rr-BA and SS-BA populations (Figure 2).

4. DISCUSSION

In this study, we demonstrated that differences in the proportion of Z7-12:Ac component in the sex pheromone blend were limited to the first day of female calling for the three FAW populations (rr-BA, rr-MG, and SS-BA) studied. The geographic strains of the FAW populations had a greater impact on the ratio of the Z7-12:Ac component than Bt resistance. Similarly, we discovered that for all Z7-12:Ac ratios examined, the geographic FAW strains also had difference in the responses of males tested in the wind tunnel. Since there is a dearth of information in the literature about the sexual interactions between FAW populations that are both resistant to and susceptible to Bt in various locations, these findings offer crucial support for integrated pest management when it comes to the use of semiochemicals to control this pest.

The survival, development, and fertility of insects resistant to Bt toxins may exhibit altered parameters associated with their life cycle, resulting from both positive and negative effects (Gassmann et al. 2009; Zhang et al., 2014; Jakka et al., 2014; Velez et al. 2016; França, 2018). Santos-Amaya et al. (2022), among others have documented that Bt resistant insects had a shortened survival rate and a delayed development time. Bt-resistant FAW males' spermatophore mass and fertility were found to have decreased, according to Ribas et al. (2022). Our findings indicate that the females' sex pheromone on the first and second day of calling did not exhibit differences in ratio of the Z7-12:Ac component correlated to resistance to Bt toxins. *Ostrinia furnacalis* (Guenée) and *Mamestra brassicae*, L., *Lobesia botrana*, (Denis and Schiffermüller), and *Grapholita molesta* (Busck) females are resistant to insecticides and showed similar outcomes (Wei & Du, 2004; Moustafa et al., 2016; Navarro-Roldán et al., 2017). This study represents the first assessment of Bt resistance's impact on FAW populations' sexual communication.

The resistant rr-BA population produced a lower ratio of the Z7-12:Ac component of their sex pheromone than the resistant rr-MG population on the first day of calling. This difference was not observed when comparing the isoline populations rr-BA and SS-BA. On the second day of calling, we did not observe differences in the ratio of Z7-12:Ac in the sex pheromone of the three FAW populations (Figure 1). Therefore, our results show that resistance to Bt was not correlated to changes the ratio of the Z7-12:Ac component produced by females, but rather the in geographic location where the FAW populations originated.

Previous studies have shown that there are some differences in the composition and ratio of sex pheromones between different geographic populations of FAW (Sekul & Sparks, 1967; Tumlinson et al., 1986; Batista-Pereira et al., 2006; Groot et al., 2008; Lima & McNeil, 2009; Cruz-Esteban et al., 2018; Sisay et al., 2024). The females from the rr-BA and SS-BA populations are descended from the same genetic lineage and were chosen from parents who were gathered in Bahia state fields. Although they slow different levels of Bt-resistance, we did not find any differences in the ratio of the Z7-12:Ac component in these populations, namely rr-BA and SS-BA (Santos-Amaya et al ., 2015). The rr-MG population, which was collected in a field located in the state of Minas Gerais, however had a different Z7-12:Ac ratio than the rr-BA resistant population from Bahia.

Z7-12:Ac is a crucial secondary component of the FAW sex pheromone for attracting males in the field (Tumlinson et al. 1986; Unbehend et al. 2013, 2014), and its presence in the mix can increase male capture by up to tenfold in pheromone traps (Andrade et al., 2000). The ratio of the Z7-12:Ac component in the female sex pheromone found in this study varied between 1.2 and 1.9%. Similar results have been reported in FAW populations from Brazil (Batista-Pereira et al., 2006), Africa (Haenniger et al., 2020; Sisay et al., 2024), and Mexico (Cruz-Esteban et al., 2018). Different results were observed in FAW populations in China (Jiang et al., 2022) and the United States (Tumlinson et al., 1986) with a variation between 0.5 to 5%. Therefore, the production of the Z7-12:Ac component by the female may vary according to geographic location as we found in our results.

Sexual communication in moths is typically influenced by variations in the blend of sex pheromones of females and the corresponding response of males (Cardé & Baker, 1984; Roelofs & Bjostad, 1984; Löfstedt et al., 1991). Our results showed that even for males that arrived close to the source and for those that landed on the odor source, rr-MG insects showed greater sensitivity and response to changes in the ratio of Z7-12:Ac (Figures 2 and 3). This is the first report of Bt-resistant males exhibiting differential sensitivity to sex pheromones blend ratios. Similar sensitivity effects have been documented in males of *Grapholita molesta* (Busck), *Plutella xylostella* (Linnaeus), *Trichogramma brassicae* (Bezdenko) and *Spodoptera litura* (Fabricius) resistant to insecticides (Linn & Roelofs, 1986; Delpuech et al., 1998; Wei & Du, 2004; Xu et al., 2010).

The flight speed of moths is modulated by the plume structure and by the composition and concentration of the pheromone (Witzgall & Arn, 1990; Charlton et al., 1993; Mafra-Neto & Cardé, 1994, 1995; Vickers, 2006; Cardé, 2021). It is known that the insects fly in a zigzag approach along the plume and that flight speed increases in straighter flights (Witzgall & Arn, 1990; Witzgall, 1997; Cardé, 2016). Our results show that the speed of males that arrived close to the odor source and that landed on the odor source were greater for blend with ratios of 2.5 and 3% of Z7-12:Ac (Figure 3) . In general, resistant rr-MG males had the highest speeds in most ratios tested, with the exception of the 3% ratio where rr-MG males that landed on the odor source had their speed reduced by half when compared to rr-BA and SS-BA males. Therefore, this may have occurred because these insects follow a more linear flight path at this ratio of Z7-12:Ac. This would suggest getting to the source faster, guaranteeing your offspring.

It is known that males have a larger response range to pheromone blend variations, than the pheromone blend produced by females (Haynes, 1988; Baker, 1989; Löfstedt et al., 1991; Linn, 1997; Krokos et al., 2002). This could therefore account for the fact that rr-MG males in this study were more receptive to all the pheromone ratios examined in the wind tunnel. Additionally, we found that when the Z7-12:Ac component’s ratio in the odor source was increased, more males flew upwind, and landed.. This further indicates that the males response window and the apparent optimal ratio range lies between 2.5 and 3% Z7-12:Ac for FAW (figures 2 and 3). Similar results were documented by Unbehend et al., (2013) testing the Z7-12:Ac ratio on the attraction of corn strain males of FAW.

To produce offspring, males need to find and be able to mate with females. It is known that for polyandrous insects, the greater the number of mattings, the greater the number of descendants (Svärd & McNeil, 1994; Bergström & Wiklund, 2002; Torres-Vila et al., 2013; Lee et al., 2014). Each mating attempt increases the male's chances of rising their mating success (Löfstedt et al., 1991; Linn, 1997). Our results showed higher percentage of rr-MG males did the courtship when they reached the source (Figure 2) than rr-BA and SS-BA males, suggesting that rr-MG males have a better chance of mating in the field than the other strains.

In summary, only a reduction in the ratio of the Z7-12:Ac component in the sex pheromone of the resistant rr-BA population has been observed on the first calling day. Moreover, this reduction appears to be associated with the different geographic location between FAW populations and not with resistance to Bt. Similarly, our wind tunnel results show that male differed based on geographic location between FAW populations, independent of Bt resistance. Nevertheless, it is possible that there are fitness costs associated to resistance that may constrain these resistant insects, deserving further study. Given that the crossing of SS-BA and rr-BA individuals is anticipated to occur in the field, it might be worthwhile to look into any changes in sexual communication in the hybrids (S x r). Finally, field studies utilizing pheromone baits reflecting the ratios found in the tree populations studied would be beneficial in determining whether the male response seen in the lab will correspond with males caught by traps in the field.

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6. FIGURES FIRST CHAPTER

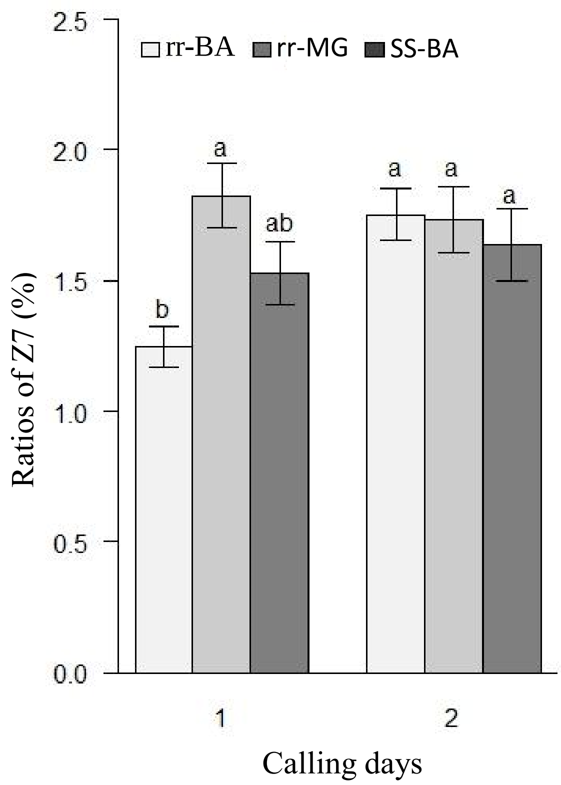

Figure 1. Ratio (mean ± standard error) of the Z7-12:Ac component in the sex pheromone titer of females from three populations of FAW during two calling days. rr-BA and rr-MG = insect populations resistant to Cry1A.105 + Cry2Ab toxins and SS-BA = insect population susceptible to these toxins. P values are given for treatments [generalized linear model (family, Gaussian)] followed by pairwise comparisons of Least Squares Means (LSMeans). Different letters indicate significant differences among treatments ($P<0.05$).

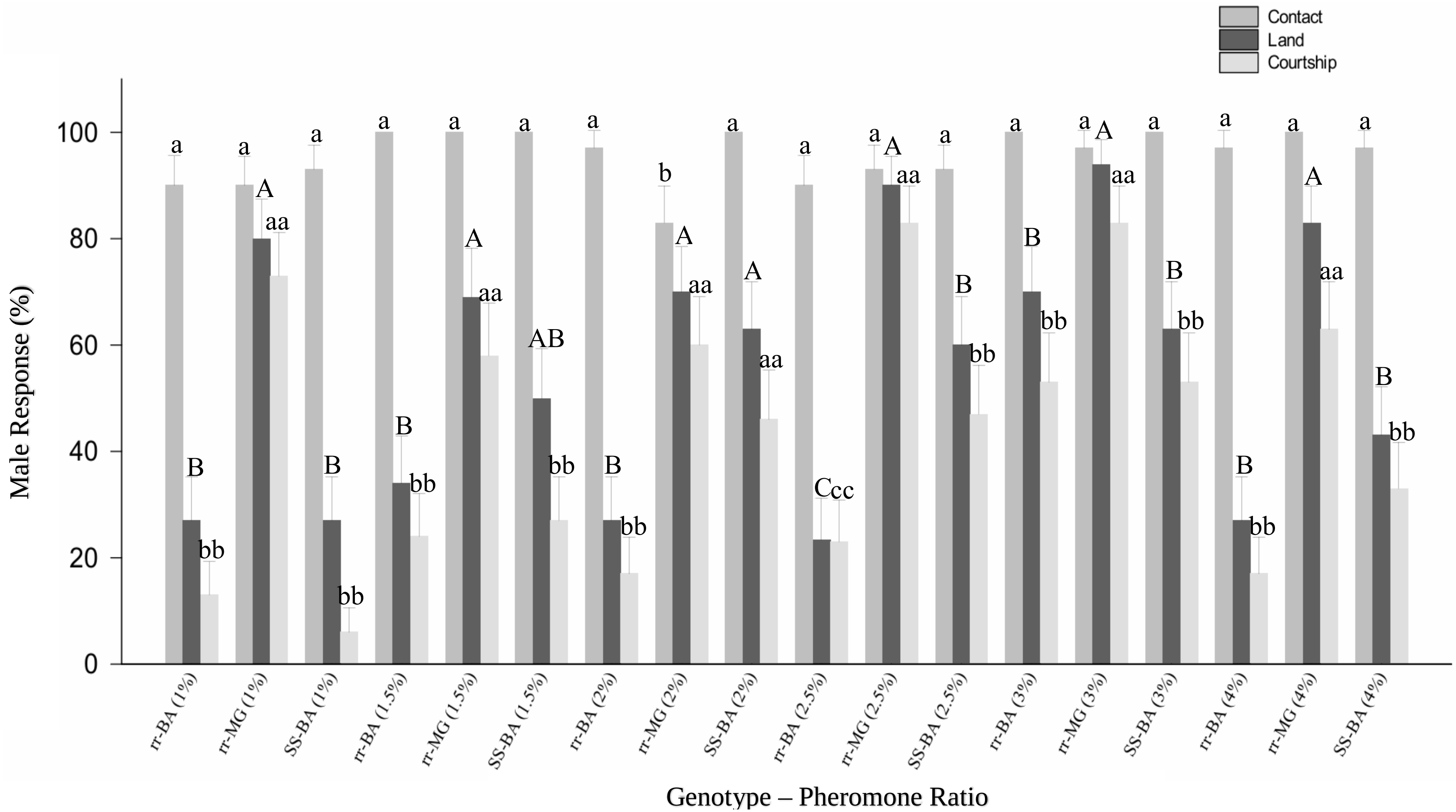

Figure 2. Percentage (mean ± standard error) of male responses from three FAW populations (rr-BA and rr-MG = insect populations resistant to Cry1A.105 + Cry2Ab toxins and SS-BA = insect population susceptible to these toxins) that arrived close to the pheromone source (5 cm) (medium gray color bars), that landed on the pheromone source (dark gray bars color), mating attempted with the odor

source (light gray bars color) containing different ratios 1, 1.5, 2, 2.5, 3, and 4% of Z7-12:Ac in the blend with Z9-14:Ac. P values are given for treatments [generalized linear model (family, quasibinomial)] followed by pairwise comparisons of Least Squares Means (LSMeans). Lowercase letter indicates the difference between contact of the males of the into each ratio of Z7-12:Ac ($P<0.05$); capital letter indicates the difference between landing of the males in each ratio of Z7-12:Ac ($P<0.05$); two lowercase letters indicate the difference between the courtship of the males in each ratio Z7-12:Ac ($P<0.05$).

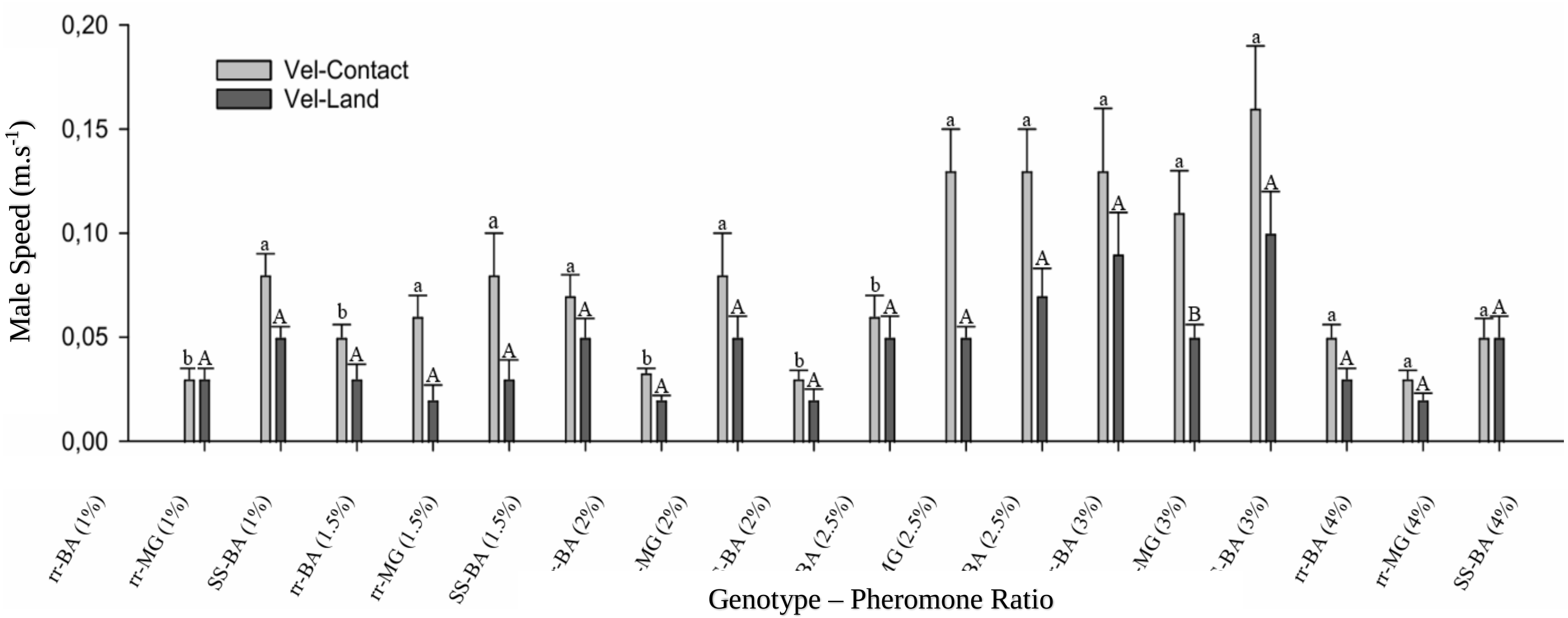

Figure 3. Speed ($m.s^{-1}$) (mean ± standard error) of males from three FAW populations (rr-BA and rr-MG = insect populations resistant to Cry1A.105 + Cry2Ab toxins and SS-BA = insect population susceptible to these toxins) that flew until they got close (5 cm) to the pheromone source (medium gray color bars), that flew until they landed at the source of the pheromone (dark gray bars color) containing different ratios 1, 1.5, 2, 2.5, 3, and 4% of Z7-12:Ac in the blend with Z9-14:Ac. P values are given for treatments [generalized linear

model (family, binomial and Gaussian)] followed by pairwise comparisons of Least Squares Means (LSMeans). Lowercase letter indicates the difference between contact of the males of the into each ratio of Z7-12:Ac (P<0.05); capital letter indicates the difference between landing of the males in each ratio of Z7-12:Ac (P<0.05)

CHAPTER 2

Paternal inheritance of hybrids affects sexual communication between male and female of the fall armyworm (*Spodoptera frugiperda)*

ABSTRACT

Costs in Bt (*Bacillus thuringiensis*) resistance can reduce fitness in resistant fall armyworm (FAW) *Spodoptera frugiperda*, and it is important to understand how these might affect sexual communication and reproductive behavior in hybrid resulting from crossing between resistant and susceptible insects to BT toxin. Therefore, evaluated the proportion of Z7-12: AC on the sex pheromone of hybrid females resultant from mating between Bt resistant and susceptible insects to plants expressing Cry1A.105 and Cry2AB toxins. In addition, we tested the flight behavior of FAW hybrid males in a wind tunnel to the different proportions of Z7-12:Ac in the pheromone blend found in hybrid females. Our results show a reduction in the proportion of Z7-12: AC in ♀rr-MG X ♂SS-BA and ♀rr-BA X ♂SS-BA hybrid females on the first calling day. This reduction seems to be inherited maternally. Similarly, we find that ♀SS-BA X ♂rr-MG and ♀rr-MG X ♂SS-BA hybrid males have a wider response window to the proportions of Z7-12:Ac for landing behaviors at source of odor and mating courtship. These were influenced by the paternal and maternal genotype inherited from the RR-MG population of FAW. These findings provide important information for integrated pest management using this semochemical in areas of refuge to decrease selection pressure, which gives rise to hybrid insects.

Keywords: Sexual communication; Hybrid; Refuges; Wind tunel; Maize.

1. INTRODUCTION

Bacillus thuringiensis (Bt) technology has been adopted for decade to control lepidopteran in the world's main commodities such as maize, soybeans and cotton (Van den Berg et al., 2013; Omoto et al., 2016). The use of Bt plants is a particularly important tool in integrated pest management, as it helps to reduce the level of economic damage and reduces the likelihood to adopt conventional chemical control (Shelton et al., 2002; Romeis et al., 2006). Chemical control can be reduced without compromising environmental quality, leading to lower production costs. However, in increasingly larger and less diverse production fields, the selection pressure for insects resistant to Bt is already a reality (Farias et al., 2014; Fatoretto et al., 2017). In 2009, the first resistant insects to CRy1F technology were reported and since then, every technology launched has been quickly losing its efficiency due to the emergence of resistant insects (Farias et al., 2014; Tabashnik et al., 2023; Orozco-Restrepo et al., 2024). Even the most current one, which is VIP3 has already lost efficiency for controlling Lepidoptera in maize plants (Bernardi et al., 2016; Amaral et al., 2020).

One of the ways to reduce selection pressure in these production areas and stop the evolution of resistance is to use the high dose refuge strategy (Carrière et al., 2015). In order to maintain susceptible insects in the field, the refuge is used. These wild insects can mate with resistant insects from Bt areas, and if the resistance is heterozygous recessive, the resulting progenie will perish while feeding on Bt plants (Gould, 1998, Tabashnik et al., 2013; Welch et al., 2015). The refuge denotes approximately 10% of the crop area with non-Bt plants, whereas to the remaining portion of the crop contains plants expressing high levels of Bt and will, therefore, cause hybrids insects extintion (Tabashnik et al., 2013; Carrière et al., 2015; Welch et al., 2015).

The fall armyworm (FAW), *Spodoptera frugiperda*, is one of the main pests controlled with Bt-transgenic crops. Is an pest of worldwide importance, because of it is highly polyphagous (Yang et al., 2022). It persists in the fields even in absence of the commodity crop because it feeds on a variety of other hosts (Montezano et al. 2018). There are already reports of *S. frugiperda* strains resistant to every commercial Bt technology introduced so far, and as this resistent population expands, it is progressively causing more crop damage (Amaral et al., 2020; Orozco-Restrepo et al., 2024). The prevalence of this resistant population might be by increasing the use refuge areas, and if this resistance has a fitness cost, for the disadvantage to reduce it's competiveness in relation to the susceptible populations(Gould, 1998; Tabashnik et al., 2013; Santo-Amaya et al., 2022; Orozco-Restrepo et al., 2024).

Fitness costs linked to development, reproduction and survival have already been reported in insects resistant to Bt technology (Santos-Amaya et al., 2017; Ribas et al., 2022; Orozco-Restrepo et al., 2024). The cost is due to the insect's potential energetic/physiological compensation for carrying resistance genes. For this, the insect may stop putting energy into some process of development, reproduction and survival in order to allocate energy to having and expressing resistance genes (Gassmann et al., 2009). There are already many studies reporting costs related to the development of these insects, but few of them address the costs related to their reproduction (Santos-Amaya et al., 2022; Ribas et al., 2022; Carrière et al., 2023).

More specifically there are no publications on the production of sex pheromone in resistant females of FAW . The sex pheromone of this specie consists of at least two components: the main component (Z)-9-tetradecenyl acetate (Z9–14:Ac) and the critical secondary component (Z)-7-dodecenyl acetate (Z7–12:Ac) (Tumlinson et al., 1986; Jiang et al., 2022). The presence of Z7-12:Ac in the female sex pheromone heightened the male response in Central and South American populations (Unbehend et al., 2014). Small changes in the proportion of secondary components can have large effects on male response in other species, as well as playing an important role in species-specific reproductive isolation (Yang et al., 2009; Uehara et al., 2015). Furthermore, changing the proportion of a pheromone's components can also influence male response (Downham et al., 2003; Chen et al., 2018). Therefore, understanding how resistance affects male attraction for the sex pheromone the proportion of the components that make up the sex pheromone and the might be important in order to optimally use pheromone-based management tools an integrated pest management programs.

In the previous chapter, we observed that the resistant rr-MG males showed hightened responses in the wind tunnel compared to susceptible SS-BA and resistant rr-

BA males. However, we still do not know whether hybrids obtained from crossing resistant and susceptible insects maintain this higher performance. Therefore, in this study we evaluated whether FAW hybrids from matings between Bt-resistant and susceptible individuals (resistant to Cry1A.105 and Cry2Ab toxins) have a paternal or maternal inheritance. To do this, we measured and compared the proportion of the Z7-12:Ac in relation to Z9-14:Ac of the sexual pheromone produced by the females of these hybrids. Then, the males were subjected to flight tests in a wind tunnel to evaluate their ability to respond to the pheromone blend ratios found in the first part of this work. Therefore, we to evaluated whether hybrid FAW from crossings between resistant and susceptible lines affected the production of sexpheromones in females, and the flight capacity of the males.

2. MATERIALS AND METHODS

2.1 Insects

We studied hybrids of three FAW populations; two resistant (hereafter referred to as rr-BA and rr-MG) and one susceptible (hereafter referred to as SS-BA) to genetically modified plants expressing Cry1A.105 and Cry2Ab Bt toxins. These three populations were collected from different regions of Brazil and have different degrees of resistance. The rr-BA and SS-BA populations were collected in maize fields in Luís Eduardo Magalhães, Bahia state, Brazil, in 2013. Santos-Amaya *et al* (2015) found that their resistance is recessive with fitness costs associated to life history and demographic parameters. The rr-MG population was collected in Cajuri, Minas Gerais state, Brazil, in 2016, and the resistance is complete and not associated with fitness costs to life-history traits (Orozco-Restrepo, 2019). Larvae Bt-susceptibility was tested by feeding the insects with Bt-expressing plants or with their isogenic, non-bt expressing counterparts and their mortality was evaluated (Santos-Amaya *et al*., 2015).

Insects were reared under laboratory under controlled conditions as previously described (27 ± 3º C, 70 ± 15% R.H., and 14:10 h 131 L/D photoperiod) (Ribas et al., 2022). Briefly, adults were maintained in PVC cages (20 cm diameter x 30 cm height) covered with paper on the inner walls for oviposition. A piece of cotton soaked in a 10%

sugar and 1% ascorbic acid solution was offered every two days ad libitum as the insect's food source. Eggs were collected every two days and stored in 500 ml plastic bags until hatching. Groups of neonates were kept in plastic cups until 2nd instar. After that, the caterpillars were individually placed in a cell of a 16-cell PVC tray (Advento do Brasil Ind. e Comércio de Plásticos Ltda, São 129 Paulo, Brazil) containing 20g of artificial diet (Greene et al., 1976).

To determine how the resistance to Bt toxins affects hybrids from the cross between resistant and susceptible insects, hybrid males and females were obtained by reciprocal crosses between susceptible females × resistant males (♀S × ♂r) and resistant females × susceptible males (♀r × ♂S). These crosses were done for both resistant populations (rr-BA and rr-MG). Therefore, we crossed females of SS-BA with males of rr-BA which offspring were named ♀SS-BA x ♂rr-BA; Females of rr-BA with males of SS-BA whichoffspring were named ♀rr-BA x ♂SS-BA; females of SS-BA with males of rr-MG which offspring were named (♀SS-BA x ♂rr-MG; and females of rr-MG with males of SS-BA and the offspring we called ♀rr-MG x ♂SS-BA. The maintenance of hybrid populations was similar to that of susceptible and resistant genotypes.

The pupae were sexed and adults used in two types of tests. First, the ratio of Z7-12:Ac of the female sex pheromone were determined for all hybrid females. Second, wind tunnel the behavioral assays with hybrid males tested their response to the amplitude of ratios of Z7-12:Ac found in the females.

2.2 Ratio of Z7-12:Ac in female moths

Gland extractions were performed in virgin females within one and two days of calling in the third hour of scotophase (Lima & McNeil, 2009). We excised sex pheromone glands located between the VII and IX abdominal segments (Sekul & Sparks, 1967) using micro scissors. The extracted glands were placed for 30 minutes in a glass vial of 1.5 mL with 15 μL of hexane and 40 ng of n-heptyl acetate as internal standard (IS). Next, the glands were removed, and the extract obtained was kept at -20◦C until analysis. For each treatment, ten repetitions of three female glands were used.

The factors under study were the four FAW hybrids populations and the two days of calling, and each three female gland extract constituted a replicate.

The pheromone gland extracts were analyzed in a gas Shimadzu GC-17A chromatograph with a flame ionization detector (GC-FID) equipped with an Rtx-5 restek bond 5% diphenyl-95% dimethylpolysiloxane capillary column (30 m, 0.25 mm i.d., and 0.25 µm film thickness, Thames Restek UK Ltd). One microliter of each replicate sample was injected in splitless mode with the injector at 250°C. The column oven was maintained at 80ºC for 2 min, then the temperature was increased to 180ºC at 30ºC/min. Finally, the temperature was increased to 250°C at 5 °C/min and held for 2 min. Helium was used as carrier gas at an 1 mL/min constant flow rate. We quantified the amount of the minor component Z7-12:Ac of the mixture based on an analytical curve at 0.1, 0.5, 0.7, 1.0, 10, 30, and 50 ng/µL with an authentic standard of Z7-12:Ac (Wang et al., 2022). The ratio of the Z7-12:Ac component was calculated in each sample analyzed by dividing the amount of Z7-12:Ac by the sum of the amount of Z7-12:Ac and Z9-12:Ac found in each FAW populations.

2.3 Male behavior in wind tunnel

The wind tunnel consisted of a Plexiglas 3 m long x 1 m wide x 1 m high, through which a fan pushes clean air, producing a laminar airflow of 0.3 m/s. Ten microliters of Sex pheromone solution for each treatment were pipetted onto filter paper (2 cm x 1 cm), located on the landing platform, 30 cm high and 200 cm from the males' release point. The bioassays were carried out at 24 ± 2◦C, 40 ± 20% RH, and 1 lux of diffused red light on the top, following the method of Vickers and Baker (1997) and Jiang et al. (2019, 2020).

Sex pheromone tested in the wind tunel were formulated with different ratios of the Z7-12:Ac component of females of the four FAW hybrids (♀SS-BA x ♂rr-MG, ♀rr-MG x ♂SS-BA, ♀rr-BA x ♂SS-BA and ♀SS-BA x ♂rr-BA). The formulations used contained Z9-14:Ac and Z7-12:Ac in the following proportions: 99:1 (1%), 98,5:1,5 (1,5%), 98:2 (2%), 97,5:2,5 (2,5%), 97:3 (3%) and 96:4 (4%). These percentages correspond to the amplitude of ratios of Z7-12:Ac found in females of the four populations as determined in the first part of this work. The dose reference of Z9-14:Ac was fixed at 70 ng, the equivalent to one female, based on our GC-FID results.

FAW males were separated at the pupa stage and left in separate rooms to avoid exposure to female sex pheromones. For acclimatization, the adult males were individually placed in small glass cylinders (15 cm high and 7 cm in diameter) and transferred to the wind tunnel room 1 h before testing. Virgin males were individually into the wind tunnel and observed for three min. The pheromone filter paper was changed after testing each male. The male's behavioral responses were recorded according to the following typical categories: (1) attraction of males until reaching close (5 cm) to the pheromone source; (2) flight speed (m/s) of males until reaching close (5 cm) to the pheromone source; (3) attraction of males until reaching the pheromone source; (4) speed (m/s) of males flight until reaching the pheromone source; and (5) courtship at the odor source. Thirty males from each population were tested for each ratio of Z7-12:Ac, and each male constituted a replicate. The wind tunnel assays were performed between the 2nd and 6th hour of scotophase using three-day-old virgin males.

2.4 Statistical analysis

Statistical analyses were performed in R (v. 4.0.0; R Development Core Team, 2020) using Analysis of Variance (ANOVA), followed by residual analysis to confirm the suitability of distributions of the tested models. They were as follows: 1) Gaussian for the titer of Z7-12:Ac produced by females; 2) binomial and Gaussian, respectively, for the percentages and speed of males that arrived close to the source and that landed on the pheromone source; 3) binomial for the percentage of males that attempted to mate. Least Squares Means (emmeans) were used compare significant differences among treatments.

3. RESULTS

3.1 Ratios of Z7-12:Ac in the female hybrids

The ratios of the compound Z7-12:Ac found in the sex pheromone glands of females from each of the four hybrids during two calling days are displayed in figure 4. We found an interaction between genotype and age ($F = 5.02$; $df = 3, 72$; $P < 0.01$). On the first calling day, females from the ♀rr-MG x ♂SS-BA (1.57 ± 0.07%) and ♀rr-BA x ♂SS-BA (1.60 ± 0.10%) hybrids produced a lower proportion of Z7-12:Ac in their sexpheromone than females of ♀SS-BA x ♂rr-MG (1.97 ± 0.16%) and ♀SS-BA x ♂rr-BA (1.79 ± 0.06%) hybrids. On the second calling day, only females of the ♀SS-BA x ♂rr-MG (1.43 ± 0.16%) hybrid showed a lower proportion of the Z7-12:Ac component of the sexual pheromone than ♀rr-MG x ♂SS-BA (1.99 ± 0.23%), ♀rr-BA x ♂SS-BA (1.90 ± 0.10%) and ♀SS-BA x ♂rr-BA (1.79 ± 0.08%) females.

3.2 Male hybrid behavior in wind tunnel

Figure 5 show the results obtained in the second part of this work, in which we evaluated the behavioral responses of males of the four FAW hybrids (♀SS-BA x ♂rr-MG, ♀rr-MG x ♂SS-BA, ♀rr-BA x ♂SS-BA and ♀SS-BA x ♂rr-BA) in a wind tunnel at proportions of 1; 1.5; 2; 2.5; 3 and 4% of the Z7-12:Ac component of the sex pheromone, determined in the first part of our work.

3.2.1 Response of male hybrids close (5 cm) to the odor source

The results related to the percentage of males that arrived close to the pheromone source showed that there was no difference between males from the four FAW hybrids when testing the proportions of 1% ($F = 2.83$; $df = 3, 116$; $P = 0.29$), 1.5% ($F = 2.08$; $df = 3, 116$; $P = 0.10$), 2% ($F = 0.95$; $df = 3, 116$; $P = 0.41$), 2.5% ($F = 0.75$; $df = 3, 116$; $P = 0.52$), 3% ($F = 0.95$; $df = 3, 116$; $P = 0.41$) and 4% ($F = 1.38$; $df = 3, 116$; $P = 0.25$) of Z7-12:Ac in the blend with Z9-14:Ac (figure 5). There was also nodifference ($F = 1.09$; $df = 2.50$; $p = 0.3391$) in the flight speed ($m.s^{-1}$) of males that arrived close to the pheromone source between males of the four FAW hybrids when testing the ratios of Z7-12:Ac in blend with Z9-14:Ac.

3.2.2 Male hybrids response to the odor source

The results related to the percentage of males that landed on the odor source showed that there was a significant effect between males of the four FAW hybrids when testing the 1% ratios ($F = 9.05$; $df = 3, 116$; $P > 0.01$). , 1.5% ($F = 4.02$; $df = 3, 116$; $P < 0.01$), 2% ($F = 12.41$; $df = 3, 116$; $P < 0.01$), 2.5 % ($F = 7.55$; $df = 3, 116$; $P < 0.01$), 3% ($F = 4.77$; $df = 3, 116$; $P < 0.01$) and 4% ($F = 6.95$; $df = 3, 116$; $P < 0.01$) of Z7-12:Ac in blend with Z9-14:Ac (figure 5). Also, using these ratios of Z7-12:Ac, the percentage of FAW hybrid males that landed on the pheromone source for the ♀rr-MG x ♂SS-BA and ♀SS-BA x ♂rr-MG hybrids was higher than for hybrids of ♀rr-BA x ♂SS-BA and ♀SS-BA x ♂rr-BA (figure 5).

3.2.3 Male hybrids courtship at the odor source

The behavior of FAW males curling their abdomen after landing on the pheromone source was designated as a courtship behavior. A significant effect was verified on the percentage males from the four FAW hybrids that performed courtship at with the pheromone source when testing the ratios of 1% ($F = 8.20$; $df = 3, 116$; $P < 0.01$), 1.5% ($F = 6.64$; $df = 3, 116$; $P < 0.01$), 2% ($F = 11.19$; $df = 3, 116$; $P < 0.01$), 2.5% ($F = 7.30$; $df = 3, 116$; $P < 0.01$), 3% ($F = 5.87$; $df = 3, 116$; $P < 0.01$) and 4% ($F = 5.33$; $df = 3, 116$; $P < 0.01$) of Z7-12:Ac in blend with Z9-14:Ac (figure 5). With these ratios of Z7-12:Ac, the percentage males from the four FAW hybrids that performed courtship atwith the pheromone source was higher for the ♀rr-MG x ♂SS-BA and ♀SS-BA x ♂rr-MG hybrids than ♀rr-BA x ♂SS-BA and ♀SS-BA x ♂rr-BA hybrids (figure 5).

4. DISCUSSION

In this study we showed that the ratio Z7-12:Ac the sex pheromone for four FAW hybrids (♀SS-BA x ♂rr-MG, ♀rr-MG x ♂SS-BA, ♀rr-BA x ♂SS-BA and ♀SS-BA x ♂rr-BA) differed on the first and second day of calling. Additionally, there was a reduction in the ratio of the Z7-12:Ac component in female hybrids, which appears to be a maternal inheritance. Likewise, we found that the male hybrids in the wind tunnel presented a wide window of response to pheromone ratios, where hybrids generated from crossing insects from the rr-MG population had the best responses for all proportions of Z7-12: Ac tested, corroborating the results found in the first chapter. These findings provide important evidence for integrated pest management with the use of this semiochemical in areas that use refuge to reduce selection pressure, which gives rise to resistant insects. These results may improve the data about the sexual communication of hybrids resulting from crossings of insects from refuge areas and areas with Bt technology plants.

Fitness costs are commonly assessed by comparing differences in development and reproduction between FAW-resistant and susceptible populations to Bt (Santos-Amaya et al, 2022; Ribas et al, 2022; Orozco-Restrepo et al, 2024). However, there are no studies evaluating how this cost in FAW Bt-resistant and susceptible males and females can affect mating communication of hybrids resulting from crosses between these populations. Thus, this is the first work that investigates the sexual communication of FAW hybrids of susceptible and resistant genotypes. Our results show that the range in the Z7-12:Ac ratio found in females on the first and second day of calling (1.43 – 1.99%) (figure 4) was similar to that found in rr-BA, rr-MG and SS-BA females (1.2 – 1.9 %) reported in the first chapter (figure 1). Similar results have been reported in other studies (Batista-Pereira et al., 2006; Schöfl et al., 2009; Cruz-Esteban et al., 2018; Haenniger et al., 2020; Sisay et al., 2024). Therefore, the crossing between resistant and susceptible insects, regardless of the sex of the parents, did not affect the range of the ratio of the Z7-12:Ac component in the sexual pheromone of female hybrids.

However, the proportion of the Z7-12:Ac component in the blend was different between the hybrids depending on the sex of the parents (figure 4). On the first day of calling, crosses originating from susceptible males (SS) resulted in hybrids female (♀rr-MG x ♂SS-BA and ♀rr-BA x ♂SS-BA) producing the lowest proportion of the Z7-12:Ac when compared to hybrids (♀SS-BA x ♂rr-MG and ♀SS-BA x ♂rr-BA) from crosses with susceptible females (SS). This reduction in the ratio of the Z7-12:Ac component in female hybrids was not influenced by resistance to Bt inherited from their parents nor by the different geographic origin of the FAW population, as observed in the previous study related in the first chapter.

Our results suggest that there may be a maternal inheritance in the proportion of the Z7-12:Ac component in the sexual pheromone of hybrid females on the first day of calling. Similar results were reported by Schöfl et al., (2009), who observed maternal inheritance when evaluating female calling initiation time and mating time for both sexes in hybrids from Florida FAW populations. These similarities found in the sexual pheromone of FAW female hybrids with females of their maternal lineage suggest that pheromone synthesis is controlled, at least in part, by the W chromosome (Groot et al., 2008; Lima et al., 2009; Schöfl et al., 2009). As female Lepidoptera are heterogametic sex (ZW) and males are homogametic sex (ZZ), a mode of maternal inheritance may be caused by the W chromosome (transmitted from the mother). Genetic research on the biology of pheromones in Lepidoptera indicated the possible involvement of autosomal and sex-linked genes, varying according to the parameter under analysis (Grula & Taylor 1979; Roelofs et al. 1987; Kost et al., 2016). In contrast, Haenniger et al., (2020) found no differences in the ratio of the Z7-12:Ac component in the sex pheromone of hybrid females from African FAW populations

Sexual communication in most Lepidoptera is modulated by females that release sexual pheromones over long distances and by males that are able to recognize and respond to these stimuli (Cardé and Baker 1984; Löfstedt, 1993; Smadja & Butlin, 2009; Wicker- Thomas, 2011). Our wind tunnel results showed that, even for male hybrids that landed on the odor source and, for those that attempted to mate, ♀rr-MG x ♂SS-BA and ♀SS-BA x ♂rr-MG hybrids showed the greatest sensitivities and responses to changes in Z7-12:Ac percentage (figure 5). This is the first report of hybrid males from the crossing of populations susceptible and resistant to Bt exhibiting differential sensitivity to sex pheromones. Similar sensitivity effects were documented in male hybrids from *Heliothis subflexa* x *Heliothis virescens* that showed a change in responsiveness to the Z9–14:Ald component (Ouero et al., 2006). On the other hand, Zhao et al., (2006) in hybrid males of *Helicoverpa assulta* x *Helicoverpa armigera* and Haenniger et al., (2020) in hybrid males from African FAW populations saw no change in the response of these males to pheromone components of these species.

This greater sensitivity in the response of ♀SS-BA x ♂rr-MG and ♀rr-MG x ♂SS-BA male hybrids is consistent with the responses observed in males from the rr-MG population presented in our first chapter (figures 2 and 3). Where rr-MG males showed a wider window of response to the proportions of the Z7-12:Ac component for the behaviors of landing on the odor source and mating attempt. Similar results were found in this chapter with male hybrids. This confirms again that the response window of males is wider than the response window of females and that males can also respond to varying mixtures of female sex pheromone components (Löfstedt et al., 199; Krokos et al., 2002; Linn et al., 2003; Batista-Pereira et al., 2006).

For sexual reproduction to occur, usually the males need to find females to mate. Polyandrous insects have multiple mating partners, and the more times a male mates, the greater his reproductive success, measured in terms of the number of offspring (Svärd & McNeil, 1994; Bergström & Wiklund , 2002; Torres-Vila et al., 2013). Each additional copulation increases the chance of that male fertilizing the females eggs, which in turn results in more offspring (Löfstedt et al., 1991; Linn, 1997). Our results show that a higher percentage of ♀SS-BA x ♂rr-MG and ♀rr-MG x ♂SS-BA hybrid males did the courtship at the landing platform (figure 5) for all Z7-12:Ac ratios studied, than ♀rr-BA x ♂SS-BA and ♀SS-BA x ♂rr-BA hybrid males. This suggests is a higher likelihood of these hybrid males mating in the field and dispersint their genotypes alleles. Our results show no evidence of fitness costs associated to the Bt resistance in ♀SS-BA x ♂rr-MG and ♀rr-MG x ♂SS-BA hybrids.

Our findings indicate that the heightened courtship observed there may be an inheritance of the rr-MG genotype in ♀SS-BA x ♂rr-MG and ♀rr-MG x ♂SS-BA hybrid males, since they come from crossings of both males and females from the rr-MG population. Similar results were found by Schöfl et al., (2009) in hybrids of FAW populations of corn and rice strain. They noticed that the time of mating and the time of oviposition had a dominant inheritance. Likewise, the early mating time reported for hybrids from African FAW populations might also be genetically inherited (Haenniger et al., 2020).

In summary, our data slows a reduction in the ratio of the Z7-12:Ac component in the sex pheromone of females of ♀rr-MG x ♂SS-BA and ♀rr-BA x ♂SS-BA hybrids but only on the first day of calling. The ratio reduction appears to be maternally inherited among FAW populations, and not associated with BT resistance. Similarly, our wind tunnel data suggests that male hybrid responses were affected by different paternal and maternal genotypes among FAW populations, not correlated to Bt resistance. Although we did not find fitness costs associated with Bt-resistance, it is possible that fitness costs may still constrain these hybrid insects, thus warranting additional studies. Development and survival parameters have been investigated in FAW populations resistant and susceptible to Bt which found resistance associated to fitness costs (Santos-Amaya et al., 2022; Orozco-Restrepo et al., 2024), thus it might be necessary to further evaluate these fitness costs in hybrid insects. Finally, field experiments testing lures loaded with different ratios of the Z7-12:Ac would provide valuable information regarding the range of male response to determine it the laboratory observed resposnses for the rr-MG populations (first chapter) and for ♀SS-BA x ♂rr-MG and ♀rr-MG x ♂SS-BA hybrids (this chapter) would be reflected on the trap captures.

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6. FIGURES SECOND CHAPTER

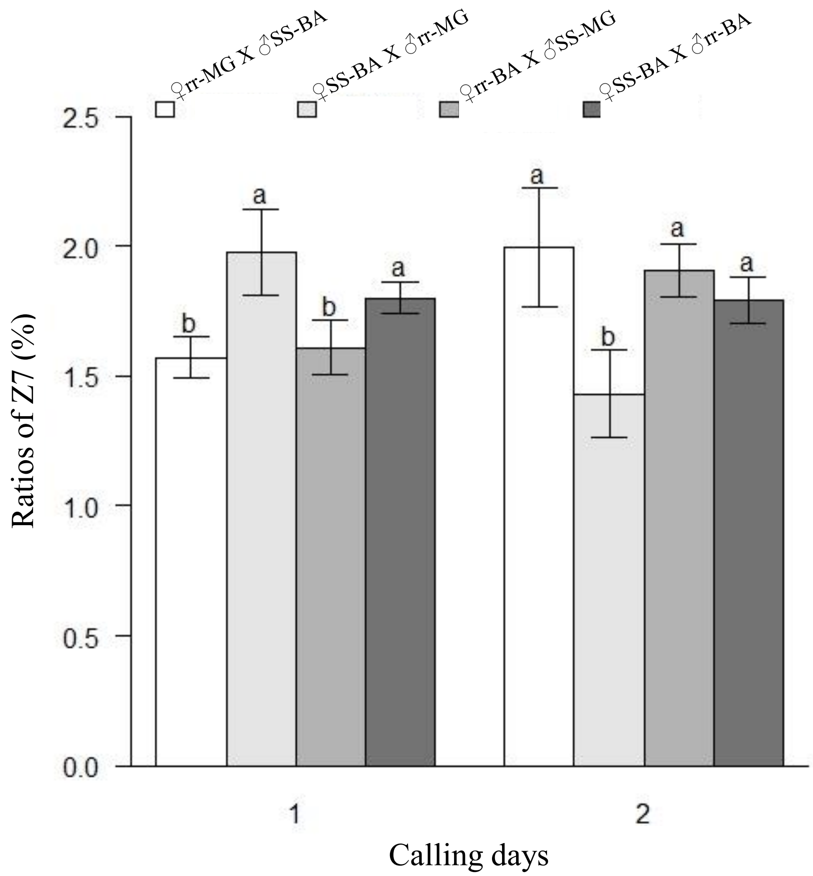

Figure 4. Ratio (mean ± standard error) of the Z7-12:Ac to (Z7-12:Ac + Z9-14:Ac) * 100 component in the sex pheromone gland of females of four hybrids of FAW during two days of calling. ♀rr-MG X ♂SS-BA (hybrid from crossing between females of rr-MG and males of SS-BA), ♀SS-BA X ♂rr-MG (hybrid from crossing between males of rr-MG and females of SS-BA), ♀rr-BA X ♂SS-BA (hybrid from crossing between males of SS-BA and females of rr-BA) and ♀SS-BA X ♂rr-BA (hybrid from crossing between males of rr-BA and females of SS-BA). P values are given for treatments [generalized linear model (family, Gaussian)] followed by pairwise comparisons of Least Squares Means (LSMeans). Different letters indicate significant differences among treatments ($P<0.05$).

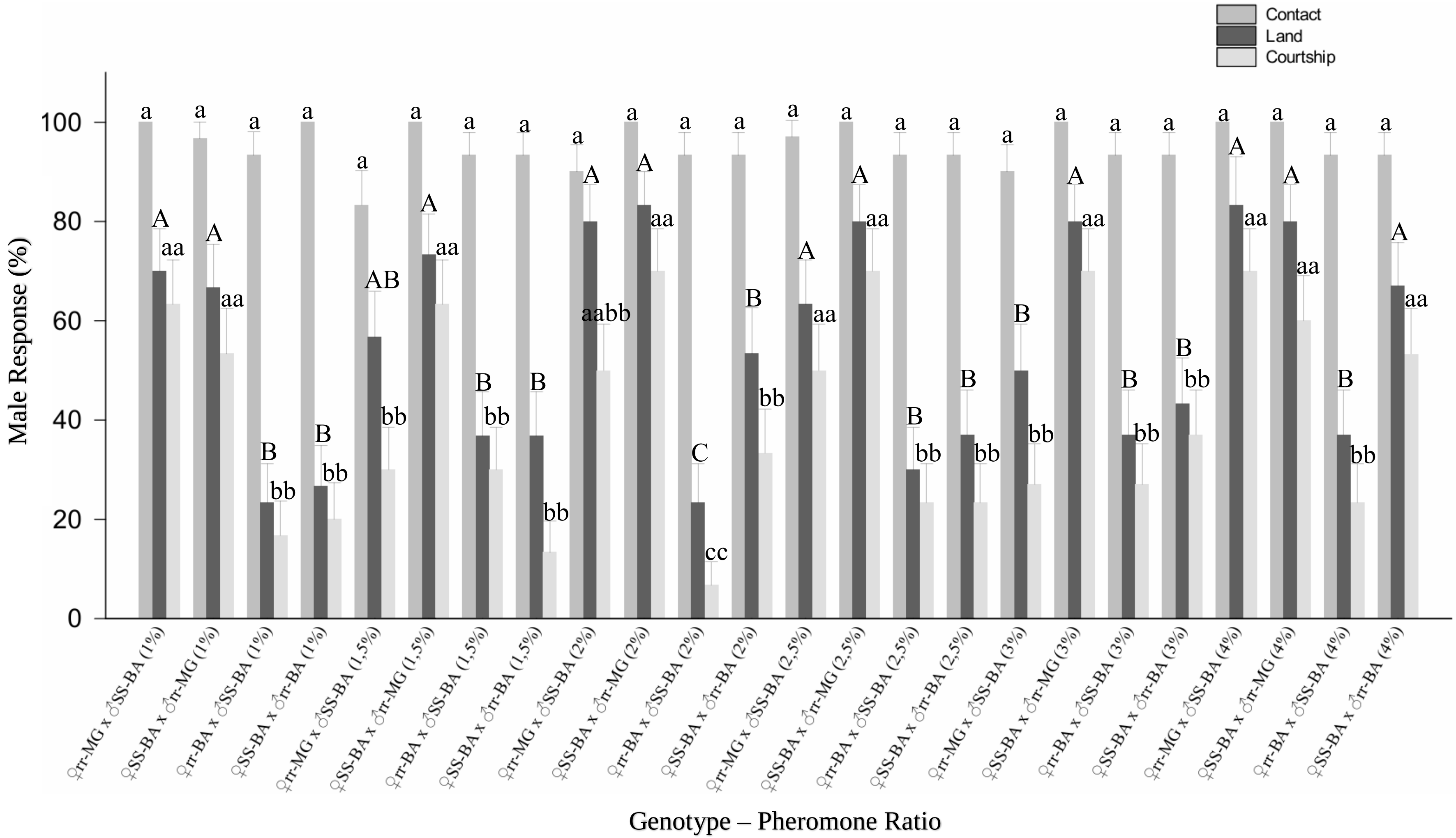

Figure 5. Percentage (mean ± standard error) of male responses from four hybrids of FAW (♀rr-MG X ♂SS-BA (hybrid from crossing between females of rr-MG and males of SS-BA), ♀SS-BA X ♂rr-MG (hybrid from crossing between males of rr-MG and

females of SS-BA), ♀rr-BA X ♂SS-BA (hybrid from crossing between males of SS-BA and females of rr-BA) and ♀SS-BA X ♂rr-BA (hybrid from crossing between males of rr-BA and females of SS-BA)) that arrived close to the pheromone source (5 cm) (medium gray color bars), that landed on the pheromone source (dark gray bars color), mating attempted with the odor source (light gray bars color) containing different ratios 1, 1.5, 2, 2.5, 3, and 4% of Z7-12:Ac in the blend with Z9-14:Ac. P values are given for treatments [generalized linear model (family, quasibinomial)] followed by pairwise comparisons of Least Squares Means (LSMeans). Lowercase letter indicates the difference between contact of the males of the into each ratio of Z7-12:Ac ($P<0.05$); capital letter indicates the difference between landing of the males in each ratio of Z7-12:Ac ($P<0.05$); two lowercase letters indicate the difference between the courtship of the males in each ratio Z7-12:Ac ($P<0.05$)

CHAPTER 3

Evaluation of sexual pheromone blends on the attractiveness of fall armyworm males in different regions of Brazil in corn crops

ABSTRACT

Sex pheromones are used in agriculture to monitor and control pests such as fall armyworm (FAW) (*Spodoptera frugiperda*). The proportion of sexual pheromone components produced by females may vary according to the geographic location of the pest. These variations can affect the efficiency of pheromone traps in capturing males in corn fields in different regions. Therefore, our aim was to test how different proportions of the binary mixture Z7-12:Ac + Z9-14:Ac (2:98, 4:96, 10:90 and 12:88, respectively) affect the capture efficiency of males of FAW in sex pheromone traps positioned in different regions of Brazil. Furthermore, we tested whether the planting time (early season or second season), the corn plant phenological stage (vegetative and reproductive), and the years of data collection influenced this capture. Our results showed that traps containing 2% of Z7-12:Ac captured the greatest number of males. We also observed that geographic location significantly influenced the capture of males, with traps located in the state of Tocantins capturing more FAW males than traps from the state of Minas Gerais. Furthermore, we noticed an annual variation in the capture of males may attributed to factors such as climatic conditions and population fluctuation of the pest. Additionally, it was observed that the number of males captured was greater during the vegetative phase of corn than during the reproductive phase, suggesting a greater oviposition preference of FAW moths for younger corn plants than for the older ones. This information is important for the development of sex pheromone traps in integrated pest management, emphasizing the need to consider not only the attractiveness of pheromone traps, but also other aspects of the agricultural environment that may influence the effectiveness of its control.

Keywords: Sex pheromone traps; (Z)-7-dodecenyl acetate; *Spodoptera frugiperda*; Behavioral control; Integrated pest management.

1. INTRODUCTION

The use of Integrated Pest Management (IPM) is an important tool for a more sustainable and efficient cultivation in terms of maintaining the environment, because of the increase in cultivated areas and increasingly intensified agriculture (Stern et al., 1959 FAO, 2024). IPM consists in the use of many types of management used by itself or together to control pests in crops (Pedigo et al., 1986; Elliott et al., 1995). Some of the most currently control methods used in IPM are chemical, genetic, biological, cultural and behavioral (Day et al. 2017). Additionally, there is an increasing requirement for more sustainable technologies that pollute the environment less and cause less damage to the system (Busch et al., 2020). Therefore, producers have aggregated chemical control with other controls in an effort to increasingly reduce the application of chemical pesticides.

Another problem related to the use of chemical pesticides, which also occurs for some technologies such as transgenics plants with Bt (*Bacillus thuringiensis*), is resistance. Certain chemical compounds and the use of transgenics in large-scale monocultures for pest control may cause these organisms to became resistant to these technologies and lose their ability to control them effectively (Farias et al., 2014; Fatoretto et al., 2017). This is a subject that is currently much discussed regarding the use of transgenics, as even the latest technology launched to control *Spodoptera frugiperda*, which is the main pest of corn crops, no longer shows any effect in controlling this pest in crops (Bernardi et al., 2016; Amaral et al., 2020).

S. frugiperda, better known as fall armyworm (FAW), is the main pest of corn and has been causing more and more problems for this crop, because transgenics which is the main current control tool for this insect, is losing its efficiency (Gonçalves et al., 2024; Orozco-Restrepo et al., 2024). In addition to transgenics, chemical molecules are used to control this pest and behavioral and biological control are currently being increasingly applied (Day et al. 2017). The behavioral control of this pest consists of using the sexual pheromone of this species to attract or sexually disrupt males (El-Ghany, 2019). In the attract-and-kill technique, males are attracted by the synthetic pheromone and become trapped in a glue trap from which they can no longer escape and die (Phillips, 1997; El-Ghany, 2019). The sexual confusion technique consists of flooding the system with the sexual pheromone, in such a way that the male becomes confused and is unable to find the female in the area and mate for generate offspring (Barclay & Judd, 1995; Mafra-Neto et al., 2014). These traps can also be used to monitor this pest in crops with the aim of controlling the advancement of the population of these insects within the field and decision-making system (Stokstad, 2017; Hendrichs et al., 2021).

This species has three main components in the composition of its sexual pheromone, (Z)-9-tetradecenyl acetate (Z9-14:Ac), (Z)-7-dodecenyl acetate (Z7-12:Ac), and (Z)- 11-hexadecenyl acetate (Z11-16:Ac) (Groote et al., 2008; Lima & McNeil, 2009; Allison & Cardé, 2016). These compounds may vary in their proportion depending on the breed of the species, geographic distribution of the species and resistance to insecticides (Lima & McNeil, 2009; Allison & Cardé, 2016). Therefore, it is important to study the local population where you intend to control pests using this type of control.

Commercial FAW traps are formulated with the three female sex pheromone components: Z9-14:Ac, Z7-12:Ac, and Z11-16:Ac (Malo et al., 2001; Bratovich et al., 2019). However, it is known that the determining components of the pheromone for the male to find the female are Z9-14:Ac and Z7-12:Ac (Tumlinson et al., 1986; Groot et al., 2008; Jiang et al., 2022; Wang et al., 2022). In laboratory tests using a wind tunnel, we saw that females from different locations, resistant and susceptible to Bt Cry1A.105 + Cry2Ab toxins may have different proportions of these components in their sex pheromone. Additionally, we saw that males from these populations respond differently to variations in the proportions of these two components.

There are no studies that investigate how changing the proportion of the Z7-12:Ac component in pheromone traps can affect the capture of males accross different regions of Brazil. Therefore, we aim to investigate how different proportions of the binary mixture Z7-12:Ac + Z9-14:Ac (2:98, 4:96, 10:90 and 12:88, respectively) affect the efficiency of FAW male captured in sex pheromone traps. Additionally, we investigate the potential influence of planting time (early season or second season), corn phenological stage (vegetative and reproductive), geographic location, and year on this capture. This data is essencial for optimizing FAW control and monitoring in Brazilian corn crops, with the goal of enhancing the efficacy of integrated pest management techniques.

2. MATERIALS AND METHODS

2.1 Field bioassays

Field bioassays were carried out in corn fields in the states of Minas Gerais and Tocantins in the years of 2022/2023 and 2023/2024 both conducted during the early season period (summer corn) and the second season period (second harvest corn). In 2022/2023, three bioassays were conducted on corn season in the regions of Viçosa – MG (20°44'38"S and 42°50'37"W), Cajuri – MG (20°46'29"S and 42°49'15"W) and Gurupi – TO (11°50'00"S and 48°54'21"W); and two bioassays on the second season of corn were conducted in Porto Firme – MG (20°41'19"S and 43°04'20"W) and Gurupi – TO (11°51'51"S and 48°56'54"W). The experiments in Viçosa and Cajurí were carried out with hybrid corn expressing Bt Cry1A.105 + Cry2Ab proteins in areas of 8 hectares. For Porto Firme and Tocantins the experiments were carried out with hybrid corn expressing Bt proteins Cry1A.105 + Cry2Ab + Vip3 in areas of 10 hectares. In 2023/2024, three bioassays were conducted on early season of corn and three on second season corn in Coimbra – MG (20°50'57"S 42°46'06"W), Cajuri – MG (20°46 '21"S and 42°48'44"W) and Gurupi – TO (11°48'12"S and 49°00'47"W). The experiments in Coimbra and Cajurí were carried out with hybrid corn expressing Bt Cry1A.105 + Cry2Ab proteins in areas of 10 hectares. In Tocantins, experiments were carried out with hybrid corn expressing Bt proteins Cry1A.105 + Cry2Ab + Vip3 in areas of 10 hectares.

The corn crops were not irrigated and a recommended management was used to cultivate the plants (Borem et al., 2017). In each bioassays, the damage triggered by fall armyworm in the vegetative and reproductive stage of corn was evaluated. Furthermore, traps with sex pheromones containing different ratios of the critical component Z7-12:Ac were used to evaluate the attractiveness of males during the vegetative and reproductive stages of corn.

2.2 Assessment of FAW damage in corn

2.2.1 Vegetative stage

To determine the damage caused by foliar feeding by *S. frugiperda* caterpillars in the vegetative stage of corn, an evaluation was carried out using the visual scale developed by Davis (Davis et al. 1992), which assigns a score to each plant according to severity damage caused by caterpillar feeding. This scale ranges from 0 to 9, with 0 representing no damage and 9 representing almost complete destruction of the plant. Visual assessments were performed when plants were at the V8 stage of development. In each test, eighty sample points were evaluated. Each sampling point consisted of six corn plants. Throughout the field, the sampling points were distributed in parallel lines, with each line positioned fifteen meters away from the next. Within each line, sampling points were evenly spaced, with each point located fifty meters away from the next on the same line.

2.2.2 Reproductive stage

To determine the damage caused by *S. frugiperda* caterpillars in the reproductive stage of corn, an assessment was carried out by directly counting insects present on corn cobs in the R2 (water blister grain) stage of the plant development. In each test, twenty sample points were evaluated. Each of these sampling points contained twenty corn cob for analysis. The sampling points were distributed throughout the field with a uniform spacing of 50 meters between each one.

To assess the presence or absence and number of *S. frugiperda* caterpillars inside the corn cob, we opened the ear with our hands at the evaluation site and counted it.

2.3 Synthetic pheromone

The synthetic compounds of Z7-12:OAc and Z9-14:OAc were donated from a commercial sources (Bedoukian Research Inc, Danbury, United States). The purity of synthetic compounds (>97%) was determined with a Shimadzu GC-2010 plus gas chromatograph (Tokyo, Japan) equipped with a flame ionization detector. We prepared standard solutions of the compounds in hexane HPLC grade (Sigma-Aldrich, Toluca, Mexico).

2.4 Sex pheromone traps

To test male attractiveness, we formulated *S. frugiperda* sex pheromone baits containing different proportions of the critical Z7-12:Ac component. For this, we used delta-type traps measuring 30 cm in length, with a triangular cross section of 12×12×18 cm at each end. An adhesive floor (18x18 cm) was added to the base of the trap to retain trapped moths. A sex pheromone tablet was placed in the center of the adhesive floor. The pellets were loaded with 20 µl of hexane containing 400 µg of Z9-14:OAc and Z7-12:OAc in the ratio of 98:2, 96:4, 90:10 or 88:12, respectively (Cruz-Esteban et al ., 2020).

The experimental design in the field bioassays was completely replicated randomized in five blocks, each block containing four treatments. The blocks were arranged in parallel lines with approximately 50 m between them within the corn field. Traps set up in the vegetative stage were placed at a height of 1 m above the ground, while traps set up in the reproductive stage were placed 1.5 m above the ground (Cruz-Esteban et al., 2020). The distance between the traps was 50 m and they were all placed in the same orientation, in the direction of the wind (Lewis and Macaulay 1976). The traps remained in the field for a period of seven days and were then collected and the number of adults trapped on the adhesive floor was determined.

2.5 Assessment of abiotic factors

Daily temperature (average, maximum, minimum), relative humidity (average, maximum, minimum), rainfall, pressure and wind speed data were obtained from the Instituto Nacional de Meteorologia database (INMET, 2024). This data are available in the supplementary material.

2.6 Statistical analysis

All analyses were performed using the R statistical program (v. 4.0.0; R Development Core Team, 2020). Always considering the factors under study according to the assay conducted, we analyzed each variable recorded using generalized linear models (GLM) with the adequate error distribution. They were as follows: 1) Poisson for the number of insects captured per trap in the vegetative and reproductive bioassays; and for the number of larvae inside the cob; 2) binomial, for the percentages of damage (Davis scale) in the vegetative maize stage. We used the least squares means (emmeans) to compare the significance of treatment differences.

Prior to applying the statistical methods used here, we performed residual analyses to confirm the model's error distribution and suitability.

3. RESULTS

3.1 Assessment of FAW damage in the vegetative stage of corn plants

We found that the location (cities), the year of data collection (2022/2023, 2023/2024), and the collection period (early season and second season) influenced our results (F= 169.697; DF= 1.4882; P<0.001) when we evaluated the average level of damage caused by FAW in the vegetative stage using the visual scale developed by Davis. Overall, the average Damage level was higher in the year 2023/2024 than in the year 2022/2023 and the early season presented more damage than the second season for the two years of evaluation. Regarding the cities evaluated, in the 2022/2023 early season, Viçosa – MG and Cajuri – MG presented similar average damage levels, and presented a higher average damage level than Gurupi – TO. In the early season of 2022/2023, Gurupi – TO presented a higher average level of damage than Porto Firma – MG. In the early season and second season of 2023/2024, Coimbra – MG showed the highest average level of damage, followed by Gurupi – TO and finally Cajuri – MG.

3.2 Sex pheromone traps in the vegetative stage of corn plants

Analysis of the total number of adult insects caught in FAW sex pheromone traps in the vegetative stage of corn revealed that factors such as data collection year (2022/2023, 2023/2024), collection period (early season and second season), percentage of Z7-12:Ac component (2, 4, 10 and 12 percent) of the pheromone, and location (cities) all affected our findings (F= 13.4631; DF= 1, 337; P<0.001). In general, the number of insects captured was greater in 2023/2024 than in the year of 2022/2023 and the number of insects captured was greater in the second season than in the early season for both collection years. Regarding the collection cities, in the early season of 2022/2023, Gurupi – TO had the highest captured rate of males when compared to Viçosa – MG and Cajuri – MG and in the second season of the same year, Gurupi – TO was also higher than Porto Firme – MG. For the year 2023/2024, Gurupi – TO had a higher rate of adult collection in sex pheromone traps both in the early season and in the second season when compared to the locations of Cajuri – MG and Coimbra – MG. Finally, during the second season of 2023/2024, more adults were found on the traps in Cajuri – MG than in Coimbra – MG.

3.3 Assessment of FAW damage in the reproductive stage of corn plants

The evaluation of the total number of FAW caterpillars found on the corn cob revealed that our results ($X^2 = 2392.4$; DF = 2, 9270; $P<0.001$) were influenced by the location (cities), the year of data collection (2022/2023, 2023/2024) ($X^2 = 2758.6$; DF = 1, 9272; $P<0.001$), and the collection period (early season and second season). Overall, the total number of caterpillars was greater in the year of 2023/2024 than in the year of 2022/2023. The early season had more caterpillars on the corn cob than the second season in the year of 2022/2023 and the second season had more caterpillars on the corn cob than the early season in the year of 2023/2024. Regarding the cities evaluated, in the early season of 2022/2023, Viçosa - MG had more caterpillars on the corn cob than Gurupi - TO and Cajuri - MG. Additionally, Gurupi - TO presented more caterpillars than Cajuri – MG. In the second season of that same year, Gurupi - TO had more caterpillars on the corn cob than Porto Firme - MG. In the early season of 2023/2024, Gurupi - TO had 25% more caterpillars in the corn cob than Coimbra - MG and also higher than in Cajuri - TO. In the second season of this same year, Coimbra - MG had more caterpillars on the corn cob than Gurupi - TO followed by Coimbra - MG.

3.4 Sex pheromone traps in the reproductive stage of corn plants

According to our analysis of the total number of adult insects caught in FAW pheromone traps during the reproductive stage of corn, the following factors affected our findings: the location (cities), the year (2022/2023, 2023/2024), the collection period (early and second season), and the percentage of the Z7-12:Ac component (2, 4, 10 and 12 percent) of the pheromone (F= 13.4631; DF= 1, 337; $P<0.001$). In general, the number of insects captured was greater in 2022/2023 than in 2023/2024 and the number of insects captured was greater in the second season than in the early season for both collection years. Regarding the collection cities, in the early season of 2022/2023, Gurupi - TO had the highest captured rate when compared to Viçosa - MG and Cajuri - MG and in the second season of this same year the adults captured in Gurupi - TO was also higher than Porto Firme – MG. For the year 2023/2024, the captured was similar in the three cities for both early season and second season (Cajuri – MG, Coimbra - MG and Gurupi - TO).

When we compared the overall amount of moths captured in the sex pheromone traps when the plants were in the vegetative and reproductive stages, we saw that a larger amount of adults were captured when the plant was in the vegetative stage than in the reproductive stage ($F= 1386.5$; $DF= 1, 404$; $P<0.001$).

4. DISCUSSION

Our results showed a significant variation in the total of FAW males captured in pheromone traps when different ratios of the Z7-12:Ac component were used. Overall, the traps containing 2% and 4% of Z7-12:Ac captured the greatest number of males. Furthermore, the total of males captured was greater during the vegetative stage of corn than in the reproductive stage. This suggests a preference of FAW adults for the early growth stage of corn crops. Additionally, we observed a change in the number of males captured throughout the collection years. We note that the year 2023/2024 resulted in a larger catch of adults than prior year, 2022/2023. This annual dissimilarity can be ascribed to factors such as climatic conditions and differences in the pest population.

It is known that male moths have a large response window in relation to pheromone production by females (Haynes, 1988; Baker, 1989; Löfstedt et al., 1991; Linn, 1997; Krokos et al., 2002). Our field results show that there is an ideal ratio, which seems to occur with traps containing 2% of Z7-12:Ac component, despite the width of the male's response window (Figure 8). Similar results were observed in FAW males from populations in Mexico and China, which showed preference for pheromone lures with 1% and 2% of Z7-12:Ac (Cruz-Esteban et al., 2018; Wang et al., 2022). Similarly, populations of FAW males from United States were more attracted to pheromone lures containing 2% Z7-12:Ac than those with 4% and 10% Z7-12:Ac (Unbehend et al., 2013). These studies indicate a consistency in the preference of FAW males for certain ratios of the critical component Z7-12:Ac in sex pheromone traps, highlighting the importance of correct traps formulation to optimize monitoring and management of this pest.

Additionally, our results showed a reduction in the capture of FAW males in traps during the maize reproductive stage compared to the vegetative stage (Figure 8). Similar results showed a reduction in the capture of FAW adults as corn plants matures and this have been reported in several regions such as Venezuela (Salas et al., 2001), Brazil (Melo et al., 2011), and Kenya (Sisay et al., 2011). This suggests that, for effective management of FAW, it is necessary to position the pheromone traps during the initial stage of crop growth, as also seen by Malo et al., (2001). The decrease in captures males in pheromone traps at more advanced stages of corn plants can be attributed to the reduced preference of FAW moths to oviposit on old and well-developed plants. This behavior is analogous with previous studies that have documented a greater oviposition preference of FAW moths for younger corn plants (Harrison, 1984).

We also noticed a difference in the total of males captured according to geographic location and planting season (early season or second season). In 2022/2023, traps in Tocantins captured more FAW males than in Minas Gerais during the early season and second season (figure 8). This difference can be attributed, in part, to the different climatic conditions between the two states during the early season and second season in this period. For example, the average daily temperatures in early season were 22.3°C in Minas Gerais and 25.3°C in Tocantins, while in the second season they were 17.15°C in Minas Gerais and 25.7°C in Tocantins, reflecting differences of 3° and 7.55°C between locations (Table 1 to 4). Similarly, Pair et al. (1986) and Murua & Virla (2004) showed that colder temperatures were a possible cause of the decrease in FAW population in corn crops.

In the following year (2023/2024) the capture of FAW males was once again higher in Tocantins during the early season, with average temperatures of 24.22°C in Minas Gerais and 26.15°C in Tocantins (Table 1, 5 and 6). However, the temperatures recorded in these locations do not seem to have been responsible for the variation in males catch in pheromones traps, since the variation was very small, a similar result was found by Rojas et al. (2004). In general, we observed a lower number of adults captured in traps fixed during the early season, which occurs in the rainy season. A greater peak in captures occurred in the second season, which occurs at the end of the rainy season. Similar results were reported by Raulston et al., (1986), Mitchell et al., (1991) and Silvain & Hing (1985), where the largest catches occurred during the rainy season. Furthermore, it is important to note that the corn areas in Tocantins are extensive and are not surrounded by native or cultivated trees, unlike the other experimental sites in Minas Gerais. This can represent a barrier both to insects and to the dispersion of pheromones. Rojas et al., (2004) showed that the most significant factor that influenced the capture of FAW in pheromone traps was the availability of areas with corn, sorghum and soybean cultivation, observing greater capture of FAW males in areas with more intense cultivation.

Regarding the damage caused by FAW infestation, appears to be no relationship between vegetative and reproductive damage with the capture of males during the two years of evaluation and during the plant seasons (see Figures 6, 7 and 8). Murua & Virla (2004) observed similar results in a field experiment with corn in Argentina, indicating that the percentage of attack by FAW caterpillars during the summer was not related to the capture of adults in the same period. In contrast, Silvain et al., (1985) identified a significant correlation between moth captures and the relative abundance of caterpillars in field tests carried out in French Guiana. These observations highlight the complexity in the relationship between damage caused by FAW caterpillars and the effectiveness of monitoring strategies based on captured adult. Therefore, the pheromone traps cannot be evaluated only by the simple presence of visible damage to plants but also by factors beyond, such as abiotic factors.

Our study revealed a significant variation in the number of FAW males captured throughout the years. We observed that in 2023/2024 the traps captured a greater number of adults than in 2022/2023. Similar results were observed in studies carried out in Argentina, Mexico, and the United States (Pair et al., 1986; Melo et al., 2002; Murua & Virla, 2004; Rojas et al., 2004; Bratovich et al., 2019). This annual variation can be influenced by several factors, such as seasonal climatic variations, changes in the distribution of the pest population, and even fluctuations in the availability of food resources or environmental conditions that affect the activity of FAW adults. These results highlight the importance of considering not only specific management strategies, such as pheromone proportions of important components in traps, but also temporal variability before implementing integrated pest management to control this pest.

In summary, the results elucidated that the pheromone trap containing 2% of Z7-12:Ac was the most efficient for attracting FAW males to the traps. Furthermore, we identified that the efficiency of the capture was significantly influenced by several factors, including the location of the traps, the phenological stage of the crop (vegetative and reproductive), the planting time (early season or second season) and the different years. It is important to highlight that our results did not indicate a relationship between the damage caused by FAW caterpillar infestation and the capture of males. This suggests that the intensity of crop damage was not directly related to the number of males captured in pheromone traps. These findings are crucial for the development of integrated pest management strategies, emphasizing the importance of considering not only the attractiveness of pheromone traps, but also other aspects of the agricultural environment that may influence the efficacy of control measures.

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6. FIGURES THIRT CHAPTER

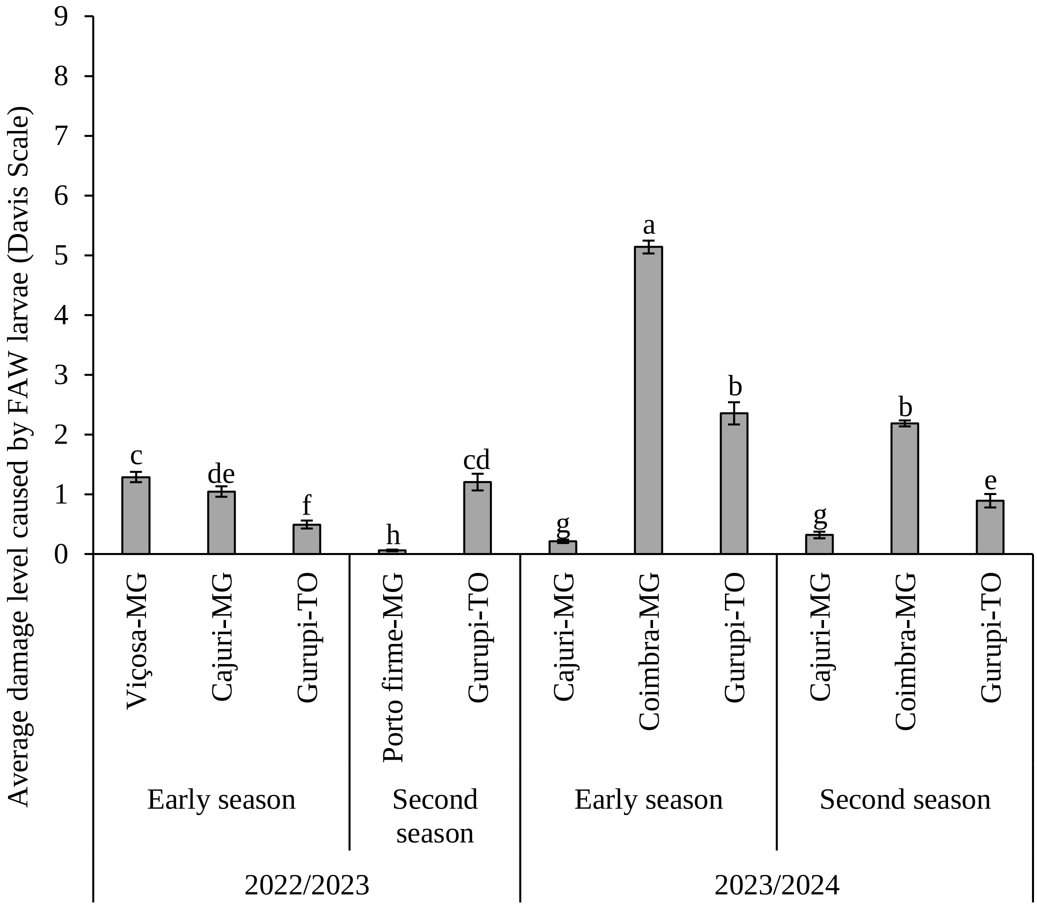

Figure 6. Average level of damage (Davis scale) on corn plants at the vegetative stage in different regions of Brazil (Viçosa-MG, Cajuri-MG, Gurupi-TO, Porto Firme-MG, Coimbra-MG) during the early season and second season of corn crops in the years of 2022/2023 and 2023/2024. P values are given for treatments [generalized linear model (family, Quasibinomial)] followed by pairwise comparisons of Least Squares Means (LSMeans). Different letters indicate significant differences among treatments ($P<0.05$).

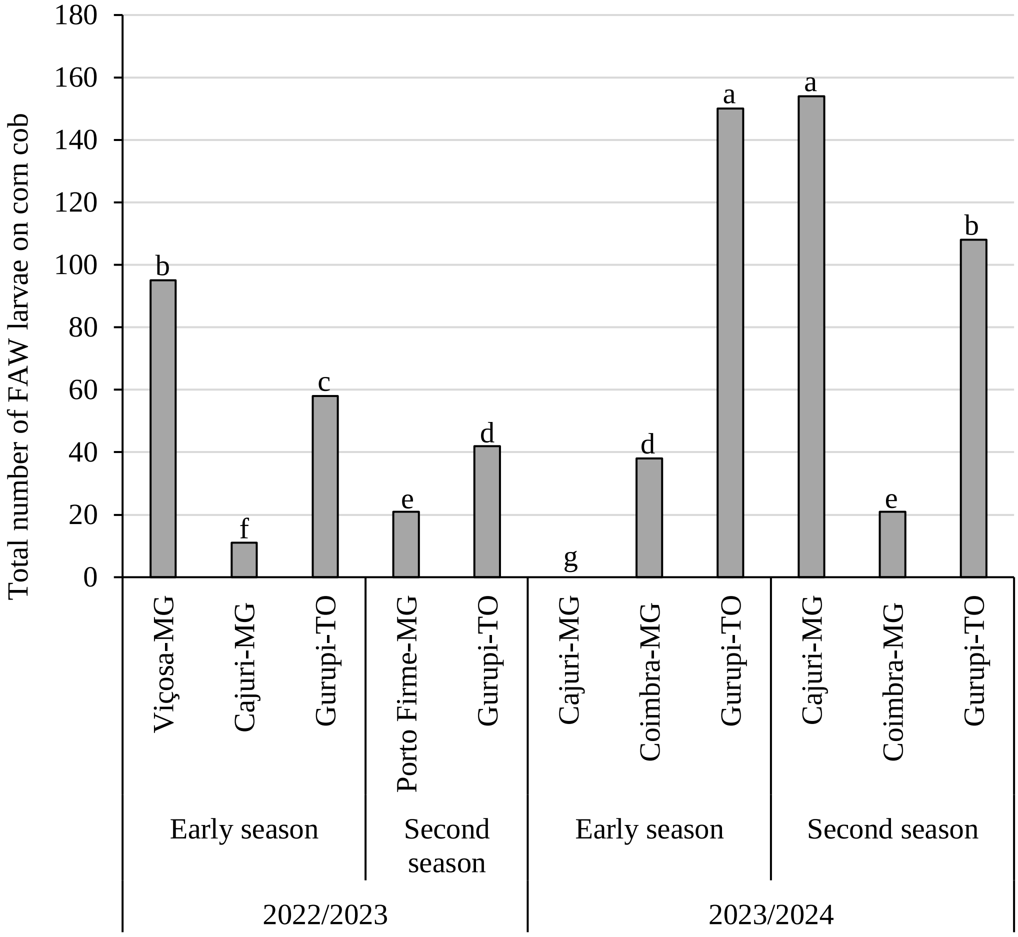

Figure 7. Total number of *Spodoptera frugiperda* larvae on corn cobs in different regions of Brazil (Viçosa-MG, Cajuri-MG, Gurupi-TO, Porto Firme-MG, Coimbra-MG) during early season and second season of corn crops in the years of 2022/2023 and 2023/2024. P values are given for treatments [generalized linear model (family, Poisson)] followed by pairwise comparisons of Least Squares Means (LSMeans). Different letters indicate significant differences among treatments ($P<0.05$).

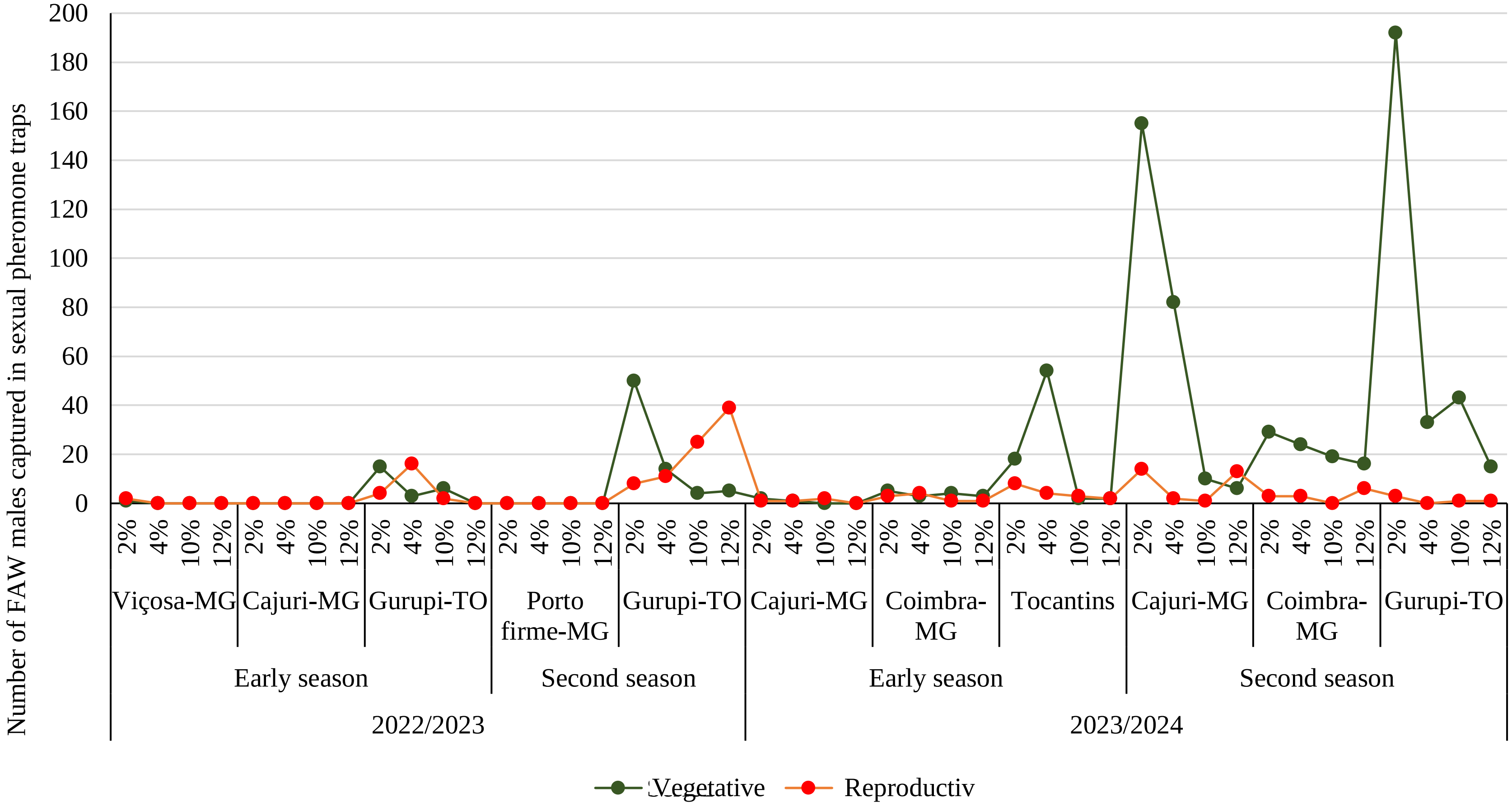

Figure 8. Total number of *Spodoptera frugiperda* males captured by traps with 2%, 4%, 10%, and 12% of the critical component Z7-12:Ac in different regions of Brazil (Viçosa-MG, Cajuri-MG, Gurupi-TO, Porto Firme-MG, Coimbra-MG) during early season and second season of corn crops in the years of 2022/2023 and 2023/2024. The green line represents the number of males captured during the vegetative stage of corn, and the orange line represents the number of males captured during the reproductive stage of corn. P values are given for treatments [generalized linear model (family, Poisson)] followed by pairwise comparisons of Least Squares Means (LSMeans). Different letters indicate significant differences among treatments ($P<0.05$).

7. TABLES THIRT CHAPTER

Table 1. Average temperature, humidity, pressure, wind and rainfall data from the day traps were set up until the day they were dismantled in Tocantins state (Gurupi city), Gurupi station in different years (2022/20223) and season (early season and second season) (Data taken from the national meteorology institute: https://portal.inmet.gov.br/).

			Temperature			**Humidity**					
		Corn stage	Mean	Maximum	Minimum	Mean	Maximum	Minimum	**Pressure**	**Wind (m/s)**	**Rainfall**
2022/2023	Early season	Vegetative	25.3	25.9	24.8	84.9	87.6	82.1	979.3	1.0	0.5
		Reproductive	25.3	25.9	24.7	83.6	86.5	80.7	979.2	1.1	0.3
	Second season	Vegetative	25.9	26.5	25.3	82.7	85.6	79.8	978.4	1.0	0.1
		Reproductive	25.5	26.3	24.8	73.4	76.5	70.4	981.5	1.2	0.0
2023/2024	Early season	Vegetative	26.0	26.5	25.4	83.2	85.8	80.6	981.4	1.2	0.4
		Reproductive	26.3	26.9	25.7	83.7	86.2	81.1	978.8	1.2	0.5
	Second season	Vegetative	26.4	27.0	25.8	85.7	88.2	83.1	979.4	0.9	0.2
		Reproductive	26.0	26.9	25.2	72.5	75.8	69.2	980.4	1.2	0.0

Table 2. Average temperature, humidity, pressure, wind and rainfall data from the day traps were set up until the day they were dismantled in Minas Gerais state (Viçosa city), Viçosa station in the year of 2022/20223 and early season (Data taken from the national meteorology institute: https://portal.inmet.gov.br/).

			Temperature			**Humidity**					
		Corn stage	Mean	Maximum	Minimum	Mean	Maximum	Minimum	**Pressure**	**Wind (m/s)**	**Rainfall**
2022/2023	Early season	Vegetative	21.8	22.4	21.2	82.2	85.2	79.2	931.4	1.0	0.2
		Reproductive	23.0	23.5	22.6	83.3	86.1	80.5	933.7	1.0	0.6

Table 3. Average temperature, humidity, pressure, wind and rainfall data from the day traps were set up until the day they were dismantled in Minas Gerais state (Cajuri city, property number one), Viçosa station in the year of 2022/20223 and in the early seasons (Data taken from the national meteorology institute: https://portal.inmet.gov.br/).

			Temperature			**Humidity**					
		Corn stage	Mean	Maximum	Minimum	Mean	Maximum	Minimum	**Pressure**	**Wind (m/s)**	**Rainfall**
2022/2023	Early season	Vegetative	22.2	22.8	21.6	81.5	84.5	78.4	931.5	0.9	0.1
		Reproductive	22.3	22.7	21.9	85.2	87.7	82.7	934.0	1.1	0.5

Table 4. Average temperature, humidity, pressure, wind and rainfall data from the day traps were set up until the day they were dismantled in Minas Gerais state (Porto firme city), Viçosa station in the year of 2022/20223 and in the second seasons (Data taken from the national meteorology institute: https://portal.inmet.gov.br/).

			Temperature			**Humidity**					
		Corn stage	Mean	Maximum	Minimum	Mean	Maximum	Minimum	**Pressure**	**Wind (m/s)**	**Rainfall**
2022/2023	Second season	Vegetative	16.6	17.2	16.1	82.7	85.3	80.0	938.9	0.4	0.0
		Reproductive	17.7	18.2	17.2	86.5	88.6	84.5	939.9	0.6	0.0

Table 5. Average temperature, humidity, pressure, wind and rainfall data from the day traps were set up until the day they were dismantled in Minas Gerais state (Coimbra city), Viçosa station in the year of 2023/20224 in different seasons (early season and second season) (Data taken from the national meteorology institute: https://portal.inmet.gov.br/).

			Temperature			**Humidity**					
		Corn stage	Mean	Maximum	Minimum	Mean	Maximum	Minimum	**Pressure**	**Wind (m/s)**	**Rainfall**
2023/2024	Early season	Vegetative	26.1	26.8	25.4	63.9	67.4	60.3	932.8	0.8	0.0
		Reproductive	24.6	25.1	24.1	80.3	83.1	77.4	935.0	1.1	0.6
	Second season	Vegetative	23.4	23.8	22.9	84.2	87.0	81.4	935.0	0.7	0.2
		Reproductive	22.2	22.6	21.9	89.2	91.2	87.3	936.4	0.4	0.2

Table 6. Average temperature, humidity, pressure, wind and rainfall data from the day traps were set up until the day they were dismantled in Minas Gerais state (Cajuri city, property number two), Viçosa station in the year of 2023/20224 in different seasons (early season and second season) (Data taken from the national meteorology institute: https://portal.inmet.gov.br/).

			Temperature			**Humidity**					
		Corn stage	Mean	Maximum	Minimum	Mean	Maximum	Minimum	**Pressure**	**Wind (m/s)**	**Rainfall**
2023/2024	Early season	Vegetative	21.6	22.2	21.0	76.5	79.5	73.5	935.4	0.9	0.1
		Reproductive	24.6	25.1	24.1	80.5	83.3	77.6	935.2	1.1	0.6
	Second season	Vegetative	23.1	23.6	22.6	82.0	84.8	79.2	937.6	0.9	0.2
		Reproductive	20.9	21.3	20.4	86.3	88.6	84.0	937.8	0.5	0.1

GENERAL CONCLUSIONS

- On the first calling day, a reduction in the ratio of the Z7-12:Ac component in the sex pheromone of females of the resistant rr-BA population was observed.
- Male responses were affected by the different geographic location between FAW populations and not due to Bt resistance. rr-MG insects showed a larger response window to changes in the Z7-12:Ac ratio.
- Hybrid males (♀SS-BA x ♂rr-MG and ♀rr-MG x ♂SS-BA) generated from crossing insects from the rr-MG population had the best responses for all ratios of Z7-12: Ac.
- Sex pheromone traps with 2% and 4% Z7-12:Ac captured more FAW males than 10% and 12%.
- The capture of males in traps was greater in the vegetative phase of corn than in the reproductive phase
- The total number of males captured was different according to geographic location, traps in Tocantins captured more FAW males than in Minas Gerais
- However, additional work is needed to figure out if the captured males in sex pheromone traps are resistant or suscetible to bt toxins.