

**University of São Paulo
“Luiz de Queiroz” College of Agriculture**

**Computational modeling to describe the dynamics of fall armyworm
Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae) in *Bt* crop
areas**

Adriano Gomes Garcia

Thesis presented to obtain the degree of Doctor in
Science. Area: Entomology

**Piracicaba
2017**

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Bachelor in Biological Sciences

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1. Insetos pragas 2. Ferramentas de decisão 3. Programas computacionais
4. Cultivos transgênicos I. Título

DEDICATION

This thesis is dedicated to my beloved Josemeri, Grega and Zorro

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To Josemeri, Grega and Zorro for all your love, support and patience during this long road. Thank you for helping me to find my life's purpose and for bringing so much joy and happiness into my life.

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EPIGRAPH

But Moses said to God, "Who am I that I should go to Pharaoh and bring the Israelites out of Egypt?"

And God said, "I will be with you. And this will be the sign to you that it is I who have sent you: When you have brought the people out of Egypt, you will worship God on this mountain."

Exodus 3: 11-12

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RESUMO

Modelagem computacional para descrever a dinâmica populacional de *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) em áreas com cultivos *Bt*

Spodoptera frugiperda é um dos principais insetos-praga do hemisfério ocidental, causando diversos danos a cultivos de interesse econômico, como milho e algodão. Uma das estratégias mais comuns de controle é o uso de plantas *Bt*, contudo sua aplicação intensa e indiscriminada levou à evolução de resistência em algumas populações. O estabelecimento de refúgio pode representar uma opção viável para atrasar esse processo. Refúgios são áreas nas quais cultivos não-*Bt* são plantados de forma a fornecerem insetos suscetíveis para a população. Neste estudo, eu desenvolvi inicialmente um modelo para descrever a evolução de resistência de populações de *S. frugiperda* em diferentes configurações de refúgio (mistura de sementes, blocos e faixas) e tamanhos (20 – 50%), considerando o movimento larval. Eu demonstrei que para a mistura de sementes, na maioria dos casos, maiores taxas de movimentação larval implicavam em maiores proporções de insetos resistentes na população, independente do tipo de resistência testada, enquanto áreas com configurações em faixas apresentaram tendência oposta. Na segunda parte deste trabalho, eu obtive dados para validar o modelo proposto. O desenvolvimento de *S. frugiperda* em folhas de milho foram determinadas em 5 temperaturas constantes variando de 14°C a 30°C. Estes dados também foram utilizados para estimar o número de gerações do inseto em 42 localizações no estado da Flórida de 2006 a 2016, que foram interpolados e representados em mapas, utilizando SIG (Sistema de Informações Geográficas). Eu observei que condados mais ao sul apresentaram maior número de gerações e que *S. frugiperda* é capaz de passar o inverno abaixo de latitudes ~29°N. Utilizando os dados obtidos no experimento, um novo modelo foi proposto, considerando um intervalo maior de condições e permitindo ao usuário definir a área de cultivo, as exigências térmicas da população estudada, as funções de oviposição e viabilidade, a taxa de migração, a taxa de movimentação larval e a frequência do alelo de resistência. O modelo foi verificado com dados de monitoramento de 2012 a 2015, coletados em uma área de cultivo localizada na Flórida, distante apenas 70 km da área que forneceu a população para o experimento laboratorial. Eu também utilizei o modelo para estimar o número de adultos para 2016 e para duas situações hipotéticas que consideraram possíveis cenários envolvendo o aquecimento global (médias de temperaturas de 2016 + 1°C e + 2°C). O modelo se ajustou aos dados de monitoramento com sucesso e indicou que os aumentos simulados na temperatura média poderiam produzir surtos quase duas vezes maiores que os níveis de *S. frugiperda* estimados para 2016. O modelo pode ser usado para estimar a dinâmica populacional de *S. frugiperda* em uma determinada área de acordo com a composição e disposição dos cultivos, investigar os efeitos das mudanças de temperatura nos níveis populacionais, definir áreas de refúgio apropriadas para o manejo da evolução de resistência, definir os melhores períodos para plantação e colheita durante o ano de forma a reduzir a população da praga, entre outros.

Palavras-chave: Insetos pragas; Ferramentas de decisão; Programas computacionais; Cultivos transgênicos

ABSTRACT

Computational modeling to describe the dynamics of fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) in *Bt* crop areas

Spodoptera frugiperda is one of the main insect pests of the New World, and causes extensive damage to economically important crops such as cotton and corn. One of the most common control strategies is the use of *Bt* plants; however, their intense and indiscriminate use has led to the evolution of resistance in some populations. The establishment of a refuge can be a viable option to delay this process. Refuges consist of areas where non-*Bt* crops are planted, in order to provide susceptible insects to the population. In this study, I initially developed a model to describe the evolution of resistance of fall armyworm populations in different refuge configurations (seed mixture, blocks, and strips) and sizes (20% – 50%), considering the larval movement. I demonstrated that with a seed mixture, in most cases, the higher the rate of larval movement, the higher the proportion of resistant insects in the population, regardless of the type of resistance tested; meanwhile, strip configurations showed the opposite trend. In the second part of this study, I obtained data to validate the proposed model. The development of the fall armyworm on corn leaves was determined at five constant temperatures ranging from 14°C to 30°C. These data were also used to estimate the number of generations of fall armyworms at 42 locations in the state of Florida, from 2006 to 2016, which were interpolated and represented on maps, using GIS (Geographic Information System). I observed that counties farther south had the highest numbers of generations, and that fall armyworms should be able to overwinter as far north as ~29°N. Using the data obtained in the experiment, a new model was proposed, covering a wider range of conditions and allowing the user to define the crop area, the thermal requirements of the population studied, the viability and oviposition functions, the migration rate, the rate of larval movement and the frequency of alleles for resistance. The model was verified with monitoring data collected in a crop area in Florida from 2012 – 2015, only 70 km from the area that provided the population for the experiment. I also used the model to estimate the number of adults for 2016 and for two hypothetical situations that considered possible scenarios involving global warming (mean temperatures in 2016 + 1°C and + 2°C). The model succeeded in fitting the monitoring data, and indicated that the simulated increases in mean temperature could produce outbreaks nearly twice as large as the levels of fall armyworm estimated for 2016. The model can be used to estimate the population dynamics of *S. frugiperda* in a particular area, according to the crop composition and disposal, to investigate the effects of temperature changes on the levels of fall armyworm populations, to define appropriate refuge areas in order to manage the evolution of insect resistance, and to define the best periods for plantation and harvesting during the year to reduce pest populations, among others.

Keywords: Insect pest; Decision tools; Computational programs; Transgenic crops

1. INTRODUCTION

Several questions concern farmers and entomologists when dealing with insect pests: What is the best control strategy? How to implement this strategy? Is it safe for people and for the environment? Agriculture has become an international activity and the economic foundation of many countries, where food is produced not only to supply internal demands, but for the whole world (CERVANTES-GODOY; DEWBRE, 2010). Unfortunately, answering many of the questions about improvement of agriculture requires time and resources that are usually not available. Models can provide reliable and relatively rapid responses (TENG; DE VRIES, 1992; JONES et al., 2017). Interest in using models to study insect pest dynamics first arose in the 1950s, aiming to define the intensity and frequency of chemical pulverizations, motivated by the rise of the concept of pest management (STERN et al., 1959). Modeling has become an important part of the process to choose the best economically and environmentally sound management strategy. Norton (1976) emphasized the important role of models as tools to aid in reaching decisions, although Bertsekas (1976) cautioned about the necessity to consider stochastic events in the modeling process due to the unpredictability inherent in biological systems. For instance, stochasticity can be related to unpredictable outbreaks of some insect pests (COOKE; CARROLL, 2017). Even so, deterministic models were able to provide useful results for insect-management plans involving the introduction of sterile males (KNIPLING 1955, PROUT 1978). Comins (1977) adapted a logistic model to define the quantity of pesticides to be used and the size of refuge areas to avoid the development of resistant insects. In the 1970s, the use of parasites to control pest populations was also studied using models, coupling non-linear differential equations to classify members of a host population according to their state, e.g., susceptible and infected, and describing their dynamics over time (ANDERSON; MAY, 1978, 1981; MAY; ANDERSON, 1978).

Currently, the technology has evolved following the evolution of computers, allowing diverse new modeling approaches and enlarging the role of models in pest control (JONES et al., 2017). Modeling is the representation of reality; therefore, the computational models aim to describe entomological problems using the facility and dynamism provided by computers. Computational models have proven to be useful tools for agriculture because they constitute a laboratory *in silico*, where experiments can be done to anticipate the effect of a natural or anthropic variable on the insect dynamics (MICHALSKI; CARBONELL;

MITCHELL, 1985; FERREIRA; GODOY, 2014). Bone et al. (2006) coupled a cellular automata model and fuzzy logic to describe the spatial distribution of the mountain pine beetle *Dendroctonus ponderosae* (Coleoptera: Scolytidae) and simulate the susceptibility levels of trees to this beetle. Cellular automata models were also used to study habitat management, to optimize the control of the cucurbit beetle *Diabrotica speciosa* (Coleoptera: Chrysomelidae) (GARCIA et al., 2014) and to evaluate the invasion potential of the tomato leafminer *Tuta absoluta* (Lepidoptera: Gelechiidae) in Africa, considering temperature, relative humidity and yield of tomato production (GUIMAPI et al., 2016).

One of the insect pests of great interest to model is the fall armyworm *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepodoptera: Noctuidae). *S. frugiperda* is a polyphagous pest that occurs in the western hemisphere from Argentina to Canada (SPARKS, 1979), and attacks several crops, including corn, cotton and soybeans (BARROS; TORRES; BUENO, 2010). In Brazil, the fall armyworm is one of the main insect pests of corn and cotton (MARTINELLI et al., 2006). In the United States, it is concentrated mainly in the southeast due to the climate conditions (HOGG; PITRE; ANDERSON, 1982). Young larvae begin to damage leaves by scraping the leaf stem, and completely consume the leaves during the last larval instars (VILARINHO et al., 2011; REDAELLI; OURIQUE, 2015).

One of the main strategies of fall-armyworm control involves the use of transgenic crops altered to express genes from the bacterium *Bacillus thuringiensis* (*Bt* plants). The increasing use of *Bt* plants, together with the low level of commitment to using refuge areas, have raised concerns. Intensive use of these plants has favored the evolution of insect resistance to the plants in the field, making some of the commercially available technologies unviable (JAMES, 2013; TABASHNIK; BRÉVAULT; CARRIÈRE, 2013).

One approach to delay the evolution of insect resistance to *Bt* toxins is the use of refuge areas. These consist of areas where non-*Bt* crops are planted in order to promote the survival of susceptible individuals, preventing an increase in the proportion of resistant insects when resistance is a recessive character (SISTERSON et al., 2005; CROWDER; CARRIÈRE, 2009). In the United States, proportions of non-transgenic refuge areas totaling 20% have been recommended for transgenic-corn fields in the Corn Belt and 50% for transgenic-corn fields in the Cotton Belt (ENVIRONMENTAL PROTECTION AGENCY, 1998). In Brazil, proportions of 10% for non-transgenic crops in transgenic-maize fields, 20% in transgenic-soybean fields, and 5% to 20% in transgenic-cotton fields are recommended (MINISTRY OF AGRICULTURE, LIVESTOCK AND FOOD SUPPLY, 2014).

Therefore, it is not surprising that the dynamics of *S. frugiperda* in transgenic and non-transgenic crops have already been simulated using computational models. Cerda and Wright (2004) developed a population-based model to investigate optimal refuge configurations and proportions to manage the resistance in hypothetical populations. They found that the larger the refuge sizes, the lower the rates of increase in the frequency of resistance; however, larval movement could produce an opposite trend. Carroll et al. (2012) used a probabilistic model to compare different refuge configurations, concluding that seed mixtures were more effective in delaying resistance evolution across a range of conditions.

My primary goal in this thesis was to create a new approach to simulate the spatio-temporal dynamics of *S. frugiperda*. First, differently from most of the studies in this area, I used a spatially explicit model that allowed a detailed investigation of the spatial distribution of *S. frugiperda*. Additionally, I used an individual-based model, whose greatest advantage is the ability to represent the individual-level variations among insects during their life cycle, allowing a more realistic representation of the study case (DEANGELIS; GRIMM, 2014). My intention here was to create a computational tool able to represent in detail the design and characteristics of a small-scale area. This type of model is appropriate for this representation because it details the variability of individual characteristics (JORGENSEN; CHON, 2009). I also combined a larger set of conditions than previous models that investigated the evolution of resistance, providing a more complete set of results. The theoretical model and the results of this proposal are described in Chapter 2 of this dissertation.

The second stage of this study was the model validation. According to Rykiel (1999), validation is not a test of a scientific theory, nor is it a statement of the veracity of a scientific understanding. Validation merely means that a model is acceptable for its purpose, since it meets certain requirements. A partnership was established with the USDA in order to test if the model provided acceptable results. A fall armyworm population from Florida was studied in the laboratory in order to obtain data to use as input for the model. With these data in hand, I decided to conduct an additional analysis to map strategic areas for the development of fall armyworms in Florida and compare the results to previous studies that used different approaches (SNOW; COPELAND, 1969; WOOD; POE; LEPPLA, 1979; WADDILL et al., 1982). Based on the thermal requirements of *S. frugiperda*, the number of generations of fall armyworm was estimated at 42 locations in Florida, from 2006 to 2016, and represented in interpolation maps using a Geographic Information System (GIS). The results of this study are described in Chapter 3.

Finally, in Chapter 4, the model developed in Chapter 2 was improved and adapted with the data obtained in Chapter 3. Initially, my proposal was to create a theoretical model providing useful information about the refuge configuration and size in order to manage the evolution of resistance of *S. frugiperda* (Chapter 2, GARCIA et al., 2016). However, in Chapter 4, the potential of the model was expanded. One of the improvements was that all equations and rules related to insect development have become temperature-driven. This allowed me to consider the effect of climate changes and seasonality on the population dynamics. Additionally, the results provided by the model were verified with data from the field, which increased its reliability. Although insects are considered poikilotherms and temperature is one of the most important variables in their development and geographic distribution (LUGINBILL, 1928; ROSE; SILVERSIDE; LINDQUIST, 1975; BARFIELD; MITCHELL; POE, 1978; YOUNG, 1979), most models do not consider the influence of temperature variation on insect development.

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2. PREDICTING EVOLUTION OF INSECT RESISTANCE TO TRANSGENIC CROPS IN WITHIN-FIELD REFUGE CONFIGURATIONS, BASED ON LARVAL MOVEMENT

ABSTRACT

The selection pressure imposed by the widespread use of transgenic technologies can lead to the evolution of insect resistance, and the availability of refuge areas that allow susceptible homozygous insects to survive is a key factor in delaying the evolution of resistance in agricultural landscapes. Different strategies to exploit refuge areas exist, but several insect-related ecological traits may directly affect the efficiency of refuges in slowing the development of resistance. Insect larval movement is one such trait that may affect the management of resistance, depending on the refuge strategy adopted. We developed a computational model to simulate how larval movement would affect the spatio-temporal dynamics of the evolution of resistance of insect pests to *Bt* crops, under different refuge configurations. In order to test the model, we used population data for *Spodoptera frugiperda*, one of the main target pests for control with *Bt* toxins. Simulations were run for spatial arrangements composed of three refuge configurations (seed mixture, blocks, and strips), with sizes ranging from 20% to 50% for two types of resistance (incomplete and complete) and three rates of larval movement (proportion of larvae moving per time step), equal to 0, 0.1 and 0.5. We demonstrated that with a seed mixture, in most cases the higher the rate of larval movement, the higher the proportion of resistant insects in the population in an area, regardless of the type of resistance tested. Strip configurations showed the opposite trend. In a block configuration, the number of resistant larvae was highest at an intermediate dispersal rate (0.1). We concluded that larval movement is an important variable affecting the evolution of resistance to *Bt* crops, but its effect depends on the type of resistance and the configuration and size of the refuge.

Keywords: Cellular automata; Transgenic crops; Resistance management; Refuge areas; Insect ecology

2.1. Introduction

Increased concern regarding the side effects caused by the indiscriminate use of pesticides to the environment and non-target organisms has motivated the development of new pest-control strategies (THACKER, 2002). One of these technologies involves the use of transgenic crops, most of them genetically altered to express genes from the bacterium *Bacillus thuringiensis* (*Bt* plants). The increasing use of *Bt* plants, together with the low level of compliance with refuge requirements, have raised concern. The lack of compliance with guidelines for the use of refuges has favored the evolution of insect resistance to *Bt* plants in the field, and has led to the rapid nullification of some of the commercially available technologies (JAMES, 2013; TABASHNIK; BRÉVAULT, CARRIÈRE, 2013).

One strategy to delay the evolution of insect resistance to *Bt* toxins is the use of refuges. This consists of planting non-*Bt* crops in order to promote the survival of susceptible insects that will outnumber the resistant ones, preventing an increase in the proportion of the resistant phenotype when resistance is a recessive trait (SISTERSON et al., 2005; CROWDER; CARRIÈRE, 2009). Different countries have adopted different requirements for refuge areas. For instance, in the United States, the Environmental Protection Agency (EPA) has determined that the optimum proportions of non-*Bt* refuge areas are 20% for *Bt*-maize fields in the Corn Belt and 50% for *Bt*-maize fields in the Cotton Belt, based on scientific criteria and practicality for growers (ENVIRONMENTAL PROTECTION AGENCY, 1998). In Brazil, the Ministry of Agriculture, Livestock and Food Supply recommends 10% non-*Bt* plants in *Bt*-maize fields, 20% in *Bt*-soybean fields, and 5% to 20% in *Bt*-cotton fields (MINISTRY OF AGRICULTURE, LIVESTOCK AND FOOD SUPPLY, 2014).

Usually, the refuge is structured as a block or a set of strips of non-*Bt* and *Bt* crops (CARROLL et al., 2013). An alternative to the structured refuge is the use of seed mixtures of non-*Bt* and *Bt* crops (RIB) to ensure growers' compliance with refuge requirements (CARROLL; HEAD; CAPRIO, 2012; CARROLL et al. 2013). Although RIB appears to be a plausible solution for managing the evolution of insect resistance to *Bt* crops, several important issues related to the effect of insect larval movement on the evolution of resistance remain to be resolved. Larval movement can expose more larvae to sublethal doses of *Bt* toxins, increasing selection for *Bt* resistance either if the target pest moves as early instars from *Bt* to non-*Bt* crops, or if it moves from non-*Bt* to *Bt* crops as older instars (BINNING et al., 2014; HEAD et al., 2014).

Spodoptera species (Lepidoptera: Noctuidae) are among the main target pests for control with *Bt* toxins in maize and cotton crops, and insect-resistance management is essential to ensure the effectiveness of this control (HUANG et al., 2014). *Spodoptera frugiperda* (J. E. Smith, 1797) is the main species occurring in the New World, with a distribution ranging from Argentina to Canada (SPARKS, 1979), where *Bt* plants are extensively cultivated. Because of the heavy use of *Bt* plants, field-evolved resistance to *Bt* maize has already been documented in Puerto Rico (VÉLEZ et al., 2013) and Brazil (FARIAS et al., 2014; OMOTO et al., 2016).

Ecological modeling can be a useful tool to investigate the influence of larval movement and other ecological factors on the evolution of resistance (SISTERSON et al., 2005). Theoretical ecological models that include spatial heterogeneity and insect fitness on different hosts (e.g., *Bt* or non-*Bt* crops) can provide insights on this evolution. Cellular automata (CA) are an interesting choice for developing these ecological models, because all relevant within-plot characteristics of a crop can be included in the model (GARCIA et al., 2014). The discrete nature of CA models permits one to represent the presence or absence of the different stages of the insect pest at a specific position of the lattice, by a binary variable whose state can change over time through simple stochastic rules that mimic an insect life cycle (HIEBELER, 2005; GARCIA et al., 2014). Additionally, a combination between CA and individual-based approaches may provide a realistic sense of movement since CA models focus on the spatial conformation of the variables through transitions rules while individual-based models focus on the variability of individual characteristics (JORGENSEN; CHON, 2009).

In order to investigate the effect of larval movement of *S. frugiperda* on the evolution of resistance under different *Bt* refuge configurations, we developed a CA model. We ran simulations testing three different rates of larval dispersal under three refuge configurations (seed mixture, strips, and blocks), and with refuge areas ranging in size from 20 to 50% of the total cultivated area. We also considered the type of resistance (complete or incomplete) to *Bt* maize, since this attribute may influence the selection of the resistant phenotype. According to Tabashnik et al. (2013), incomplete resistance occurs when resistant insects developing on *Bt* crops are at a disadvantage compared with those that develop on non-*Bt* maize. In each simulation, we determined the proportion of resistant larvae per time step. We hypothesized that larval movement would differently affect the evolution of resistance to *Bt* crops, for complete or incomplete resistance mechanisms, under different configurations and proportions of refuges.

Many different studies have focused on the evolution of insect resistance to transgenic crops, including considerations of spatiality. A model based on a reaction-diffusion system of partial differential equations (PDEs), combining a spatial demographic model of population dynamics with classical genetic theory, was used to study the relationship between refuge size, pest movement, and resistance evolution in the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Crambidae), and showed that contiguous refuge areas are more efficient than a large number of small refuge patches (TYUTYUNOV et al., 2008). A stochastic model was developed to simulate the rate of resistance evolution of *Helicoverpa armigera* (Lepidoptera: Noctuidae) to Cry1AC in India, integrating genetic and ecological parameters (KRANTHI; KRANTHI, 2004). They were able to define the time spans necessary to reach a resistant allele frequency of 0.5 for different proportions of *Bt* cotton in the total area. Kang et al. (2014) developed a spatial explicit model of population dynamics and genetics of *Diatraea saccharalis* (Lepidoptera: Crambidae) to investigate the evolution of *Bt* resistance in a landscape composed of *Bt* and non-*Bt* maize, sorghum, and rice fields, and concluded that the landscape heterogeneity may complicate the management of *Bt* resistance in *D. saccharalis*. In order to study specifically the resistance evolution in *S. frugiperda*, Téllez-Rodríguez et al. (2014) developed a two-patch population model to investigate the effect of a preference for oviposition on *Bt* or non-*Bt* crops on resistance evolution. They concluded that the avoidance of damaged leaves (which led females to lay eggs on *Bt* plants rather than on plants in the refuge areas) rendered the refuge strategy ineffective. However, none of these previous modeling investigations considered larval movement.

Among the spatial models that have been proposed to study the relationship between larval movement and the evolution of insect resistance, Cerda and Wright (2004) developed a population-based model to test the effects of different refuge proportions (5% – 50%) and different refuge configurations (border, central, equidistant random) on the frequency of resistant alleles in hypothetical populations. They reported that the larger the refuge sizes, the lower the rates of increase in the frequency of resistance; however, higher rates of movement produced an opposite trend. Carroll et al. (2012) developed a probabilistic model to compare RIB and a structured refuge when larval movement in a hypothetical population was considered, and concluded that RIB was more effective in delaying resistance evolution across a range of conditions. Both these studies illustrate the importance of studying larval movement and its implications for resistance evolution.

Differently from most of the work in this area, we used a spatially explicit model that allowed a detailed investigation of the effects of several variables on the spatial distribution of

S. frugiperda. In addition, we used an individual-based model rather than a population-based model. The greatest advantage of this approach is the ability to represent the individual level-mechanisms and the variations among individuals during their life cycle (larval, pupal and adult stages; and different reproductive stages, i.e. pre-oviposition and oviposition), allowing a more realistic representation of the phenomenon under study (DEANGELIS; GRIMM, 2014). We also combined a larger set of conditions that were not previously modeled, to investigate the evolution of resistance, such as the type of resistance (complete and incomplete) with refuge configuration and refuge size, providing a more complete set of results.

2.2. Model assumptions

Spodoptera frugiperda is a holometabolous insect. Only larvae are exposed to the selection pressure imposed by the contact with *Bt* toxins, as only this stage feeds actively on *Bt* crops. Therefore, plants are damaged only by larvae, while adults are responsible for laying eggs on the leaves. In order to take this into account, a stochastic cellular automata consisting of a grid of 604 x 604 sites representing one plant available in each cell was proposed to simulate the population dynamics of larvae/pupae (immature stage) and adults, following a similar method to that of Garcia et al. (2014). Regarding the immature stage, a cell could be either empty or occupied by only one larva. This assumption was based on the mean number of larvae developed per maize plant, as reported by Farias et al. (2001). Although females lay clusters with hundreds of eggs on host plants, only one larva survives per plant, due to environmental pressure and larval cannibalism. Regarding adults, a cell could be empty or occupied by a maximum number of 10 adults, here set as the plant (cell) carrying capacity. Both stages were connected by adult emergence and oviposition. We assumed unlimited feeding resources. We also used a parallel update with fixed boundary conditions, i.e., the first and last rows and columns of cells were kept constant over time (both were equal to 0). Each time step t corresponded to one day, and each cell of the CA represented 1 x 1 m of the crop system. We also associated an age-counter with each insect represented in order to follow its chronological age. The immature stage was divided into larval and pupal stages, in order to represent the period of larval mobility. The rules are:

(1) Immature (larvae and pupae) population dynamics

(a) A cell occupied by an immature insect could become empty with probability $[1 - \mu(i)] + \sigma(i)$ due to mortality or adult emergence per day, respectively. Both parameters were dependent on the chronological age i of the immature insect.

(b) An empty cell could become occupied by a larva if an adult (in a Moore neighborhood of radius 2 plus the central cell) laid eggs in it. Per-capita oviposition probability per day was $\phi(a)$, and was dependent on the chronological age a of the adult.

(2) Adult population dynamics

(a) A cell would become empty of adults if all adults died. The mortality for an individual female per day is described by the probability $\gamma(a)$, depending on the chronological age a of the adult.

(b) An empty cell could be occupied per day with probability $\sigma(i)/2$ if a larva in the corresponding cell developed into a female adult. The fraction $1/2$ was related to the sex ratio, and the probability was dependent on the chronological age i of the immature.

We considered an initial infestation in the center of the lattice, totaling 1600 individuals (40 x 40 cells). We chose $f = 0.01$ as an initial frequency of the resistance allele. Therefore, 16 cells of the total of 1600 were randomly chosen to allocate resistant individuals. The parameter $\mu(i)$ (immature viability) was used to calculate the daily probability of immature mortality $[1 - \mu(i)]$ as well as larval viabilities on *Bt* maize in the cases of either complete or incomplete resistance. A summary of the model parameters is presented in Table 1.

Table 1. Model parameter units and description.

Parameter	Unit	Description
$\mu(i)$	day ⁻¹	probability per time that an immature insect survives
$\sigma(i)$	day ⁻¹	probability per time that a pupa becomes an adult
$\gamma(a)$	day ⁻¹	probability per time of death of a female adult
$\phi(a)$	day ⁻¹	probability per time of oviposition by a female adult
f	-	frequency of resistant allele

All probability functions are described in eqs. (1)-(4). They were proposed by using data on non-*Bt* maize plants from *S. frugiperda* populations provided by Barros et al. (2010).

For CA1, we have:

Survival of immature insects

$$\begin{cases} \mu(i) = 0.98 \text{ if } 0 < i \leq 15 \text{ (corresponding to the larval stage)} \\ \mu(i) = 0.96 \text{ if } 15 < i \leq 25 \text{ (corresponding to the pupal stage).} \end{cases} \quad (1)$$

Metamorphosis (pupa becoming adult)

$$\begin{cases} \sigma(i) = 0 \text{ if } i < 25 \\ \sigma(i) = 1 \text{ if } i = 25 \text{ (at this age, all pupae developed into adults).} \end{cases} \quad (2)$$

For CA2, we have:

Mortality of adult insects

$$\begin{cases} \gamma(a) = 0 \text{ if } a \leq 11 \\ \gamma(a) = 0.1(a - 11) + 0.5 \text{ if } 11 < a \leq 21 \\ \gamma(a) = 1 \text{ if } a > 21. \end{cases} \quad (3)$$

Oviposition by a female adult

$$\begin{cases} \phi(a) = 0 & \text{if } a \leq 3 \\ \phi(a) = 0.7 \left[\left(\frac{1}{e^{0.3(a-1)} - 0.3} \right) - \left(\frac{1}{e^{0.3a} - 0.3} \right) \right] & \text{if } a > 3. \end{cases} \quad (4)$$

Since we used an individual-based model, each individual in the lattice was tested by these equations depending on whether it was an immature or an adult stage. For instance, in case of an immature, a random rational number between 0 and 1 was chosen by the computer, and if this number was less than 0.98, the immature was considered to survive to the next time step. Otherwise, the individual would die.

According to Elmo et al. (2006), there is no preferential direction for adult dispersal. The distance traveled by an adult at each time step was calculated by using data for adult movement in maize fields (VILARINHO et al., 2011). Therefore, in eq. (5), P is the probability that an adult will travel over a distance S per time step in the lattice if the number of insects in the cell destination is less than the carrying capacity defined previously (10 adults) (Figure 1).

Adult movement

$$\begin{cases} P = 1 & \text{if } S \leq 5 \text{ (all adults disperse over 5 meters per day)} \\ P = -0.49 \ln(S) + 1.8 & \text{if } 5 < S \leq 35 \\ P = 0 & \text{if } S > 35 \end{cases} \quad (5)$$

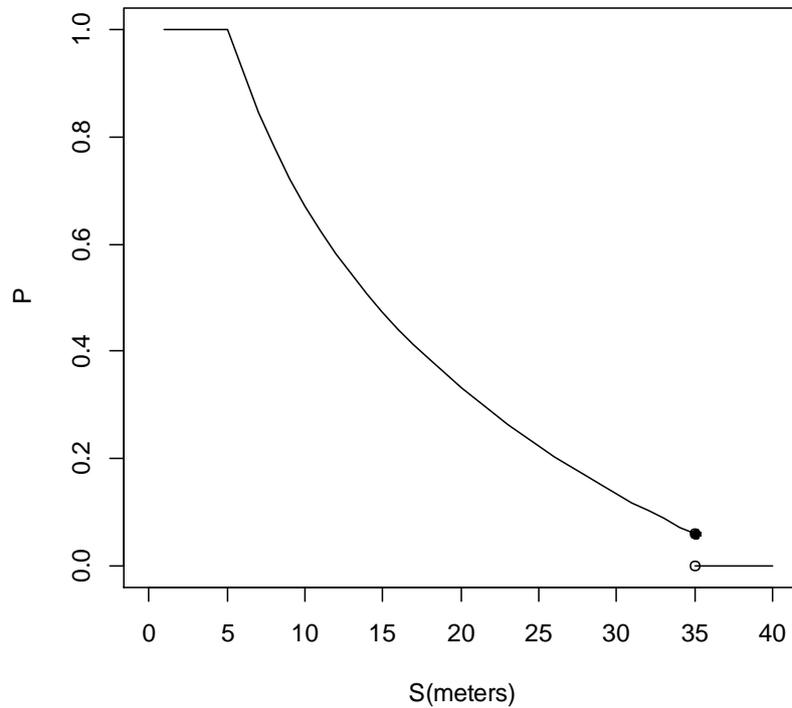


Figure 1. Graphical representation of the equation system (5). P represents the probability that an adult will travel over a distance S per day.

Larval dispersal was implemented as a random movement (diffusion) into a Moore neighborhood of radius 1 (3x3 neighborhood), because experimental evidence suggests that larvae disperse over only short distances. This means that on each day, a larva can move to any of the neighbor cells that are directly in contact with its cell. We considered that only a given proportion of all larvae could move in each time step (rate of dispersal). By increasing the proportion of larvae moving in each time step, larval dispersal in the lattice was increased. For instance, assuming a rate of dispersal equal to 0.1 means that only 10% of larvae (chosen randomly) are moving in the lattice per time step.

2.2.1. Modeling the genetic component

We considered a recessive and autosomal resistance to *Bt* maize (R gene), determined by a single locus, and the existence of three different phenotypes (SS – susceptible homozygote, SR – susceptible heterozygote, RR – resistant homozygote) (VÉLEZ et al., 2014; HUANG et al., 2014). In order to determine the genotype of each individual, we used

the Hardy-Weinberg equation created by Hardy (1908) to study the genetic variation in a population for a single gene locus. It is expressed by:

$$p^2 + 2pq + q^2 = 1, \quad (6)$$

where p is the frequency of the S allele ($p = 1 - q$) and q is the frequency of the R allele. In eq. (6) p^2 represents the frequency of the genotype SS, $2pq$ determines the frequency of SR, and q^2 the frequency of RR.

The genetic contribution of females, but not males, to the offspring was known. The reason is that in the proposed model, the adult population is composed only of females since they are responsible for laying eggs, and as such cause the population to grow.

Thus, we needed to use eq. (6) to estimate the conditional probabilities for each genotype, considering that we knew only the contribution of the parental female. For instance, if the mother is SS, we know the probability that the offspring genotype will be RR is equal to 0. However, since the father's genotype is unknown, the offspring can be either SS or SR. For the offspring to be SS, the father would be SS (p^2) or SR, and the probability that allele S will be transmitted to the offspring is given by $(0.5 \times 2 \times pq)$. For the offspring to be SR, the father would have to be SR and the R allele be transmitted to the offspring $(0.5 \times 2 \times pq)$, or the father would be RR (q^2). The complete conditional probabilities are shown in Table 2.

Table 2. Conditional probabilities of the occurrence of each offspring genotype, depending on the mother's genotype.

Mother genotype	Offspring genotype		
	SS	SR	RR
SS	$p^2 + pq$	$pq + q^2$	0
SR	$0.5(p^2 + pq)$	$0.5(p^2 + q^2) + pq$	$0.5(q^2 + pq)$
RR	0	$p^2 + pq$	$q^2 + pq$

2.2.2. Fitness cost associated with resistance

Gassmann et al. (2009) defined fitness cost as a condition in which fitness is increased by the presence of certain alleles under a stress condition (e.g., resistance to *Bt* crops), but fitness is reduced when individuals carrying these alleles are freed from this stress condition (e.g., resistance to *Bt* crops in the absence of *Bt* crops). The existence of fitness costs associated with resistance to *Bt* toxins has been intensively discussed for *S. frugiperda*. No fitness cost was associated with resistance in populations of *S. frugiperda* from Puerto Rico (JAKKA, 2013), but a high fitness cost was detected in populations from Florida (DANGAL; HUANG, 2015). In this study, we assumed a reduction of 20% in daily larval viability and a delay of four days in the duration of larval development in the absence of *Bt* crops when individuals carried at least one copy of the resistance allele (RR or SR genotypes) (DANGAL; HUANG, 2015). According to Barros et al. (2010), the daily larval viability on leaves of non-*Bt* maize is 0.98; therefore, larval viability was reduced to 0.78 daily. The delay in larval development was considered by changing the domain of functions (1) and (2) (eqs. (7) and (8)).

$$\begin{cases} \mu(i) = 0.78 \text{ if } 0 < i \leq 15 + 4 \text{ (corresponding to the larval stage)} \\ \mu(i) = 0.96 \text{ if } 15 + 4 < i \leq 25 + 4 \text{ (corresponding to the pupal stage)}. \end{cases} \quad (7)$$

$$\begin{cases} \sigma(i) = 0 \text{ if } i < 25 + 4 \\ \sigma(i) = 1 \text{ if } i = 25 + 4 \text{ (at this age, all pupae developed into adults)}. \end{cases} \quad (8)$$

2.3. Simulations

To measure the effect of larval dispersal and refuge configuration on the proportion of resistant larvae, we constructed several spatial arrangements composed of *Bt* maize and non-*Bt* maize in refuge areas with different sizes and configurations. Three refuge configurations were tested in the simulations: seed mixture, blocks, and strips (Figure 2). The proportions of the refuges with non-*Bt* maize ranged from 20 to 50%. *Bt* maize was considered to produce a high-dose event, which, according to the US Environmental Protection Agency guidelines, should kill 99.99% of the susceptible individuals in the field, with a minimum mortality of 95% of the heterozygotes (US ENVIRONMENTAL

PROTECTION AGENCY, 1998). When resistance was complete, we considered that the survival of *Bt*-resistant larvae (RR) in *Bt* maize areas was the same as that of homozygote larvae (SS) in non-*Bt* maize. On the other hand, when resistance was incomplete, larval survival of the RR genotype in *Bt* maize was reduced by 50%; therefore, the survivorship of the RR genotype in non-*Bt* maize was higher even in the presence of a fitness cost associated with resistance. Thus, in the case of incomplete resistance, resistant and susceptible larvae competed for non-*Bt* cells. Figure 3 explains the different values assumed by the parameter $\mu(i)$ during the larval stage in the different simulated conditions. A summary of the initial conditions used in our simulations is shown in Table 3.

Table 3. Overview of the initial conditions used in the simulations. All possible combinations among the described variables were simulated.

Resistance type	Larval movement	Refuge configuration	Refuge size (%)
Incomplete	0	Seed mixture	20
			30
Complete	0.1	Blocks	40
	0.5	Strips	50

The use of seed mixtures as a refuge strategy has been a concern, as some investigators have argued that larvae carrying a resistance allele may use refuge areas to reach advanced instars, and become large enough to survive *Bt* toxins (sub-lethal dose) (ONSTAD et al., 2011; YANG et al., 2014; SORGATTO; BERNARDI; OMOTO, 2015). In order to test this possibility, we considered that *Bt* toxins would affect only larvae less than six days old (earlier than 4th instar). For each proportion of non-*Bt* area and each refuge configuration, we tested three different rates of larval dispersal (0, 0.1 and 0.5). Each simulation was conducted during 300 time steps and repeated 50 times. Therefore, the results presented in the next section are the mean values of the proportion of resistant larvae in these 50 repetitions. All means were compared by using the Tukey's test at 5% probability to determine if they were significantly different.



Figure 2. Refuge strategies designed for simulations of resistance evolution. a) seed mixture, and refuge areas structured in b) blocks, and c) strips. Non-*Bt* maize is shown in gray, and *Bt* maize in black. Spatial arrangements with 40% refuge areas are illustrated.

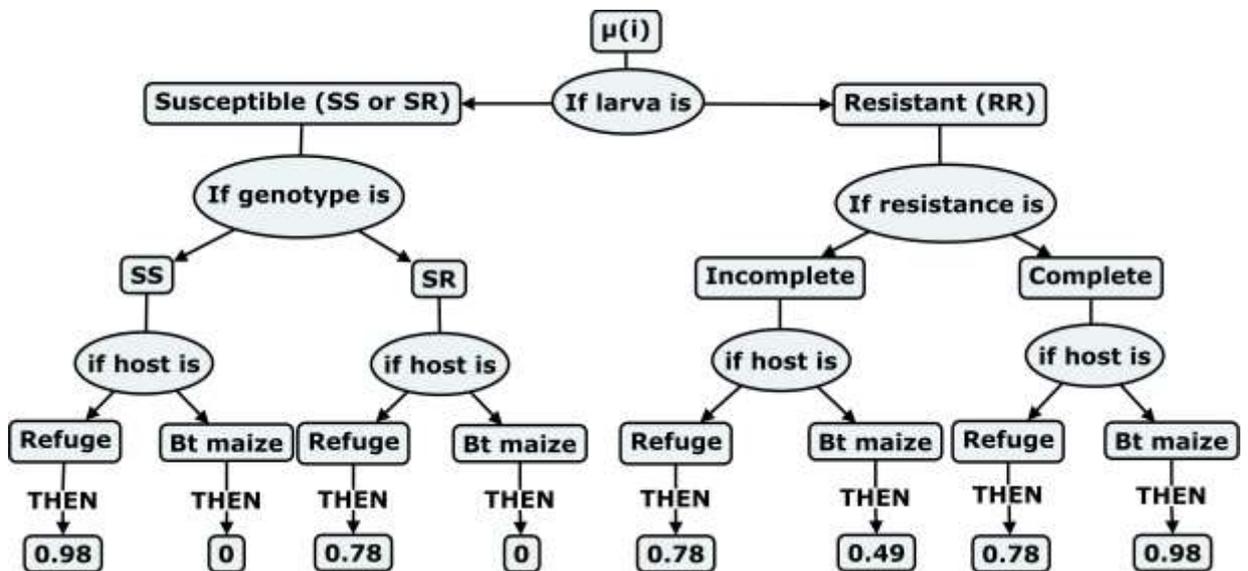
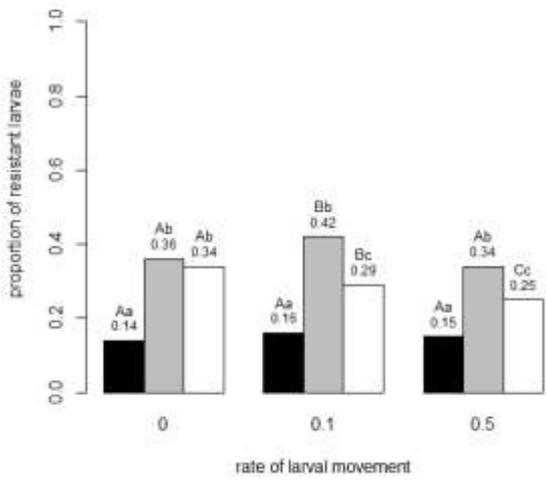


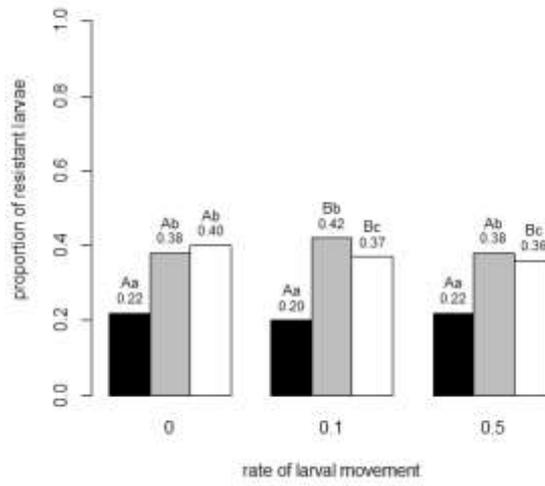
Figure 3. Diagram explaining the values assumed for $\mu(i)$ during the larval stage in different simulated conditions. The standard value of the parameter $\mu(i)$ during the larval stage is equal to 0.98 as described in eq. (1).

2.4. Results

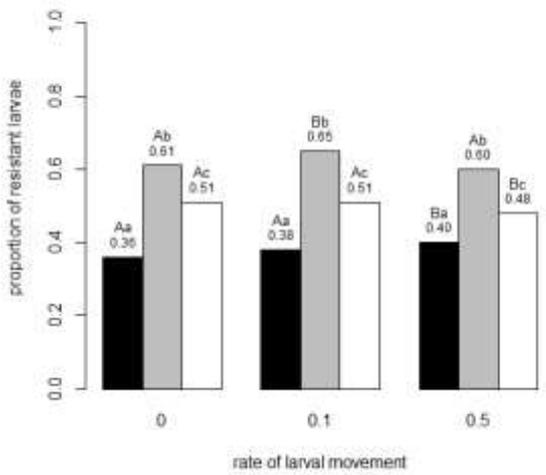
In the proposed model, the efficiency of resistant larvae in exploiting different areas depended on three main factors: resistance type, rate of larval movement, and refuge size. The proportion of resistant insects affected by these factors in each simulation is shown in Figure 4.



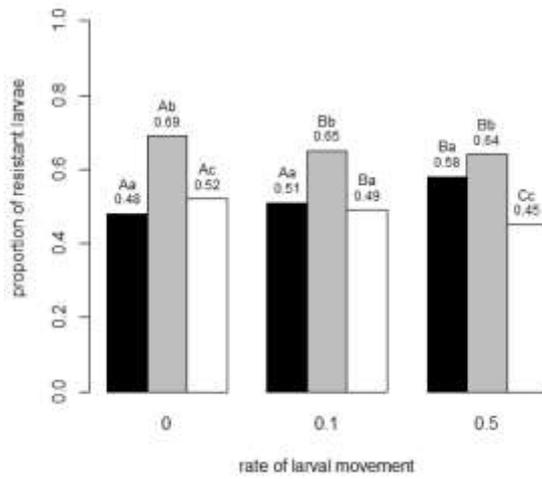
a) Incomplete resistance (50%)



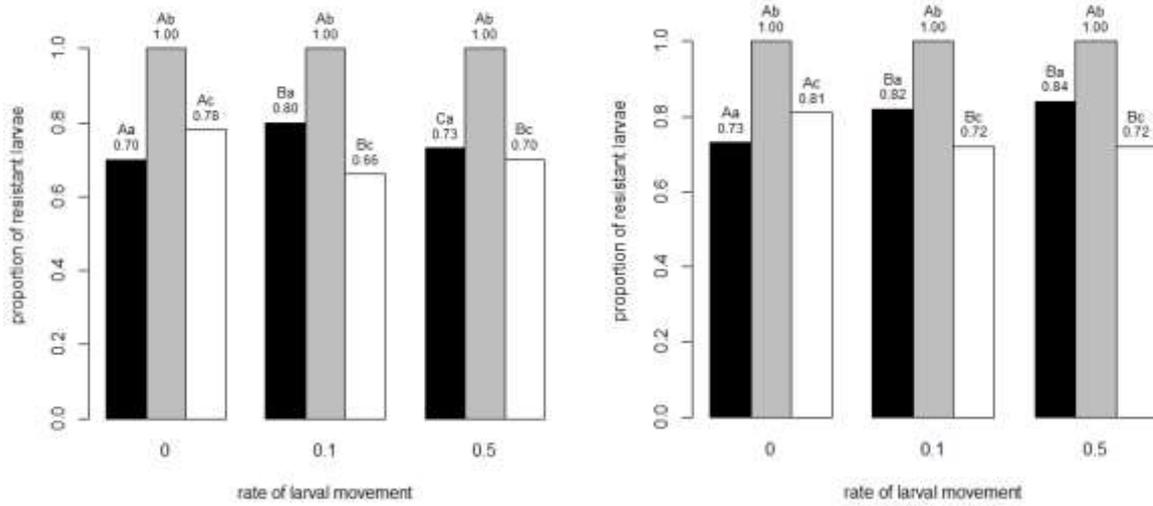
b) Complete resistance (50%)



c) Incomplete resistance (40%)

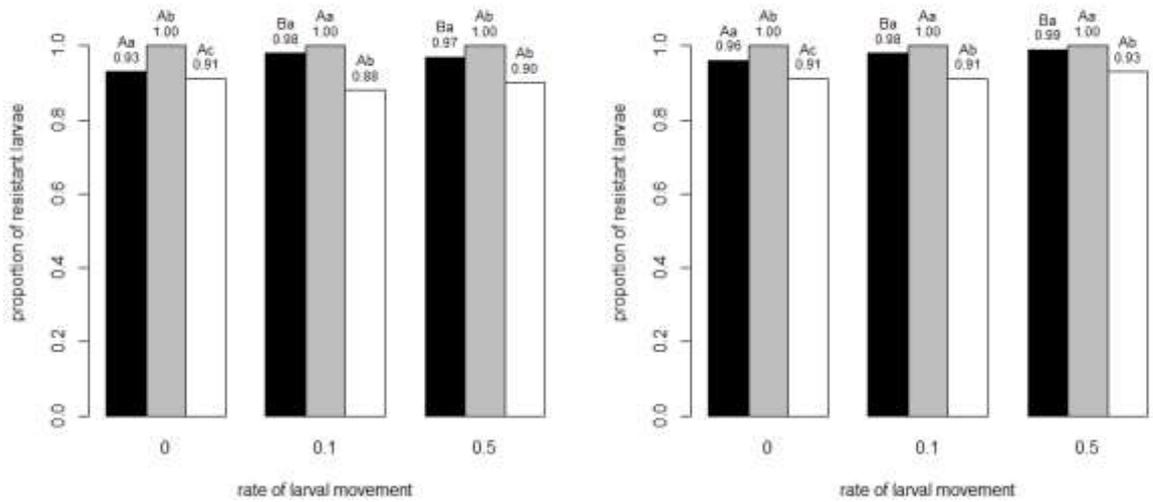


d) Complete resistance (40%)



e) Incomplete resistance (30%)

f) Complete resistance (30%)



g) Incomplete resistance (20%)

h) Complete resistance (20%)

Figure 4. Proportion of resistant larvae measured at $t = 300$ in each spatial arrangement for different refuge sizes and resistance types. Seed mixture in black, blocks in gray, and strips in white. Different uppercase letters above different dispersal rates from the same refuge configuration and different lowercase letters above different refuge configurations from the same dispersal rate indicate significant differences at the 5% level (Tukey's HSD, $p < 0.05$).

2.4.1. Seed mixture configuration

The spatial distribution of *S. frugiperda* larvae in the seed-mixture configuration is shown in Figure 5a. No significant difference in the proportion of resistant insects was found for the 50% seed mixture, because *Bt* and non-*Bt* areas were distributed homogeneously and in the same proportion, reducing the effect of larval movement on the proportion of resistant larvae. When the seed mixture with non-*Bt* maize was reduced to 40%, with either complete or incomplete resistance, the higher the proportion of larvae dispersing to neighbor cells, the higher was the proportion of resistant insects in the population (Figures 4c and 4d). This pattern also occurred with a 30% refuge area, when resistance was complete (Figure 4f). With a 30% refuge area and when resistance was incomplete, larval movement still increased the proportion of resistant larvae (Figure 4e). However, an intermediate dispersal rate (0.1) increased this proportion more than a high dispersal rate (0.5). When the refuge size was reduced to 20% (Figures 4g and h), the dominance of resistant insects was higher and the patterns of resistance evolution observed in the refuge size of 30% began to disappear since the spatial configuration became more homogeneous.

2.4.2. Block configuration

The simulations of the evolution of resistance to *Bt* crops when using refuge areas structured in a block configuration, arranged in two separate areas, showed a spatial polarization between an area occupied preferentially by SS larvae (non-*Bt* block) and another area occupied by RR larvae (*Bt* block) (Figure 5b). Heterozygous (RS) larvae predominated at the boundaries of the *Bt* and non-*Bt* areas, since crossings between SS and RR genotypes occurred in a high proportion in this area. In this refuge arrangement, larval movement affected the evolution of resistance only in areas with 40 and 50% refuge area, regardless of the type of resistance (Figures 4a-d), whether incomplete or complete. When the refuge area was reduced to 30 and 20% of the total, non-*Bt* blocks were strongly reduced, resulting in a more homogeneous area, and resistant larvae completely dominated the population.

When resistance was incomplete, the highest proportion of resistant larvae occurred when the movement rate was equal to 0.1, with refuge sizes of 40 and 50% (Figures 4a and 4c). The same pattern was found in a refuge size of 50%, when resistance was complete (Figure 4b). The highest proportion of resistant individuals carrying complete resistance to *Bt* maize in a refuge size of 40% was observed in the absence of larval movement (Figure 4d).

2.4.3. Strip configuration

In this refuge configuration (Figure 5c), the highest proportion of resistant larvae occurred in the absence of larval movement in most of the refuge sizes tested, regardless of the type of resistance (incomplete or complete) (Figure 4). Larval movement benefited susceptible genotypes, accelerating the occupation of non-*Bt* strips (horizontal movement). In a refuge size of 20%, the spatial configuration was sufficiently homogeneous to reduce the effect of larval movement, and the results were not significantly different.

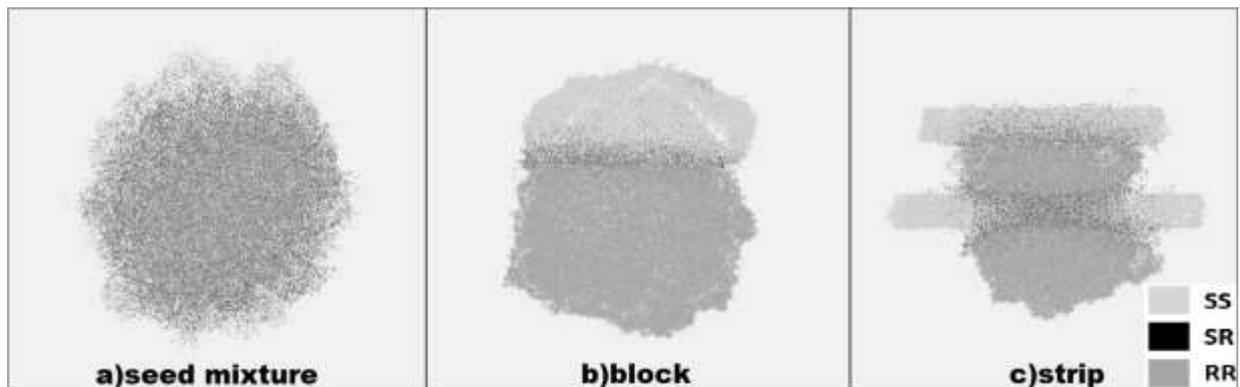


Figure 5. Pattern of larval distribution observed in each refuge configuration after 300 time steps. Insect genotypes are indicated in the legend. For these simulations, we defined spatial arrangements with 40% refuge areas, complete resistance and rate of larval movement equal to 0.5.

2.5. Discussion

The increase in the proportion of resistant larvae as larval movement increased in the seed mixture with complete (30 and 40%) and incomplete (40%) resistance occurred because susceptible larvae moved to their neighbor cells, which were mostly composed of *Bt* maize, resulting in their death, reducing their number and increasing the proportion of resistant larvae (Figure 6). As the SS larvae moved and died, non-*Bt* cells became unoccupied and free for colonization by RS (heterozygous) or RR (homozygous) larvae, which increased in the population. Such a pattern has been intensively discussed previously, and accelerated resistance due to larval movement in spatial arrangements composed of *Bt* and non-*Bt* crops has also been modeled (CARROLL et al., 2012). In a field study, Head et al. (2014) also observed that a low number of *Diabrotica speciosa* (Coleoptera: Chrysomelidae) larvae were able to move from *Bt* to non-*Bt* areas when a seed mixture was used as a refuge strategy. According to a field study conducted by Wangila et al. (2013), either heterozygotes or resistant larvae (in the case of incomplete resistance) might remain in non-*Bt* maize until they

grow large enough to enhance their survival chances in *Bt* maize, increasing their number in the population.

In the case of incomplete resistance (30%), a dispersal rate of 0.1 resulted in the highest proportion of resistant insects, because in this case, survival of resistant larvae in non-*Bt* maize was higher than in *Bt* maize. When the dispersal rate was too high, larvae moved too rapidly and increased their probability of reaching a cell with *Bt* maize, resulting in the death of RS genotypes or reduced survival of RR genotypes.

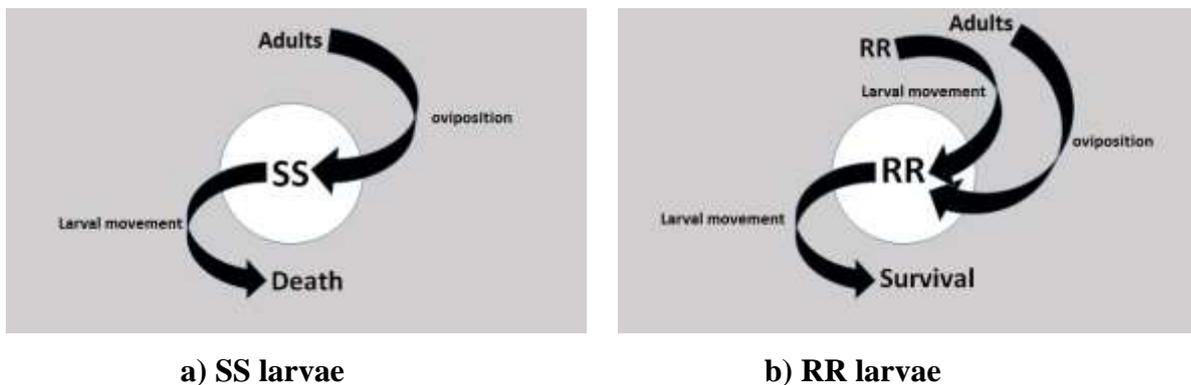


Figure 6. Scheme of larval movement over the seed-mixture configuration. a) Susceptible larvae (SS) are able to survive only in non-*Bt* cells (white). If they move to *Bt* cells (gray) before reaching the 4th instar, they die. b) Resistant larvae survive in either non-*Bt* cells or *Bt* cells.

The polarization pattern observed in the block configuration occurs because the border between *Bt* and non-*Bt* crops is limited to only one contact zone between them, which allows susceptible individuals to become isolated from resistant ones in non-*Bt* areas (Figure 7a). An intermediate movement rate (0.1) accelerated the evolution of resistance in this configuration because at this rate, dispersal was sufficiently rapid to maintain the flow of different genotypes between cells near the border between *Bt* and non-*Bt* blocks (SS larvae moved and died in the *Bt* block), but low enough to prevent them from moving out of the border. At a higher rate of movement (0.5), susceptible and resistant larvae moved in opposite directions far from the border, reducing the mortality of susceptible larvae and allowing the densities of resistant (RR) and susceptible (SS and SR) larvae to increase without affecting each other (spatial isolation). In a refuge size of 50%, when resistance was complete, resistant larvae were favored by remaining in *Bt* cells instead of competing with susceptible larvae for non-*Bt* cells, because of the associated fitness cost; therefore, the evidence of the relationship between larval movement and the proportion of resistant individuals shown was weaker. The exception occurred when resistance was complete and refuge areas occupied 40% of the total

area, because *Bt* maize occupied most of the area, enhancing the survival of resistant larvae. The survival of susceptible larvae increased at higher movement rates as they moved far from the *Bt* block, and at the same time the survival of resistant larvae decreased because they moved to non-*Bt* cells (reduced survival in case of complete resistance, due to fitness cost). Onstad et al. (2011) obtained similar results when comparing refuge strategies, e.g. a refuge structured in blocks against a seed mixture. They reported that seed mixtures eliminate the spatial isolation between the susceptible and resistant phenotypes, differently from block configurations. In addition, crosses between susceptible and resistant insects were only observed along the boundaries of *Bt* and non-*Bt* blocks. Mating between *Bt*-resistant adults took place more frequently in refuge areas structured as blocks than in areas with seed mixtures, reflecting the expectations of Carroll et al. (2012).

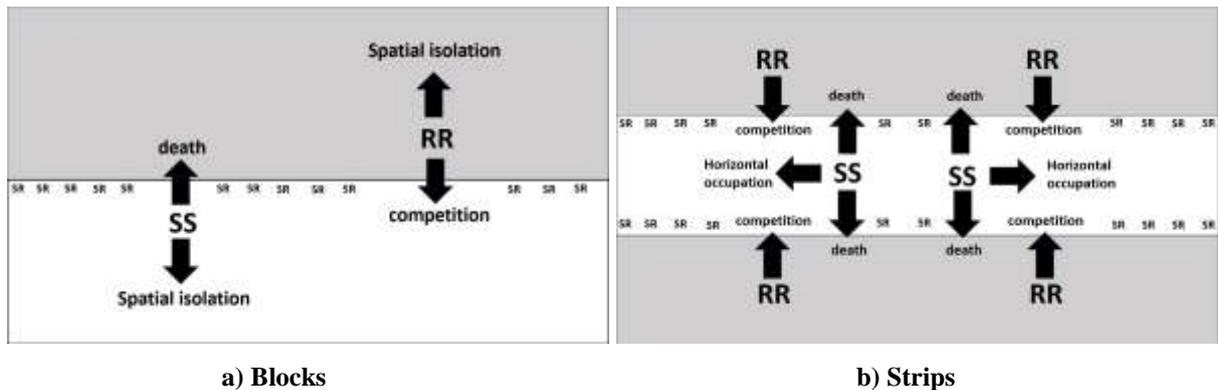


Figure 7. Scheme of larval movement over the spatial configuration: blocks (a) and strips (b). Non-*Bt* maize in white and *Bt*-maize in gray. Heterozygotes (SR) are concentrated at the boundaries between *Bt* and non-*Bt* maize.

In a strip configuration, the more rapid occupation of non-*Bt* strips by susceptible larvae prevented resistant genotypes from occupying these areas (Figure 7b). The competitive advantage of susceptible larvae over resistant larvae in non-*Bt* strips severely affected the resistant insects in the case of incomplete resistance, because the competition for non-*Bt* cells was more intense, since the resistant larvae reached their highest survival rate in these cells. With a refuge size of 20%, the spatial configuration was sufficiently homogeneous to reduce the effect of larval movement. Comparing the refuge structured in strips to the other refuge strategies tested, we observed that the strip configuration maintained the heterogeneity in the area (preventing mating between *Bt*-resistant adults), and continuous areas of non-*Bt* maize arranged as strips allowed susceptible insects to persist.

Comparing the three configurations, we can conclude that the border between *Bt* and non-*Bt* crops plays an important role in resistance evolution. The block configuration has the smallest border zone between *Bt* and non-*Bt* blocks, and was the least effective arrangement for delaying evolution. For either complete or incomplete resistance, the population becomes completely dominated by resistant individuals when the refuge area is less than 40%. The resulting reduction of borders between *Bt* and non-*Bt* blocks favors the isolation of genotypes, reducing the intercrossing that would “dilute” the resistance allele. On the other hand, the seed mixture provides the largest border of contact between *Bt* and non-*Bt* areas, since they are randomly distributed. This is the best arrangement to delay resistance evolution, in either incomplete or complete resistance, when the refuge area is higher than 30%. However, as the proportion of refuge is reduced, the strip configuration becomes the most suitable. This occurs, in part, because of the effect of larval movement. However, this is also observed when the refuge area is 20% and the larvae are not moving. We can conclude that for the seed mixture, at this proportion (20% refuge), the non-*Bt* cells are so dispersed and scarce in the lattice that the effect is similar to a homogeneous *Bt* crop.

Our findings indicate that the best option for farmers is to arrange refuge areas in order to maximize the borders of contact between *Bt* and non-*Bt* plants, but it is also necessary to consider the effect of larval movement on the system. The ideal arrangement would maintain continuous refuge areas to allow the maintenance of SS individuals, but it would also have the largest possible border zone between refuge and *Bt* crops. In our case, the strip configuration satisfied both of these requirements better than the other two arrangements. The block configuration offered a continuous refuge, but the contact between the different crops was reduced to one boundary line. On the other hand, the seed configuration offered the largest contact surface between *Bt* and non-*Bt* crops, but it restricted the SS larvae to isolated “islands” of refuge where exiting these islands would lead them to death and free those cells for occupation by RR larvae.

Although the spatial structure in agricultural systems is more complex, involving different crops and even interspecific interactions among insects, including other pests and beneficial species, our model gives an overall view of the role of the variables studied, such as the spatial configuration, resistance type, and larval movement, on the evolution of resistance in insects. In the field, the complexity of each structure would make such a study impossible, considering all the particularities of each case. Therefore, the computational approach is advantageous, as it allows for the investigation of the general characteristics of a particular system. Moreover, modeling is a continuous process and the successful validation of a

theoretical model requires a thorough understanding of all variables involved. According to Rykiel (1996), validation is not a test of a scientific theory, nor is it a statement of the veracity of a scientific understanding. Validation signifies that a model is acceptable for its purpose since it meets the predetermined requirements. In this stage, a specific landscape can be structured in our simulation, considering all of its particularities, to allow for local comparisons.

2.6. Conclusions

In general, larval movement led to an increase in the proportion of resistant insects in the population for the seed-mixture configuration, because it favored the occupation of non-*Bt* cells by resistant larvae. The opposite trend was observed for the strip configurations, because larval movement favored the occupation of non-*Bt* strips by susceptible larvae, which limited the movement of resistant ones. In the case of the block configurations, the highest proportion of resistant larvae was observed at a rate equal to 0.1, because it was high enough to maintain the flow of RR and SS genotypes between *Bt* and non-*Bt* blocks, but low enough to prevent their movement out of these blocks, leading to spatial isolation.

We also combined these results with the effect of the border zone between *Bt* and non-*Bt* areas on the evolution of resistance. In order to successfully fulfill the objectives of a refuge implementation, it is necessary to establish continuous refuge areas (maintenance of SS individuals) as well as large border zones to promote crossing between resistant and susceptible insects. The arrangement that best fulfilled these conditions was the strip configuration. Indeed, as observed in our results, the strip configuration was most effective in delaying the evolution of resistance.

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3. DELIMITING STRATEGIC ZONES FOR THE DEVELOPMENT OF FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE) ON CORN IN THE STATE OF FLORIDA, BASED ON THERMAL REQUIREMENTS AND GIS MODELING

ABSTRACT

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith), cannot survive prolonged periods of freezing temperatures, thereby limiting where it can overwinter in North America. Climate change is anticipated to reduce the frequency of freeze days in Florida over the decades, with the potential consequence of a significant expansion of the overwintering range, which in North America was assessed as south of $\sim 27^{\circ}\text{N}$ in the last century. To assess this possibility, the development of the fall armyworm on corn leaves, one of the main host plants in the United States, was determined at five constant temperatures ranging from 14°C to 30°C . Based on the development time, the thermal constant and the lower threshold temperature were used to estimate the number of generations of fall armyworm at 42 locations in the state of Florida, from 2006 to 2016. Maps were constructed to provide a visual description of the interpolated data, using GIS (Geographic Information System). The highest number of generations was observed in the counties farther south, an area that showed the highest temperatures during the years and plays a strategic role in maintaining fall armyworm populations in corn fields. Additionally, we conclude that in the absence of freeze periods, fall armyworm should be able to overwinter as far north as $\sim 29^{\circ}\text{N}$.

Keywords: *Spodoptera frugiperda*; Climate change; Spatial distribution

3.1. Introduction

Spodoptera frugiperda (J. E. Smith) (Lepidoptera: Noctuidae) is a major insect pest that damages many field and vegetable crops in the Western Hemisphere, from southern Argentina to southern Canada (SPARKS, 1979). One of its main host plants is corn (*Zea*

mays L.), which is planted as field corn in northern Florida and sweet corn in southern Florida (FLORIDA CORN INSECT IDENTIFICATION GUIDE, 2016). Since insects are poikilothermic animals, they need to develop strategies to survive more extreme temperatures at higher and lower latitudes (LEE; DELINGER, 1991). In particular, the fall armyworm does not diapause and its overwintering range is restricted to regions where larval development is possible throughout the year (BARFIELD et al., 1978, WOOD; POE; LEPPLA, 1979). Although it is important to understand the relationship between insect distribution and temperature in order to develop pest-management plans, most studies detailing the effects of temperature on the development of fall armyworm populations in North America are more than 25 years old (HOGG; PITRE; ANDERSON, 1982, ALI; LUTTRELL; SCHNEIDER, 1990). The results have been generally consistent with significant deleterious effects becoming apparent above 28°C and below 20°C, regardless of the diet of the armyworms (BARFIELD et al., 1978, ALI; LUTTRELL; SCHNEIDER, 1990). Given these constraints, it is not surprising that climatic conditions influence the geographic distribution of fall armyworm in the continental United States, and it is necessary to know the effects on current populations, which may have changed in recent decades.

Snow and Copeland (1969), using insect survey reports composed of larval collection data and data from captures of adult males in traps, defined areas where the generations are continuous. Waddill et al. (1982) monitored the seasonal abundance of fall armyworm in Florida, concluding that they were most abundant in the south during the spring and more numerous at locations farther north during the summer and fall. These observations led to the conclusion that the northern limit for fall armyworm overwintering in North America is ~27°N, unless winters are unusually mild (SNOW; COPELAND, 1969; WOOD; POE; LEPPLA, 1979, WADDILL et al., 1982). Several hypotheses have been proposed to describe the seasonal survival strategies of fall armyworm, however many of the experiments needed to test these ideas have not been completed (BARFIELD; STIMAC; KELLER, 1980).

An important technological development since these studies is GIS (Geographic Information System) modeling, which allows representation of the relationship between temperature and spatial distribution (ELIGIO et al., 2015). GIS users can computationally analyze geospatial data, including collection, analysis, manipulation, and representation of the data set (BYRNE; PICKARD, 2016). This approach has been used successfully in different studies involving the spatial dynamics of fall armyworm. Clark et al. (2007) applied GIS to map gene flow, using genetic data from Mexico, the United States, Brazil, Puerto Rico and Argentina. Westbrook et al. (2016) developed a GIS model based on cumulative degree-days

for simulated development of corn plants and larval fall armyworm, and correlated this with corn distribution data to describe migration.

In organisms such as fall armyworm where body temperature is determined by the environment, the development rate generally changes with temperature. A surrogate value for the lowest temperature that can sustain development is the lower developmental threshold, also referred to as basal temperature (T_b). The estimation of the number of generations geographically, based on the threshold temperature and thermal constant, has been widely applied in entomological studies, providing useful results (NAVA et al., 2007; REDAELLI; OURIQUE, 2015).

In the present study, we evaluated the development time of the egg, larval and pupal stages, and determined fecundity and adult longevity of *S. frugiperda* fed on corn leaves at five different temperatures (14, 18, 22, 26, 30°C) in order to identify the effects of temperature on the development of a current fall armyworm population in the United States. These experiments will update development parameters and identify possible changes in the thermal requirements of fall armyworm over the last 26 years. Then, the thermal constant and the lower temperature threshold were used to estimate the number of fall armyworm generations at constant temperatures for 42 locations in the state of Florida from 2006 to 2016. These values were interpolated, creating surface maps of the predicted number of generations of *S. frugiperda* in Florida, in order to identify the locations with the potential for high population development of this insect pest. This analysis identifies locations in Florida at high risk for becoming significant reservoirs of overwintering fall armyworm if the number of freeze days declines, using a methodology that has not been applied previously to *S. frugiperda* populations.

3.2. Material and methods

3.2.1. *Spodoptera frugiperda* colony

Fourth and fifth-instar larvae of *S. frugiperda* were collected from corn plants at the Plant Science Research and Education Unit, Citra, FL (29°24'42.9"N, 082°6'35.34"W), in September 2016. They were maintained on corn leaves ('Trucker's Favorite') under laboratory-controlled conditions (25±1 °C, 70 ± 10% RH), and a photoperiod of 14h:10h [L:D] until pupation. Emerging adults were fed a 10% water and honey solution and the eggs were collected daily.

3.2.2. Biology of *S. frugiperda* on corn leaves at different temperatures

The experiment was conducted at the USDA - ARS, Center for Medical, Agricultural and Veterinary Entomology laboratory in Gainesville, FL. Five Biochemical Oxygen Demand (BOD) climate chambers were programmed under five different temperatures (14°C, 18°C, 22°C, 26°C and 30° ± 1°C) and a photoperiod of 14h:10h [L:D]. The experiment started after one generation of the field-collected colony was reared in the laboratory.

For studies on larval development in each temperature, 96 newly hatched larvae (< 12h) were placed individually in 32-well high-intensity polystyrene rearing trays with clear polyethylene lids (Frontier Agricultural Sciences, Newark, DE), which were lined on the bottom with filter paper. Each of the six replications per treatment was performed using 16 larvae. The insects were maintained in the rearing trays until pupation. Each larva was provided corn leaves (V4 – V8 stages) washed with a 1% sodium hypochlorite solution, rinsed in deionized water and dried on paper towels. Leaves were replaced daily, and two times a week the old leaves and frass were removed from the wells. Corn plants were grown in a greenhouse in pots filled with soil up to 10 cm from the top edge. They were sown at a density of four plants per pot.

Adult developmental metrics included the duration of the pre-oviposition and oviposition periods, fecundity and adult longevity. After the pupae emerged from the different temperature treatments, moth pairs were placed in a single-pair mating system similar to that described by Stuhl et al. (2008). These small oviposition cages consisted of a cylindrical 473-mL plastic food container (Solo Cup Co., Urbana, IL) lined with a 7.6-cm coffee filter (Bunn, Springfield, IL). Holes (5 mm) were made in the bottoms of the containers to allow for airflow, and one hole (1.5 cm) was made in each lid (Solo, ML8) so that a braided cotton roll (Richmond Dental, Charlotte, NC) could be inserted. The cage was inverted and placed over a 177-mL container (S306, Sweetheart Products Group, Owings Mills, MD), which held a plastic soufflé cup (Solo, P100) with a 10% honey solution. This system allowed for adult nourishment by absorption of liquids.

Because only one female oviposited at 18°C and no adults were collected at 14°C, all eggs examined for the embryonic development time were from females reared at 26°C. The eggs were exposed to the temperature regimes within 12 h after oviposition and held until neonates hatched. For each temperature, 12 replications with 50 eggs each were allocated in Petri dishes lined on the bottom with filter paper, and maintained in each of the BOD climate chambers.

3.2.3. Determination of strain identity

Fall armyworm can be subdivided into two "host strains" with different host-plant preferences that are morphologically indistinguishable, but genetically distinct (NAGOSHI; SILVIE; MEAGHER, 2007). The molecular markers indicative of the "corn-strain" typically predominate in larvae collected from corn and sorghum, while the "rice-strain" markers are the majority in larvae from turf grass and alfalfa. Twenty-seven adults each that completed development at 22, 28, and 30°C, were analyzed for their host strain. Unambiguous results were obtained for 69 specimens. Strain determination was completed by analysis of a portion of the Triosephosphate isomerase (*Tpi*) gene as previously described (MURÚA et al., 2015). Genomic and mitochondrial DNA for use in polymerase chain reaction (PCR) amplifications was isolated from individual specimens using Zymo-Spin III columns (Zymo Research, Orange, CA) as described previously (NAGOSHI; SILVIE; MEAGHER, 2007). PCR amplification of *Tpi* sequences with appropriate primers produces an approximately 500-bp fragment that includes a variable-length intron that is typically about 250 bp in length (NAGOSHI, 2012). The PCR fragment carries an *MspI* site present in both strains within the *Tpi*-412F primer region, and a second *MspI* site that is present only in the strain preferring corn. The presence of the *MspI* site results in two bands, a diagnostic 162-bp fragment and a larger one that contains the variable-length intron. Amplification of the *Tpi* gene used primers *Tpi*-412F (5'-GGTTGCCCATGCTCTTGAGTCCGGACTGAAG-3') and *Tpi*-1140R (5'-AGTCACTGACCCACCATACTG-3').

3.2.4. Statistical Analysis and GIS modeling

Treatment mean values were analyzed by ANOVA and were compared using Tukey's test ($P \leq 0.05$) with the R software (R DEVELOPMENT CORE TEAM, 2008). Prior to this analysis, the results were tested for homogeneity (BURR; FOSTER, 1972), normality and independence of the residuals (SHAPIRO; WILK, 1965). After the durations of the developmental stages at different temperatures were determined, the lower threshold temperature (T_b) and thermal constant (K) were calculated using the following linear equation (WORNER, 1992; HADDAD; PARRA; MORAES, 1999):

$$\frac{1}{D} = a + bT \quad (9)$$

where $1/D$ is the development rate (d^{-1}) and T is the temperature ($^{\circ}C$). The lower threshold temperature T_b was calculated as the ratio between angular and linear coefficients of the line $(-a/b)$ and the thermal constant (K) was obtained using the quotient $(1/b)$ (CAMPBELL et al., 1974). Using a temperature database (FLORIDA AUTOMATED WEATHER NETWORK, IFAS, UNIVERSITY OF FLORIDA, 2017) corresponding to 42 georeferenced locations in Florida (Figure 8), it was possible to estimate the number of generations (G) of fall armyworm from 2006 to 2016 in each location per year, using the following mathematical relationship (ARNOLD, 1959):

$$G = \sum_{m=1}^{m=12} \frac{M_m(T_m - T_b)}{K} \quad (10)$$

where M_m is the number of days and T_m is the mean temperature of the month m .

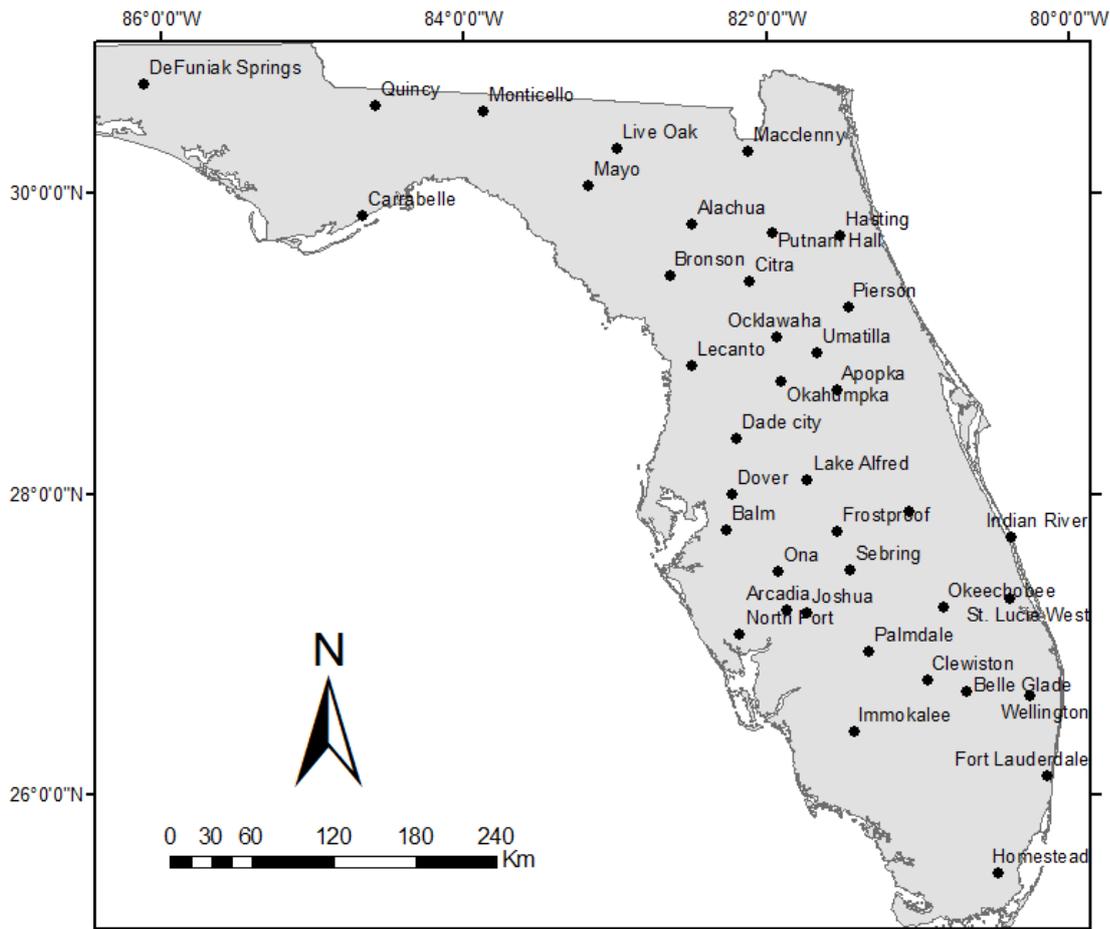


Figure 8. Locations in the state of Florida where the G values were calculated.

The ArcMap application within ArcGis 10.3 (ESRI, 2014) was used to organize and represent the values of *G* from 2006 to 2016 in shapefiles. ArcMap is the environment used to explore GIS, organizing spatial data and representing them on a map. Then, IDW (Inverse Distance Weighting) interpolation was applied to obtain a surface, providing a visual description of the data, using the Geostatistical Analyst Tool from ArcGis 10.3.

3.3. Results

3.3.1. Strain analysis

Because larvae were collected from corn we expected these to be primarily of the strain that prefers that host (corn-strain). This was confirmed using molecular markers. Of the 81 specimens tested, 64 were corn-strain, 5 were rice-strain, and 12 gave no results. During the experiment, no significant strain differences were observed, so the data were pooled.

3.3.2. Development and fecundity of *S. frugiperda* at different temperatures

Fall armyworm completed its development at all temperatures except 14°C. Temperature significantly influenced the development cycle of the insect from egg to adult. The incubation period of eggs was 13.2 days at 14°C and 2.0 days at 30°C, with significant differences among the results for all temperatures used ($F_{4,2071} = 392.7$, $P < 0.001$). Larval development took 129 days at 14°C and 14.6 days at 30°C ($F_{4,241} = 4459.9$, $P < 0.001$); pupal development took 24.5 days at 14°C and 6.6 days at 30°C ($F_{3,133} = 686.1$, $P < 0.001$) (Table 4).

Table 4. Development time (days) (mean \pm SE) of the different life stages of *Spodoptera frugiperda* recorded at five constant temperatures*

Temperature (°C)	Development (days)		
	Egg	Larva	Pupa
30	2.0 \pm 0a	14.6 \pm 0.2a	6.6 \pm 0.1a
26	3.0 \pm 0b	18.3 \pm 0.2b	9.6 \pm 0.1b
22	4.4 \pm 0.1c	27.5 \pm 0.2c	14.6 \pm 0.2c
18	6.0 \pm 0d	45.6 \pm 0.5d	24.5 \pm 0.6d
14	13.2 \pm 0.1e	129.0 \pm 2e	—

*Means followed by the same letter in a column are not different from one another by Tukey's test at 5%

Fecundity was highest at 26°C and lowest at 30°C, with a mean of 1071 and 790.1 eggs per female, respectively (Table 5). Temperature inversely affected the longevity of the adults, ranging from 8.8 days at 30°C to 19.5 days at 18°C ($F_{3,55} = 2.5$, $P < 0.001$) (Table 5).

Table 5. Mean duration (\pm SE) of the pre-oviposition, oviposition period (days) and fecundity (eggs per female) of *Spodoptera frugiperda* recorded at four constant temperatures*

Temperature (°C)	Pre-oviposition	Oviposition	Longevity	Number of eggs
30	4.8 \pm 0.8a	5.2 \pm 0.4a	8.8 \pm 0.6a	790.1 \pm 105.3a
26	4.9 \pm 0.3a	8.7 \pm 0.4b	14.8 \pm 0.5b	1071.0 \pm 80.6b
22	6.0 \pm 0.6a	11.4 \pm 0.7c	18.4 \pm 1.0c	993.5 \pm 109.4ab
18	—	—	19.5 \pm 0.5 d	—

*Means followed by the same letter in a column are not different from one another by Tukey's test at 5%

At 18°C, the insect's metabolism apparently slowed, as activity was reduced and only one female successfully oviposited (total of 50 eggs). Therefore, it was not possible to compare the results with other temperatures. There were no significant differences in the pre-oviposition period, which ranged from 4.8 to 6 days ($F_{2,51} = 2.1$, $P = 0.12$) (Table 5). The oviposition period was inversely affected by temperature, ranging from 5.2 days at 30°C to 11.4 days at 22°C ($F_{2,51} = 40.5$, $P < 0.001$) (Table 5). At 14°C, the insects were not able to reach the adult stage.

Using the development time data (Table 4) and applying the linear model (eq. (9)), it was possible to obtain the values of T_b and K , comparing to the values obtained 26 years ago by Ali et al. (1990) (Figure 9; Table 6).

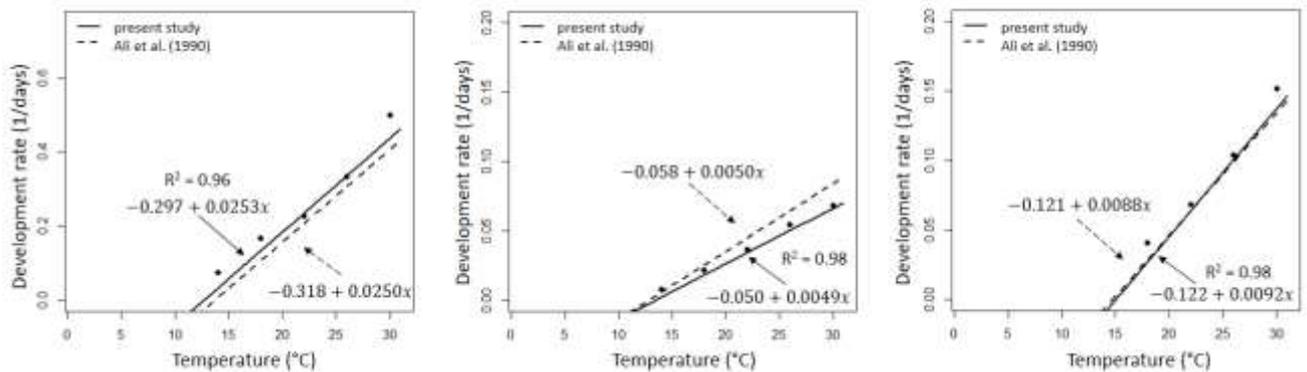


Figure 9. Rate of development versus temperature for fall armyworm a) egg masses, b) larval stage reared on corn, and c) pupae, estimated with a linear model fitted to the data for development time (continuous line). The dashed line represents the model obtained by Ali et al. (1990).

Table 6. The lower developmental threshold temperature (T_b) and thermal constant in degree-days (K) estimated for the immature stages of *Spodoptera frugiperda* in the present study and by Ali et al. (1990)

Parameter	Study	Egg	Larva	Pupa	Egg-adult
T_b	Current	11.7	12.5	14.1	13.2
	Ali et al. (1990)	12.7	11.8	13.7	12.2
K	Current	39.5	250	108.7	400
	Ali et al. (1990)	39.9	204.1	113.6	357.6

Based on the threshold temperature and the thermal constant obtained, it was possible to use the eq. (10) and produce maps representing the estimated values of G from 2006 to 2016 (Figure 10). In order to facilitate the visualization, the number of generations per year was represented in different categories: *Low* (< 7), *Medium* (7 – 9), *High* (9 – 11), *Extremely high* (> 11).

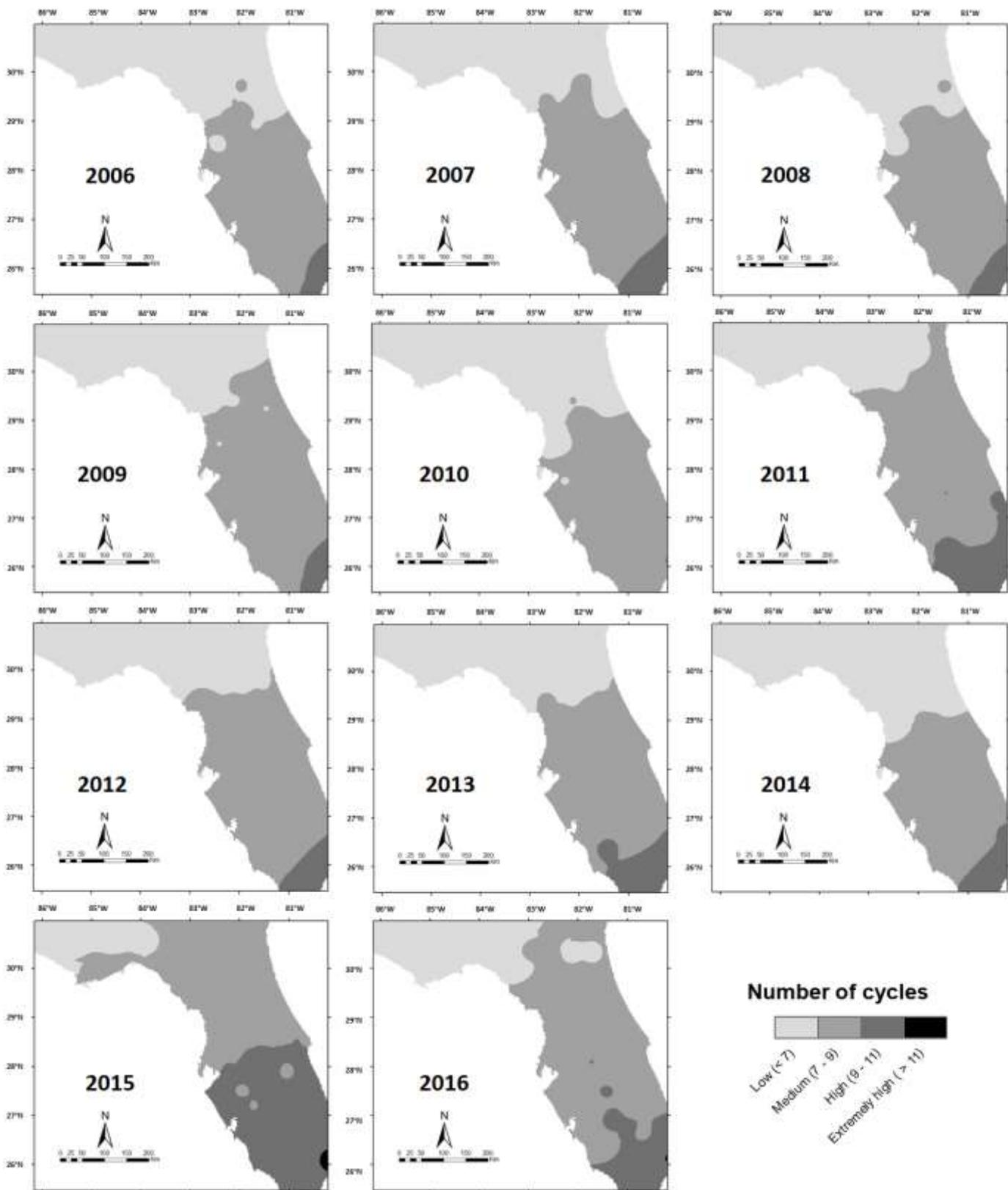


Figure 10. Interpolated maps indicating the estimated number of generations per year (G) from 2006 to 2016.

Most maps clearly showed fewer generation cycles in the north (above $\sim 29^\circ\text{N}$) and an increasing number of cycles southward. This pattern changed drastically in 3 separate years: in 2010, the zone corresponding to the category *High* disappeared; in 2015, the zones corresponding to the categories *High* and *Medium* expanded northward and a new zone corresponding to an area that exceeded 11 generations per year appeared (*Extremely High*); in 2016, the pattern observed was very similar to 2015, but the zones receded from the north.

Maps were also generated to indicate the overwintering areas where the insect generations are uninterrupted, i.e., all months show $G > 0$ (Figure 11). For all years, the line delimiting the area with uninterrupted generations is located between 28°N and 29°N , except in 2010. In 2010, the line moved abruptly southward, reaching latitude 26°N .

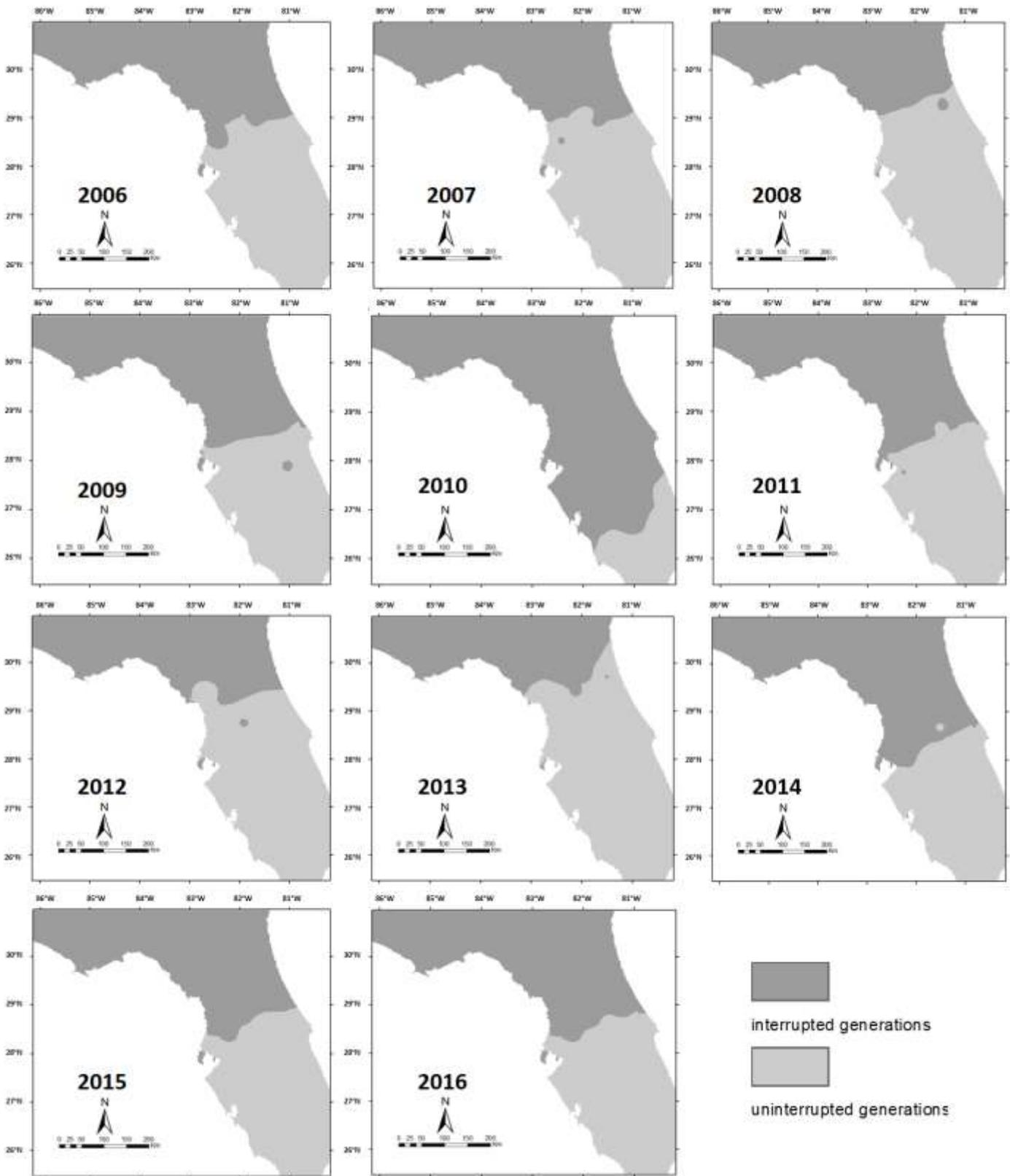


Figure 11. Maps indicating the probable overwintering areas (uninterrupted generations) based on the monthly values of G .

3.4. Discussion

Except for the pre-oviposition period, the data showed that temperature significantly affected the development, reproduction, and longevity of *S. frugiperda*. The rate of development as well as the parameters of the linear model were very similar to those found by Ali et al. (1990). Our nearly wild colony that fed on plant material required slightly longer to develop at warmer temperatures for larval development, and slightly shorter for egg development. The lower threshold temperatures and thermal constants were similar in the two studies, except for the thermal constant corresponding to the larval stage, indicating that the present population required more heat and consequently higher temperatures to pupate when compared to the population from 1990.

Ali et al. (1990) used populations that had been maintained for many generations, and therefore the differences can be explained in part by an artifact of laboratory rearing (MASON; PASHLEY; JOHNSON, 1987). However, another explanation may be the time interval separating these populations. Over these 26 years, fall armyworm populations may have undergone genetic changes, influenced by selection pressure from climate changes, leading them to adapt their metabolism to new situations that involve higher temperatures (DILLON; FRAZIER, 2013). A more detailed genetic study would be necessary to verify this hypothesis.

Regarding the maps, the temperature in Florida gradually increases toward the south, implying increased numbers of generations. In 2010, Florida faced the coldest winter in 30 years due to a combination of cold Arctic streams of air and effects of the El Niño climate phenomenon, experiencing temperatures near -4°C in east-central Florida (INCH et al., 2014). As a result, the number of insect generations decreased throughout the state. On the other hand, 2015 was the warmest year on record in Florida, followed by 2016, resulting in an increase in the number of fall armyworm generations throughout the state (UFWEATHER, 2015; NOAA 2015). If temperatures remain high, farmers may face new challenges to deal with an increase in population numbers of this insect pest.

Figure 11 indicates that locations farther north (darker area) do not provide conditions where fall armyworm populations can be maintained year-round. In these regions, the value of G was equal to 0 from December to February, i.e. the Northern Hemisphere winter. Therefore the results suggest that these populations periodically receive migratory adults from the south. Fall armyworm populations within the light-gray area on the map (uninterrupted generations) act as a reservoir of individuals for migration after colder seasons.

The occurrence of these reservoir areas in Florida was suggested by researchers in the early 1900s (WALTON; LUGINBILL, 1916; LUGINBILL, 1928), and uninterrupted or continuous generations were determined to occur south of latitude $\sim 27^{\circ}\text{N}$, unless winters were unusually mild (SNOW; COPELAND, 1969; WOOD, POE, LEPLA, 1979, WADDILL et al., 1982). This boundary was questioned by other researchers, who suggested that fall armyworms could survive winter conditions farther north (DEW, 1913; HINDS; DEW, 1915; TINGLE; MITCHELL, 1977; BARFIELD; STIMAC; KELLER, 1980). The overwintering boundary of $\sim 27^{\circ}\text{N}$ is farther south than the limit estimated from our study. In fact, we observed that some fall armyworm larvae were able to survive for more than 120 days at 14°C , although no adults were produced. We conclude that fall armyworms should be able to overwinter in latitudes between 28°N and 29°N , if host plants are available to maintain continuous generations and no drastic temperature changes occur. In 2010, for instance, temperatures dropped unusually low during the winter, which restricted the overwintering region to a small portion of the state located farther south, covering the following counties: St. Lucie, Martin, Hendry, Palm Beach, Collier, Broward, Monroe and Miami-Dade. This particular result, combined with the observation of a larger number of generations in this same area (Figure 11), allow us to conclude that this region is strategic for fall armyworm populations.

3.5. Acknowledgments

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4. A COMPUTATIONAL MODEL TO PREDICT THE LEVELS OF FALL ARMYWORM *Spodoptera frugiperda* (LEPIDOPTERA: NOCTUIDAE) (J.E. SMITH) IN CROP AREAS, CONSIDERING SPATIAL DYNAMICS, TEMPERATURE CHANGES AND GENETIC COMPOSITION

ABSTRACT

Among the lepidopteran insects, the fall armyworm *Spodoptera frugiperda* deserves special attention because of its agricultural importance. The immature stage is able to cause plant injury to cotton and corn crops as observed in North, Central, and South America, and more recently in Africa. Different computational approaches have been proposed to clarify the dynamics of fall armyworm populations, but most of them are not tested in the field and do not consider one of the most important variables that influence insect development: temperature. Our objective in this research was to develop a computational tool that covers a wide range of conditions and allows the user to define different input variables, such as crop area, thermal requirements of *S. frugiperda*, viability and oviposition functions, migration rate, rate of larval movement, and resistance to transgenic crops. In order to verify the efficiency of this tool, we tested it using monitoring data of an area located in northern Florida from 2012 to 2015. Simulations were run to estimate the number of adults in 2016 and predictions were carried out for two hypothetical situations that considered possible scenarios involving climate change. The model fitted the monitoring data well, estimating values for the resistance allele frequency (0.15), migration rate (0.48) and rate of larval movement (0.04). It also indicated that an increase of only 1°C in weekly average temperatures could almost double the levels of fall armyworm estimated for 2016, drawing attention to the possible consequences of temperature rises on pest dynamics.

Keywords: Individual-based approach; Spatial models; Resistance; Transgenic crops; Climate changes

4.1. Introduction

The fall armyworm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) is a polyphagous insect pest that occurs in the Western Hemisphere (SPARKS, 1979), attacking several crops, including corn, cotton, and soybean (BARROS; TORRES; BUENO, 2010). In Brazil, fall armyworm is considered one of the main insect pests of corn and cotton (MARTINELLI et al., 2006). In the United States, it is a severe crop pest, mainly in the Southeast (HOGG; PITRE; ANDERSON, 1982). Young larvae are usually found in the whorl in maize plants and initiate injury to leaves by scraping the foliar limb, destroying them during the last larval instars (VILARINHO et al., 2011; DOS SANTOS, 2004) and their presence can cause damage to 57% of the crop in maize fields (CRUZ et al., 1999).

One of the main strategies of insect control in these habitats is the use of transgenic crops, most of which were modified by the *Bt* (*Bacillus thuringiensis*) gene that codes for a particular endotoxin. However, resistant populations have been documented in Puerto Rico, Brazil and the United States (VÉLEZ et al., 2013; FARIAS et al., 2014; OMOTO et al., 2016). In order to manage the evolution of resistance to transgenic crops, the use of refuge areas has been recommended, i.e., non-*Bt* crops planted to promote the survival of susceptible insects (SISTERSON et al., 2005).

Because of its agricultural importance, the spatio-temporal dynamics of fall armyworm has been investigated by many different studies using computational modelling. Computational models have been found to be useful technology tools for Integrated Pest Management (IPM) programs because they constitute a laboratory *in silico* where experiments can be done to anticipate the effect of a natural or anthropic variable on the insect dynamics (FERREIRA; GODOY, 2014). For instance, the effect of different refuge proportions and configurations on the evolution of fall armyworm resistance has been intensely discussed using spatial models (CARROLL; HEAD; CAPRIO, 2012; CERDA; WRIGHT, 2004; GARCIA et al., 2016). Labatte et al. (1994) mathematically modelled the larval development using a degree-days model under various field conditions (larval damage, host plant resistance and microbial control). The spatial patterns, e.g., aggregated, uniform and random, of *S. frugiperda* in different crops were described using an individual based-model (GARCIA; GODOY, 2016).

Although insects are considered poikilotherms, i.e., animals whose internal temperature varies along with the environmental temperature, most models do not consider

the influence of temperature variation on insect development. The importance of considering temperature on the development of fall armyworm can be illustrated by the patterns of migration observed in the United States. Because of its tropical origin, fall armyworm is not able to survive extended periods of low temperatures and does not present the ability to enter diapause (LUGINBILL, 1928; BARFIELD; MITCHELL; POE, 1978). Therefore, the geographic distribution of fall armyworm is closely associated to climatic conditions. In Brazil, fall armyworm is widely distributed throughout the country because of its tropical climate, whereas in the United States, insect populations must migrate northward each spring from overwintering areas located in the south of Florida and Texas to re-infest the central and eastern United States and parts of southern Canada (SNOW; COPELAND, 1969; ROSE; SILVERSIDES; LINDQUIST, 1975; YOUNG, 1979). Considering the effects of different temperatures on the development of fall armyworm, a grid-based spatial model was able to reproduce the migration pattern in the United States using degree-days accumulated by populations throughout the year (WESTBROOK et al., 2016).

The effect of temperature on fall armyworm populations in modelling approaches is also important to respond to the increased concern regarding the impact of climate changes on the ecology of insect pests (CANNON, 1998). Overwintering areas in the state of Florida are possibly moving toward north over the years, allowing fall armyworm populations to cover a wider area during colder seasons (WOOD; POE; LEPLA, 1979; WADDILL et al., 1982; WESTBROOK et al., 2016). African countries have faced severe infestations of *S. frugiperda* populations, causing losses to crops that have been associated to fluctuations in temperature and rainfall due to global climate changes (WILD, 2017).

The present study introduces an individual-based model that describes the dynamics of *S. frugiperda* based on its thermal requirements, i.e., degree-days accumulated for insect development and temperature-driven viabilities. It also considers crop composition and its spatial arrangement, adult and larval movement, migration rate and resistance allele frequency to *Bt* corn. In order to test the model, we represented an area located in Alachua County, Florida, United States, composed of corn plants in which the presence of *S. frugiperda* has been monitored for many years. Most of the parameters were obtained through experiments in the laboratory and others were estimated using insect monitoring data in the study area and an optimization technique. Then, given a set of parameters, we ran the model using a temperature database from 2012 - 2015, verifying if the obtained results (model simulations) corresponded to the insect monitoring data obtained in field. After the model was verified, we estimated the

population dynamics and number of generations for 2016 and for two hypothetical situations: mean temperatures in 2016 + 1°C and + 2°C.

One novelty of the proposed model is in considering all equations and rules related to insect development be temperature-driven. It allows us to consider the effect of climate changes over insect populations and the influence of seasonality on population dynamics. Additionally, the results provided by the model were confronted with data from the field, increasing its reliability. We used an individual-based approach because our purpose was to create a computational tool that could be used to estimate the levels of fall armyworm in a small-scale area, representing its design and local characteristics. Individual-based models are adequate for this application because they focus on the variability of individual characteristics (JORGENSEN; CHON, 2009).

The model has potential to be used for different purposes/studies, such as estimation of population levels in a determined area, investigation of effects of temperature changes on the dynamics of fall armyworm populations, management of the evolution of insect resistance, and determination of the crop calendar in IPM plans, among others.

4.2. Methods

The overall modelling process comprised three different stages. In the first stage, the structure of the model was defined, including its rules and general structure based on the biology of the insect. The second stage involved the use of the model to simulate an area in Florida and verify the reliability of the results. In this stage, data from a laboratory experiment with fall armyworm populations was used in the modeling process and monitoring data from 2012 to 2015 was compared with model outputs. Finally, in the third stage, after model validation, the density of fall armyworm adults was estimated for 2016 and for other two hypothetical scenarios, i.e., increasing the temperatures of 2016 by 1°C and 2°C.

4.2.1. Description of the structure of the program developed

The proposed model was developed in C programming language, using a grid of cells to emulate the crop habitat to be represented. Each one of the cells corresponded to the presence of one plant in the field, which could be either empty or occupied by insects. We also associated an energy-counter with each insect that represented the energy accumulated

over time since insect development is determined by the energy accumulated according to temperature variation. Therefore, an insect should be able to accumulate a determined amount of energy (degree-days) to molt to another stage. This amount of energy is called the thermal constant (GALLO et al., 2002; PADMAVATHI et al., 2013). The thermal constant can be defined as the number of degree-days required for a development change to occur. Knowing the thermal constant and the degree-days accumulated each day, it is possible to predict the development time of hatching, larval and pupal stages, and adult emergence. Another important parameter related to the thermal requirements of insects is the lower temperature threshold, i.e., the lowest temperature in which it is possible to observe insect development (DIXON et al., 2008). Below the lower temperature threshold, the insect does not accumulate degree-days; therefore, it may die or enter diapause. Unlike other insect species, fall armyworm does not enter diapause (SPARKS, 1979).

The rules used in the individual-based model are summarized below:

(a) A cell occupied by an immature insect (eggs, larvae, pupae and adults) could become empty with a daily probability equal to $1 - \sqrt[d]{\mu(T)}$ due to mortality, where $\mu(T)$ is the viability corresponding to the insect stage and d is the duration of the insect stage (GARCIA et al., 2014; GARCIA et al., 2016). The variable d is given by $\frac{(T-T_b)}{K}$, where T is the temperature, T_b and K are the lower threshold temperature and thermal constant corresponding to the insect stage, respectively (GALLO et al., 2002) (Figure 12).

(b) An egg needs to accumulate energy equals to K_1 to hatch. A larva needs to accumulate energy equals to K_2 to pupate. A pupa needs to accumulate energy equals to K_3 for adults to emerge (GALLO et al., 2012) (Figure 12).

(c) A cell could become occupied by an immature insect if an adult laid eggs in it. Per-capita oviposition probability per day was $\phi(T)$, and it was dependent on the temperature T (GARCIA et al., 2016).

(d) A cell would become empty of adults if all adults died. We considered that mortality for an adult occurs when it accumulates an amount of energy equal to K_4 .

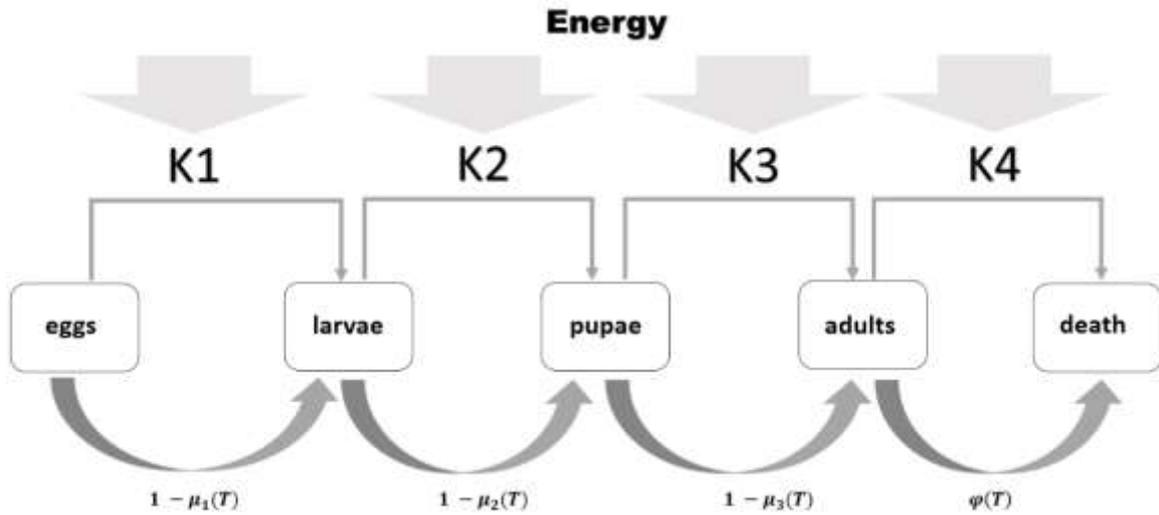


Figure 12. Schematization of the general model structure.

The distance travelled by an adult inside the simulated area at each time step had no preferential direction and was calculated as described in Garcia et al. (2016). We also associated a short-scale movement component to the larva. Each larva had a probability l to move to an adjacent plant per day using a Moore neighbourhood of radius 1 (3 x 3 cells). Each time step t corresponded to one day, and each cell represented 1 x 1 m of the crop system.

4.2.1.1. Genetic component

The genotype of each individual regarding its resistance to *Bt* corn (Cry1Fa), i.e., SS – susceptible homozygote, SR – susceptible heterozygote or RR – resistant homozygote (VÉLEZ et al., 2014; HUANG et al., 2014), was determined using the Hardy-Weinberg equation (HARDY, 1908), given by:

$$p^2 + 2pq + q^2 = 1, \quad (11)$$

where p is the frequency of the S allele ($p = 1 - q$), and q is the frequency of the R allele. Therefore, knowing the frequency of the R allele in the population, it was possible to determine the probability of an individual presenting the genotype SS (p^2), SR ($2pq$) or RR (q^2) (GARCIA et al., 2016). Regarding the fitness cost, it was assumed a reduction of 20% in

larval viability and a delay of 4 days in the duration of the larval stage in the absence of *Bt* crops when individuals carried at least one copy of the resistance allele (RR or SR genotypes) (DANGAL; HUANG, 2015).

4.2.2. Model verification

In order to verify the reliability of the model, we tested it for an actual area located in the Hague community, Alachua County, Florida (29° 46' 14" N, 82° 25' 16" W) that has been monitored with sex pheromone-baited Unitraps to verify the presence of fall armyworm adults over the last years (MEAGHER et al., 2013). In order to grid the particular study area, we used its crop calendar. Thus, assuming that each simulation is run during one year ($0 < t < 365$), the simulated area could assume different spatial crop configurations: a) from March to October ($60 < t < 300$): occupied by maize plants in a proportion of 80% transgenic plants and 20% non-transgenic plants (refuge) arranged in a block configuration; b) from November to February ($t < 60$ and $t > 300$): no crop.

The values corresponding to the thermal constants (Table 7) and equations to define the viabilities of each stage and oviposition probability per day were based on a thermal requirement study (Chapter 3 of this thesis). This study was developed using larval populations collected from corn plants at the Plant Science Research and Education Unit, Citra, Florida (29°24'42.9"N, 82°6'35.34"W), a community located only 70 km from the monitored area. The degree-days accumulated in each day were calculated using a weekly temperature database (Florida Automated Weather Network, IFAS, University of Florida, 2017) from 2012 to 2015 collected in Alachua County.

Table 7. Values of K1, K2, K3 and K4 used in the modeling process (Chapter 3).

	K1	K2	K3	K4
Chapter 3	39.5	250	108.7	180

Regarding the viability equations, we used a polynomial regression (Appendix A) in order to define the viabilities for temperatures not evaluated inside the range of temperatures studied in Chapter 3, which allowed us to estimate the viabilities for all studied range (14 – 30°C) we considered that insects are not able to survive in temperatures below the lower threshold temperature. The viability functions are described in eq. (12), (13), (14).

$$\begin{cases} \mu(T) = -0.00097T^2 + 0.0463T + 0.3117 & \text{if } T > 12.5 \\ \mu(T) = 0 & \text{if } T \leq 11.7 \end{cases} \quad (12)$$

$$\begin{cases} \mu(T) = -0.0052T^2 + 0.2681T - 2.6848 & \text{if } T > 12.5 \\ \mu(T) = 0 & \text{if } T \leq 12.5 \end{cases} \quad (13)$$

$$\begin{cases} \mu(T) = -0.0099T^2 + 0.5073T + 5.7295 & \text{if } T > 14 \\ \mu(T) = 0 & \text{if } T \leq 14.1 \end{cases} \quad (14)$$

The per-capita oviposition probability per day was given by the eq. (15) using data from the experiment described in Chapter 3 (Figure 13).

$$\varphi(T) = \frac{1}{(1 - e^{0.012E_{d-1}(T)})} - \frac{1}{(1 - e^{0.012E_d(T)})} \quad (15)$$

where E is the energy accumulated in the days d and $d - 1$.

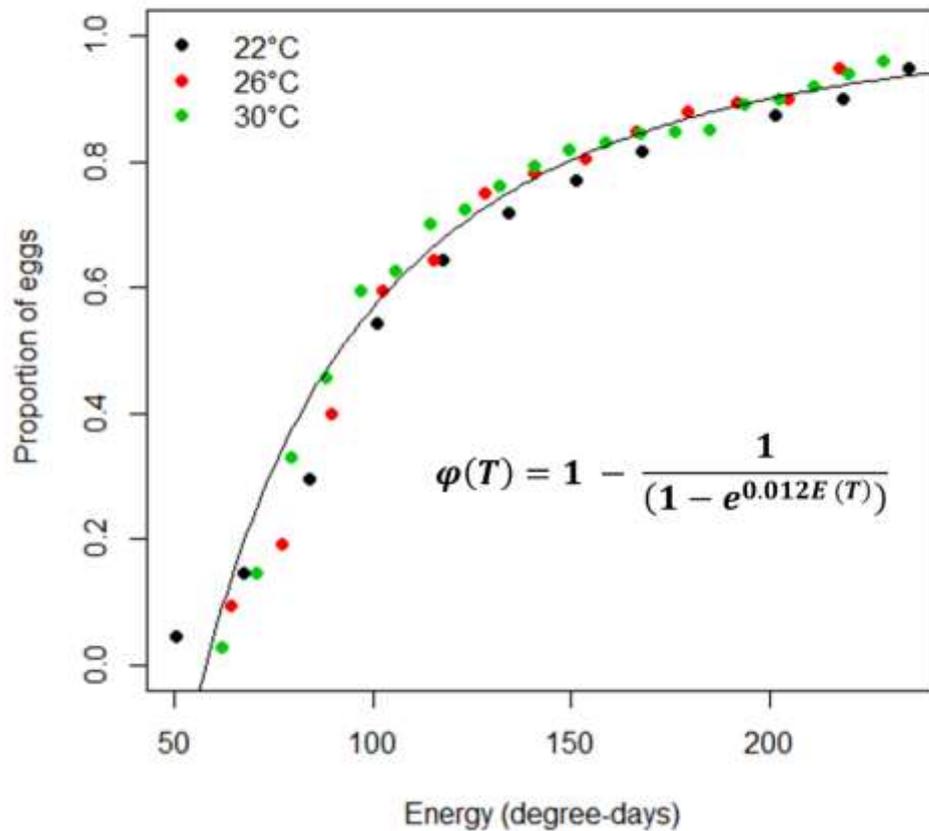


Figure 13. Proportion of eggs laid versus mean energy accumulated by adults in three different temperatures (data from the experiment developed in Chapter 3)

Another issue to be considered is that the study area is located in a region where insects are not able to overwinter, receiving individuals from warmer regions periodically (BARFIELD; STIMAC; KELLER, 1980; WESTBROOK et al., 2016). According to Meagher and Mitchell (2001) and Westbrook et al. (2016), this period corresponds approximately from the second half of April to the first half of May. Therefore, in order to represent this immigration process, we included an average migration rate higher than 0 ($m > 0$), occurring during the interval $105 < t < 135$.

4.2.2.1. Genetic Algorithm (GA)

Some parameters of the model were not available in the literature and are difficult to obtain from experimental data. Therefore, in order to reproduce the monitoring data set, a genetic algorithm was used to find the appropriate set of parameters given by $z = [m, f, l]$, corresponding, respectively, to the migration rate, resistance allele frequency to *Bt* corn associated to immigrant populations and the rate of larval movement, which promoted the best match between the model and the monitoring data.

GA is an optimization method based on the mechanisms of natural selection, whose goal is to produce new parameter sets gradually better fitted to the present conditions according to some objective function (MELANIE, 1999). In entomological research, the recent work developed by Ren et al. (2016) can be mentioned, where a GA was used to estimate the parameters of a force-based model for simulation. With this in mind, n_p is the number of parameter sets tested. A score (or fitness) is attributed to each one according to a fitness function. At each iteration of the GA algorithm, three processes may occur: reproduction, crossover, and mutation (Figure 14) (CHAMBERS, 2000).

Reproduction selects the parameter sets with the highest fitness to compose the next generation according to a fitness function. In the present study, the mean square error between the observed and the expected results (number of adults) was used as a fitness function. Crossover exchanges information between two parameter sets according to a probability 0.1 in the current study. It mimics sexual reproduction, otherwise the parents would produce an offspring identical to themselves and no increase in population fitness would be observed over the iterations. Mutation modifies randomly the value of one parameter in the set with a probability 0.1 in the current study. It allows the introduction of new values, avoiding local minimums since we were looking for a global solution. We used a number of populations equal to $n_p = 200$ and let them evolve over 100 iterations.

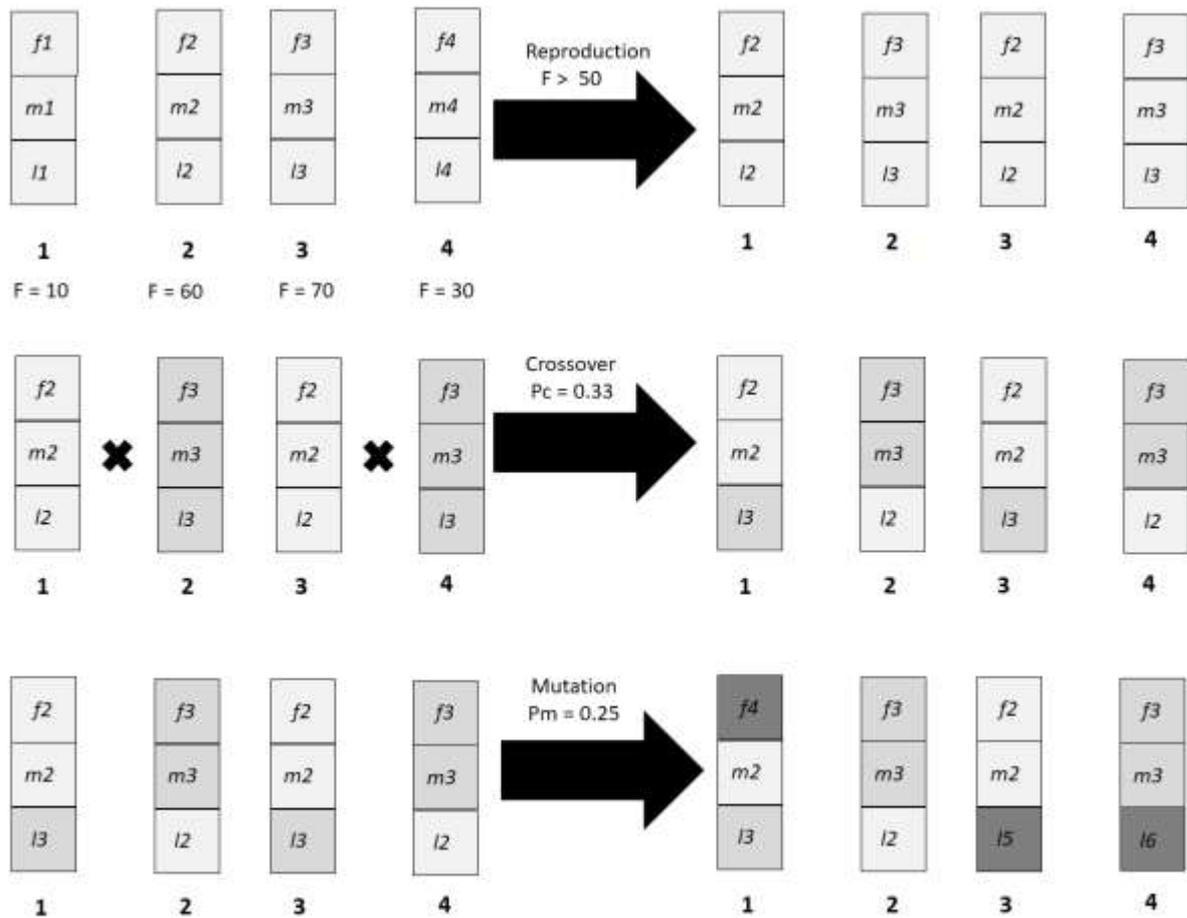


Figure 14. Exemplification of the three processes involved in a genetic algorithm. Reproduction selects the individuals to reproduce according to a fitness function F . Crossover exchanges information between two parameter sets according to a probability P_c . Mutation randomly modifies a parameter in a set according to a probability P_m .

4.3. Results

Initially the model was applied to the study area using data from 2012. The genetic algorithm found the best match between model prediction and field data, which is $z = [0.48, 0.15, 0.04]$ from the field data. Then, using these parameter values, we ran simulations for 2013, 2014 and 2015 (Figure 15).

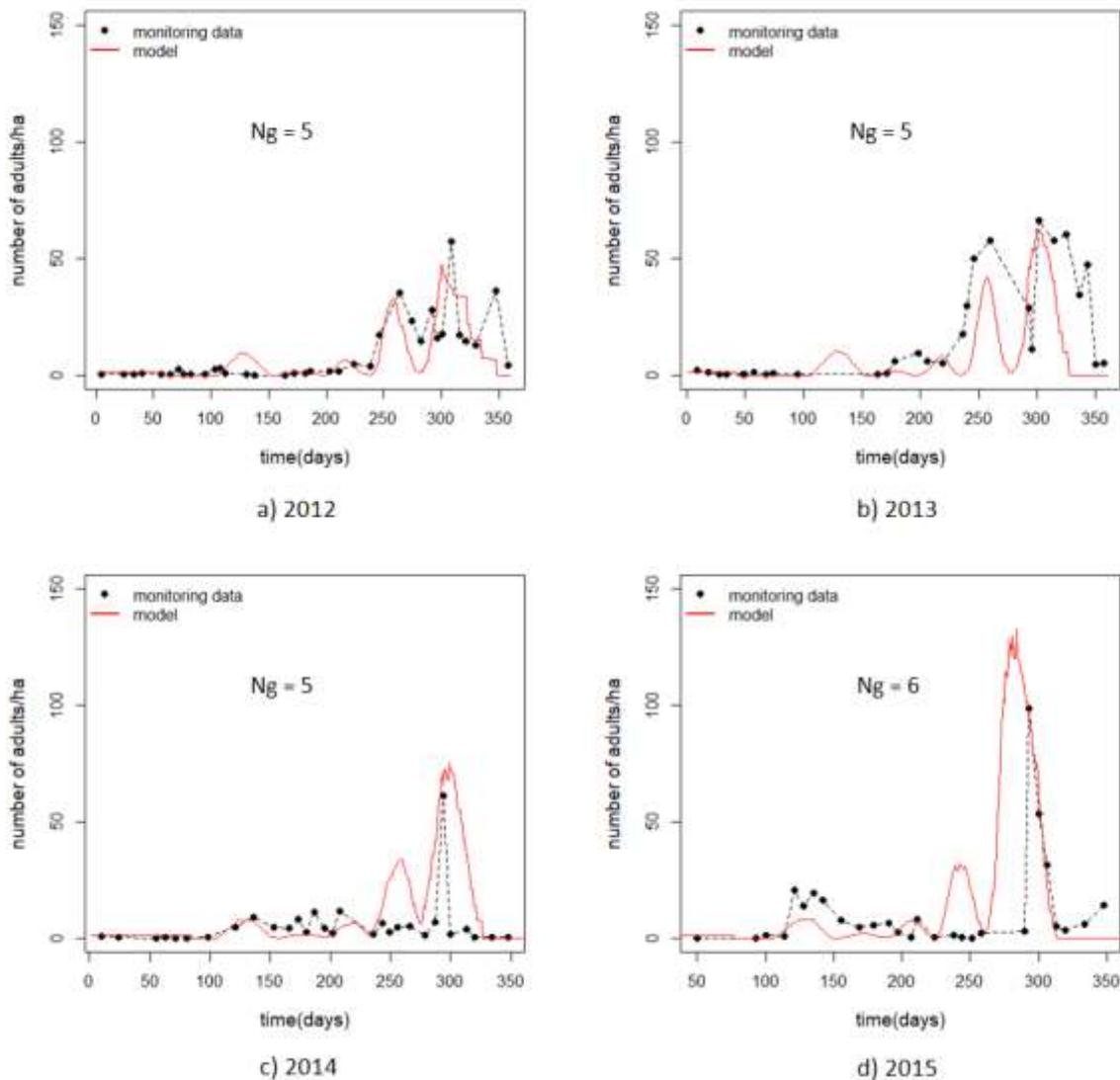


Figure 15. Field data and model simulations using the parameter set $z = [0.48, 0.15, 0.04]$ and weekly average temperatures of 2012, 2013, 2014 e 2015. Ng – Number of generations.

Since the model was able to reproduce the monitoring data for the consecutive three years with good accuracy, using the combination of parameters provided by the GA for 2012, a new question was formulated: Are the parameters obtained by the genetic algorithm not suffering significant variations during the proposed interval of time (2012-2015)? In order to test this hypothesis, we ran the genetic algorithm once more to find the combination of parameters that provide the best match of the model for each year from 2012 to 2015. Then, we calculated the mean values of the 20 best parameter set obtained from simulations of the algorithm for each year (Figure 16, Figure 17).

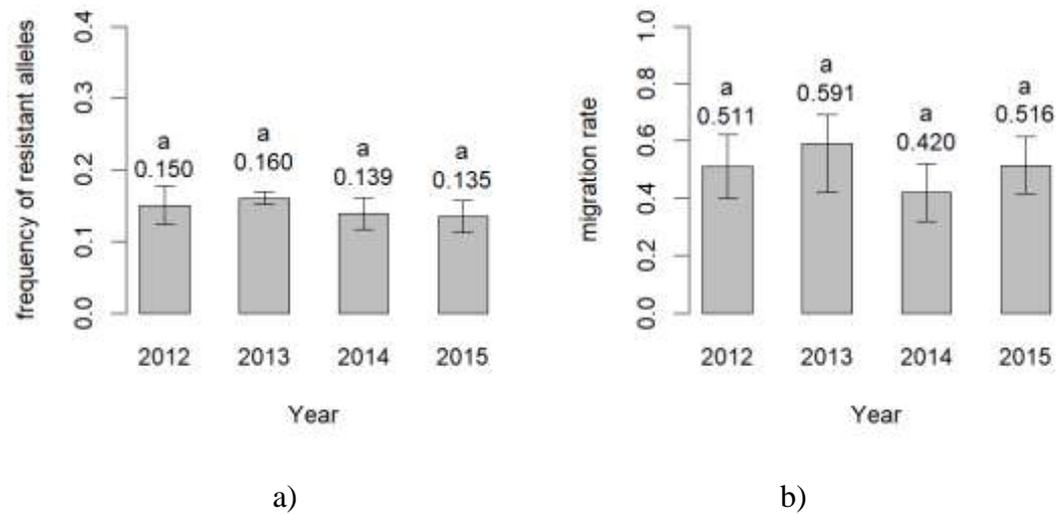


Figure 16. Mean values of m (migration rate) (a) and f (resistance allele frequency in migrant population) (b) obtained from simulations of the GA for 2012, 2013, 2014 and 2015. Different letters above the bars indicate significant differences at 5% (Tukey's HSD, $p < 0.05$).

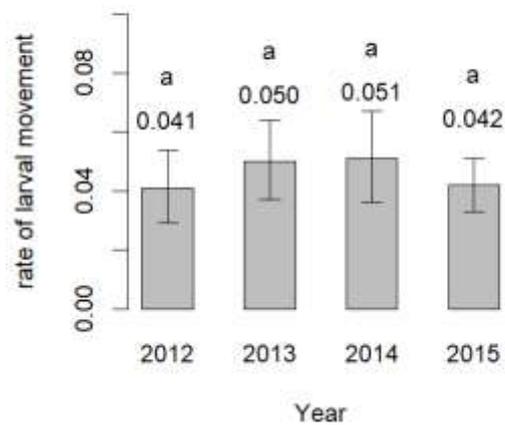


Figure 17. Mean value of l (rate of larval movement) obtained from the simulations of the GA for 2012, 2013, 2014 and 2015. Different letters above the bars indicate significant differences at 5% (Tukey's HSD, $p < 0.05$).

4.3.1. Estimation for 2016 and hypothetical scenarios

In order to investigate the possible effects of increases in the temperature on the population dynamics of *S.frugiperda*, we tested the model using climate data from 2016 and considered two hypothetical scenarios: increasing the mean temperatures of 2016 by 1°C and by 2°C. Using weekly temperature data (Florida Automated Weather Network, IFAS, University of Florida, 2017) and the combination of parameters \mathbf{z} found by the GA for 2012, we estimated the populations levels for 2016 and for each one of the hypothetical scenarios proposed (Figure 18).

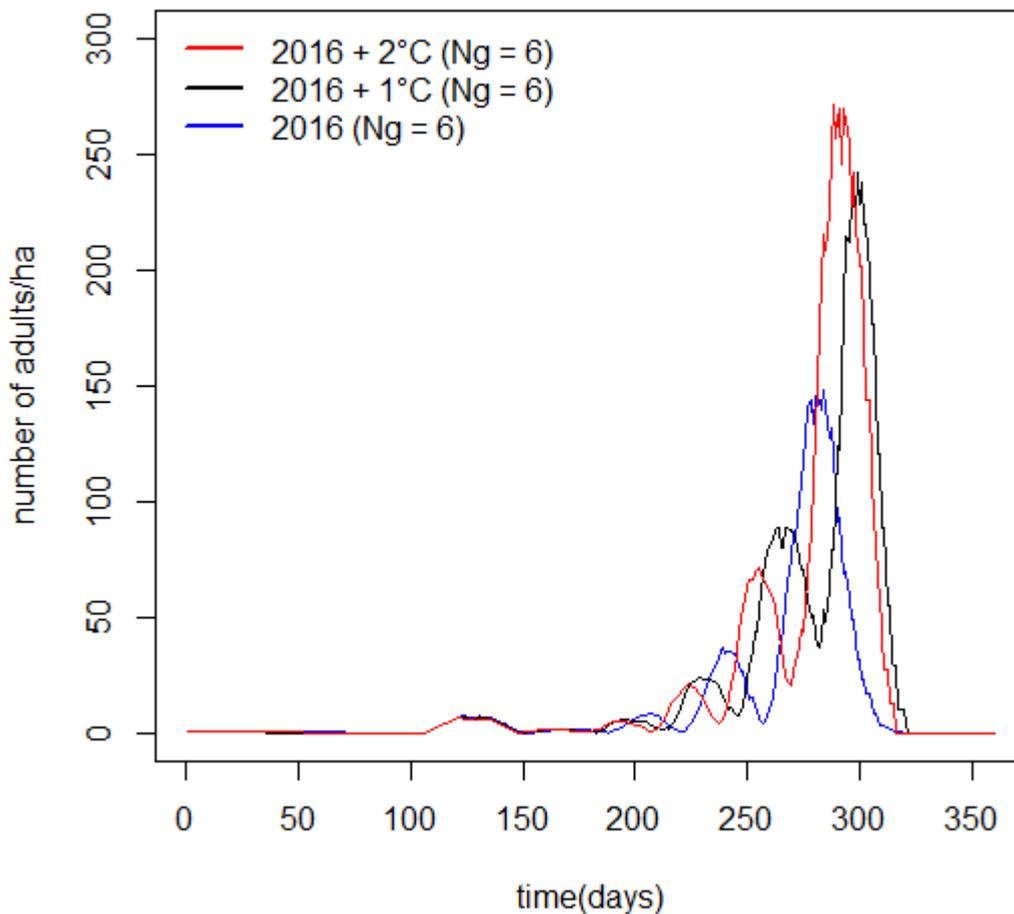


Figure 18. Populations levels estimated for 2016 and for two hypothetical situation (increasing the temperatures by 1°C and 2°C). Ng – Number of generations.

4.4. Discussion

The model successfully predicted the population distribution from 2012 to 2015, indicating a good correspondence between model results and monitoring data. The few differences between the expected and the observed data can be related to:

a) The years of 2012 and 2013 did not show an accentuated oscillation during the immigration period ($105 < t < 135$) as observed in the curves provided by the model for all years. It may be related to the absence of monitoring data in determined periods. Since we are working with discrete data, some intervals were not sampled and therefore, this oscillation was not verified because there was not monitoring data during the period. Another reason is the methodology used to model the immigration period. Since information about the specific period of immigration was scarce, we considered it occurred uniformly during a determined interval of time based on previous observations. However, migration can be ruled by different factors such as corn phenology, temperature and pluviosity (WESTBROOK et al., 2016).

b) The model was able to predict two main periods of outbreak. The first one (lower) is derived from eggs laid in mid-summer (~July) by populations from the beginning of this season when climate conditions were extremely favorable for fall armyworm development ($25^{\circ}\text{C} < T < 30^{\circ}\text{C}$) (HOGG; PITRE; ANDERSON, 1982; ALI; LUTTRELL; SCHNEIDER, 1990). The second and higher outbreak is expected in mid-autumn (~October) from eggs laid by populations at the end of summer when conditions were still optimal for fall armyworm development. In addition to these two outbreaks, a third one was observed during December, 2012, which was not predicted by the model. It was an unexpected outbreak since corn plants were not present in December. It may have occurred due to the presence of other host plants remaining in the field or an influx of moths returning from the north (PAIR et al., 1987). In 2014 and 2015, only one larger outbreak was observed, which the model was able to reproduce, but it also predicted another smaller one that was not observed in the monitoring data, which can also be explained due to the discrete nature of data since some intervals of time were not sampled. The model provided a more detailed description of population dynamics, whereas the monitoring data presented many individual “pieces” of the whole “puzzle”. However, some of these pieces were missing. Another reason for differences between the expected and observed results can be related to the nature of the model. The

modelling approach considered temperature as the driving factor moderating population dynamics, but other variables may also influence it, such as pluviosity, corn phenology or natural phenomena (BARFIELD; ASHLEY, 1987). The modelling process is just an approximation of reality, not intending to reproduce it exactly.

c) Only synchronization delays of less than five days were observed. These delays can be explained since the intervals of observation were longer than one week.

An important achievement by the model was the representation of outbreaks according to their intensities. For instance, the model was able to represent the large outbreak in 2015 (surpassing 100 adults/ ha). The year 2015 was one of the warmest on record in Florida, resulting in an increase in the number of generations of fall armyworm throughout the state (UFWEATHER, 2015; NOAA, 2015).

We did not find any study available in the literature providing values of migration rate or rate of larval movement in the field to compare to our results. Research determining the influence of rates of larval movement on the choice of refuge configurations indicated that seed mixture arrangements could represent a better alternative for lower rates of larval movement, i.e., $l < 0.1$ (GARCIA et al., 2016).

The resistance allele frequency found in our study varied between 0.12 and 0.18. Different genetic studies were conducted using fall armyworm populations from counties located in southern Florida and resistance allele frequency was estimated: Palm Beach (0.05 – 0.20), Hendry (0.01 – 0.12) and Collier (0.24 – 0.35) (VÉLEZ et al., 2013), and Collier (0.24 – 0.35) (HUANG et al., 2014). Since insects arrive in the study area from different southern locations during the migration period, the obtained resistance allele frequency (0.12 – 0.18) can be interpreted as a mean of the allele resistance frequency of populations from the many counties that provide fall armyworm moths to the simulated area. It also indicates that resistant individuals may be present in the area (HUANG et al., 2014.), information that needs to be considered in future management plans.

From 2012 to 2014, five generations of fall armyworm per year were predicted by our model. The same number was found for the same region in Chapter 3, where thermal requirements and Geographic Information System (GIS) were used to estimate the number of generations of fall armyworm from 2006 to 2016 throughout the state of Florida. In 2015 and 2016, six generations were observed during the year due to the warmer climate conditions. In

Chapter 3, seven generations for the same years were estimated. These differences are probably related to the different approaches used in both studies. Whereas in Chapter 3, a GIS-based analysis was used, considering only degree-days accumulated during the egg-adult period; the current study used a computational model that covered a wider range of parameters related to temperature, such as viability and fecundity. Additionally, the previous study did not consider parameters such as migration rate, resistance allele frequency and larval movement. Nevertheless, the values between both studies were very similar.

As indicated in Figure 16, the resistance allele frequency did not indicate statistically significant differences among years. We found no differences in the simulations because we were assessing during a short interval of time that was not enough to verify possible changes in the population. We also did not verify significant variations in the migration rate and rate of larval movement. These results may indicate that both parameters are not affected by small variations in temperature and only drastic climate changes may affect them. They can also be more tightly related to other non-studied variables, such as crop neighborhood, crop calendar, etc. A more detailed study focusing on the variables that affect both parameters would be necessary to test such a hypothesis.

The model result for 2016 was very similar to 2015, but it presented a larger insect outbreak that reached ~150 adults/ha. Before 2016, 2015 presented the warmest summer in Florida's history. However, the record was broken in 2016 with average temperatures reaching 30°C during the summer (NOAA, 2016). The gradual increase in average temperatures has raised concerns that the number of insects in the field is increasing (LIEBHOLD; BENTZ, 2011; GOERGEN, 2016). The simulations for 2015 and 2016 indicated the addition of one generation, compared to 2012, 2013 and 2014. Additionally, our simulations for the hypothetical scenarios indicated that just an increase of 1°C in weekly average temperatures could produce an outbreak 1.7 times higher than in 2016, reaching ~250 adults/ha. However, this increase in the number of insects is not proportional to the increase in temperatures indefinitely, which was observed when average temperatures increased by 2°C. The outbreak did not present a great variation compared to the simulation correspondent to an increase of 1°C. Insects are negatively affected by extreme temperatures and the optimal temperature for fall armyworm development is around 26°C as verified in Chapter 3. Therefore, it is expected that fall armyworm development will be negatively affected as temperatures rise above this value (HOGG; PITRE; ANDERSON, 1982; ALI; LUTTRELL; SCHENEIDER, 1990).

4.5. Conclusion

The model succeeded in representing the dynamics of fall armyworm populations for the simulated area and was reliable to represent the main outbreaks observed in the field. It may represent an important tool for integrated pest management programs since the model can be used either to predict the effects of the implementation of a new approach for pest control or the influence of global warming in insect dynamics. The results provided by the model also represent an alert for the effects of a gradual increase in average temperatures on the density of fall armyworm in the field.

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SUPPLEMENTARY DATA

Appendix A: Relationship between viabilities and temperatures: (a) eggs, (b) larvae and (c) pupae (data from Chapter 3). Different letters indicate significant differences at 5% (Tukey's HSD, $p < 0.05$).

