Original Research Article

Predicting evolution of insect resistance to transgenic crops in within-field refuge configurations, based on larval movement

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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.

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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 et al., 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 and 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 et al., 2012, 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 and 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 and 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 and 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. 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 604×604 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