Estimating the landscape distribution of eggs by *Helicoverpa* spp., with implications for *Bt* resistance management

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A B S T R A C T

Transgenic crops expressing insecticidal toxins of *Bacillus thuringiensis* (*Bt*) have been deployed in agricultural landscapes around the globe. While the key strategy to delay resistance is the mandatory planting of a non-*Bt* refuge crop that is preferred by the target pest, the efficacy of this resistance management strategy across different landscape contexts over time is rarely considered. Here, we develop an individual-based model to simulate the spatio-temporal distribution of a highly mobile, polyphagous, global pest, *Helicoverpa* spp, across agricultural landscapes dominated by transgenic cotton. The simulation model allows us to explore refuge 'electivity', the relative utilization of refuge habitat by female *Helicoverpa*, in relation to *Bt* cotton habitat. Refuge electivity is an emergent function of egg distributions resulting from individual moth behavior, within multiple landscapes during different seasons and crop phenology.

The individual-based model is validated against independent data collected from the field. Our findings suggest that refuge electivity is sensitive to the spatial and temporal context of the attractiveness of host crops in the landscape and the preferences of the moths. The attractiveness of mandated refuges, such as pigeon pea relative to *Bt* cotton, influences how effective they are in the landscape. Dynamics between other host crops, such as sorghum, also play an important role that varies over time and space. We use the model to identify scenarios where refuge strategies are likely to be most effective in terms of boosting susceptible populations and increasing landscape movement (genetic mixing). This dynamic approach has potential to inform better refuge design for *Bt* resistance management across a wide range of landscape contexts. For example, these findings justify the removal of sorghum as an option for mandated refuge in the Risk Management Plan (RMP) for *Bt* cotton in Australia.

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1. Introduction

Agricultural landscapes throughout the world have been transformed by the wide-scale deployment of transgenic maize, soybean, and cotton that carry the insecticidal toxins of *Bacillus thuringiensis* (*Bt*), effectively reducing field populations of a variety of lepidopteran pests. Over 76 million ha of *Bt* crops were planted globally in 2013 (James, 2013), and over 70% of global production of cotton is now genetically modified. Crucial to the continued success of these crops is the management of the evolution of resistance to the *Bt* toxins (Tabashnik et al., 2013; Jin et al., 2015). Current resistance management strategies for *Bt* crops are based

on well-established assumptions about how resistance will likely arise (Downes et al., 2010; Downes and Mahon, 2012; Mahon et al., 2012; Downes et al., 2017). However, ecological processes that may have significant bearing on resistance evolution are generally not well understood. These processes include the overall landscape movement of the pests, their relative abundance in different crops and their egg-laying behavior, and how these three factors change over time with crop attractiveness. Such complex ecological factors operate at multiple spatio-temporal scales and may have an important influence on the rate of resistance evolution (Ives, 1996; Dillon et al., 1998; Baker et al., 2016).

In some countries, a mandatory license requirement of planting transgenic seed is that a non-*Bt* host plant of the target pest is also planted, referred to as a 'refuge'. Refuges, the cornerstone of resistance evolution management in the landscape, are grown specifically to provide a non-selective environment for the pest that

reduces the force of selection for resistance (Gould et al., 1998; Ives and Andow, 2002; Tabashnik et al., 2013; Ceeney et al., 2014). A number of studies across a range of insect species have been conducted on the design and placement of refuges to prevent resistance to *Bt* (Gould et al., 1998; Frutos et al., 1999; Tabashnik et al., 2013), including various models (Alstad and Andow, 1995; Dillon et al., 1998; Carrière and Tabashnik, 2001; Ives and Andow, 2002; Cerda and Wright, 2004; Tabashnik et al., 2008; Jin et al., 2015; Pan et al., 2016) and field evaluation (Carrière et al., 2012; Baker and Tann, 2014). These studies generally conclude that refuges are beneficial. However, the effectiveness of such refuges remains uncertain (Bates et al., 2005; Baker et al., 2016), especially when deployed across dynamic agricultural landscapes and when considering pest behavior and metapopulation dynamics (Carrière et al., 2012).

Two moth species, *Helicoverpa armigera* (Hübner) and *H. punctigera* (Wallengren) (Lepidoptera: Noctuidae), are historically the most important pests of a number of crops in Australia (Zalucki et al., 1986; Fitt, 1989), and *H. armigera* now occurs worldwide (Kriticos et al., 2015). In cotton landscapes of Australia, these pests are currently very effectively managed by cotton plants expressing various *Bt* toxins (Constable et al., 2011). As part of the license agreement, refuges are mandated areas (based on a percentage of *Bt* cotton) of non-*Bt* crops attractive to both *H. armigera* and *H. punctigera*; either unsprayed non-*Bt* cotton (10%) or pigeon pea *Cajanus cajan* (L.) (5%). Crops and refuges are deployed across agricultural landscapes that change dramatically from year to year in the area of *Bt* and non-*Bt* host crops by almost three-fold differences (Ives et al., 2017). Although Australia has applied the most stringent refuge requirements of all countries in the world at 10% non-*Bt* cotton or equivalent (Tabashnik et al., 2013), resistant alleles in the pest remain present at detectable frequencies (Downes and Mahon, 2012). However, they appear to have fluctuated rather than increased over the last few years (Downes et al., 2016).

To explore the moth behaviors that affect resistance evolution we designed a simulation model of individual movement behavior of mated females of the two *Helicoverpa* species in Australia. These behaviors determine the distribution of eggs among fields over time that either contain *Bt* cotton or act as refuges (mandated and unmandated). A mechanistic, individual-based modelling approach allows for increased understanding of the main drivers of population distributions as a function of movement behaviors (Pauli et al., 2013). This has direct relevance for resistance management, because evolution of resistance to *Bt* toxins is more rapid when a greater proportion of larvae in the population are exposed (Gould, 1998; Ives and Andow, 2002; May, 1993; McGaughey and Whalon, 1992; McKenzie, 1996; Rosenheim and Tabashnik, 1990; Tabashnik, 1994). Thus, a key question is how the attractiveness, size, number, and configuration of refuges on the landscape affects the proportion of *Helicoverpa* spp. eggs in *Bt* cotton fields. We model this as a behavioral response of females to crop attractiveness in their flight and egg-laying decisions. In a population-level model of resistance evolution using the same study system and landscapes as the present study (Ives et al., 2017), periods of intense selection for resistance of *H. armigera* and *H. punctigera* could occur when crop harvesting led to sudden contractions in the proportion of refuge on the landscape, and selection events could be exacerbated if these periods occurred synchronously with large declines in the total insect population. A limitation of this study, however, is that they consider landscape structure only in a spatially-implicit manner (as do most models of this system), and the magnitude of the bursts of resistance evolution depended directly on an assumed landscape-wide redistribution of eggs with changes in the proportions of crops and refuges across the landscape. This limitation can be addressed with the spatially-explicit approach taken here.

The rate of resistance evolution depends not only on the force of selection (determined by the proportion of the population exposed to *Bt*), but also on the genetic structure of the population (Caprio, 1998; Caprio and Hoy, 1994; Caprio and Tabashnik, 1992; Sisterson et al., 2005). Specifically, the rate of resistance evolution can be divided mathematically into components for the force of selection, assortative mating (the proportion of heterozygotes in the population departing from Hardy-Weinberg equilibrium), and variation in male mating success (Ives and Andow, 2002; Ives et al., 2011). The present work does not explicitly address the effect of female movement on the genetic structure of the population, and therefore it does not lead to explicit recommendations for resistance management strategies. Nonetheless, it does address the exposure of larvae to *Bt* toxins, and hence the force of selection for resistance. Modeling movement of individuals is the first step towards building an individual-based model of resistance evolution in which the genotypes of individuals are followed over a landscape. We compare the patterns produced by the model with data from the field, to test three hypotheses about *Helicoverpa* behavior (processes) in relation to their preferences and the attractiveness of the crops:

1. Comparing the two *Helicoverpa* species (differentiated by preference for sorghum), the attractiveness of both *Bt* cotton and sorghum as well as landscape influences:

     a) The proportion of eggs laid by each species in *Bt* cotton vs sorghum.

     b) The ratio of *H. armigera* eggs to *H. punctigera* in *Bt* cotton.
2. Refuge 'electivity', measured by the relative egg load of pigeon pea compared with *Bt* cotton, depends on the attractiveness of *Bt* cotton, pigeon pea and sorghum to *Helicoverpa* spp. in the landscape.
3. The emergent simulated daily distance moved by the two *Helicoverpa* species depends on the attractiveness of cotton and sorghum as well as the proportion of pigeon pea in the landscape.

2. Materials and methods

The following model description is based on the 'ODD' (Overview, Design concepts, and Details) protocol for describing an individual-based model (Grimm et al., 2006; Grimm et al., 2010).

2.1. Model purpose

The individual-based model was written in Java, using the Repast Simphony 2.2 toolkit for agent-based modeling (North et al., 2013). The model was designed to simulate individual female moth behavior in response to landscape configuration and crop attractiveness. We then applied the model to explore emergent movement distances and egg distribution in different crops. The simulated behavioral decisions of movement and oviposition are in response to the landscape within the (user-specified) 'perceptual range' (Lima and Zollner 1996) of a female moth.

2.2. Entities, state variables and scales

The model consists of two types of agent: (1) female *Helicoverpa* moths and (2) habitat patches (crop fields) within a spatial landscape. The model spatial landscape is called a 'context' in Repast. As this model uses GIS data, we use a 'Geography' Repast context class as well as Geotools and the VividSolutions Java Topology Suite (JTS), providing spatial object model and fundamental geometric functions with OpenGIS. This is implemented in a similar manner to the Repast Simphony example 'Geography' model. The model runs on a nightly time step, within which there are a number of sub-steps relating to movement and oviposition (see below). The landscape is a torus: if the *Helicoverpa* agents cross the perimeter they re-enter on the opposite side. Using a torus maintains the number of *Helicoverpa* agents in the simulation as constant. Variation in the number