To Bt or Not to Bt?
Balancing Spatial Genetic Heterogeneity to Control the Evolution of *Ostrinia nubilalis*

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Abstract

Genetically modified corn crops have been developed to reduce the impact of potentially devastating agricultural pests such as the European Corn Borer (*Ostrinia nubilalis*). Continuous exposure to Bt toxins in genetically modified corn results in the increased prevalence of European Corn Borers that are resistant to these toxins. In this article, we first analyze the evolution of resistance in a uniform environment using a system of nonlinear difference equations. The evolution of resistance is then simulated in spatially explicit environments based on the biology of the insect and using parameters found in the literature. The optimum initial conditions and various stripe patterns on a corn field which will minimize the evolution of a resistant population are explored numerically.

1 Introduction

Insect pests are inherent problems in the agriculture industry which have plagued growers for countless years. As a result, a multitude of insecticides have been employed for their control. However, environmental and human health issues have always been a concern surrounding pesticide use. Recent technological advances, though, have allowed for a dramatic decrease in the use of externally applied insecticides. Crops have been engineered to contain exotic genes which allow them to exhibit continual expression of toxins, thus making external application of insecticides virtually unnecessary in combating infestations of some pests. The result is an approximate 90% reduction in annual insecticide use for genetically modified sweet corn, which amounts to a savings of over 700,000 pounds of insecticides annually [8]. It is estimated that $2.7 billion of the $8.1 billion spent annually on insecticides could be replaced by genetically modified crops [8].

A particular transgenic crop, known as Bt corn, has been manufactured to be resistant to the European Corn Borer (*Ostrinia nubilalis*). The European Corn Borer is the most devastating insect pest of corn in the United States and Canada. It causes damage and requires control resulting in losses that exceed $1 billion every year [13]. Bt corn provides more effective and consistent control of *O. nubilalis* than insecticides for a lower cost than insecticide application and with fewer health or environmental concerns [16]. This crop contains a gene from the naturally occurring soil bacteria *Bacillus thuringiensis*, which produces a protein lethal to European Corn Borer larvae. Various strains of genetically modified Bt corn produce crystalline proteins known as *Cry Proteins* in different forms that selectively kill groups of insects. The protein forms Cry1Ab, Cry1Ac, and Cry9C are effective against *Ostrinia nubilalis*, with Cry1Ab being the most commonly used [13]. The toxic form of the protein is activated by the insect's digestive enzymes. It binds to specific receptors on the lining of the intestine and the cells rupture, letting the gut contents leak into the body fluid. Most larvae die within two or three days of initial ingestion [13].

A characteristic of organisms in naturally occurring biological systems is the ability to adapt for survival. Studies show that there exists substantial genetic diversity for minor resistance genes to be present in wild populations of *O. nubilalis* [16]. If this gene happened to emerge in an environment comprised entirely of Bt corn, the individuals with the genetic trait that makes them resistant would survive, while all the normal insects would perish. The greater the duration of exposure and the higher the proportion of exposed individuals, the faster the process of resistance development occurs, as the intensity of selection is increased [14]. Much to the dismay of growers, selection for the resistant insect would occur, and the survivors would proliferate, producing an entirely resistant population.

The resistant allele has been assumed to be recessive; however, studies have demonstrated that insects with one resistant allele (R) and one susceptible allele (S), or heterozygotes, are not completely susceptible to Bt [1]. Instead they display a range of survival rates depending on the degree of plant toxicity. Incompletely dominant Bt resistance can therefore be effectively dominant at some Bt concentrations [1]. Major incidences of resistance can be associated with the loss of affinity of the

toxin produced by the Cry protein for the toxin receptors in the gut of the insect [1]. Disrupted or altered enzyme activity in the gut of the insect is a factor which could make the allele phenotypically display incomplete dominance [1]. Levels of codominance are determined by the portion of heterozygotes which survive Bt exposure.

The U.S. government recognizes the magnitude of the problem of selective evolution that could accompany widespread use of genetically modified crops. As a result, the U.S. Environmental Protection Agency (EPA) and the U.S. Department of Agriculture (USDA) have proposed guidelines to manage insecticide resistance [8]. A strategy necessary for the control of insect resistance is known as the *high dose/structured refuge strategy* [8]. This strategy consists of planting Bt corn with high levels of toxin expression along with sufficient normal corn to provide an adequate number of susceptible adult insects to dilute the frequency of resistant genes. Planting refuges, which are proportions of crop acreage designated to contain only non-Bt corn, have also been shown to be economically beneficial [16]. Studies show that Bt cultivars must produce a toxin concentration strong enough to kill most insects which are heterozygous for the resistance gene [15]. According to the Scientific Advisory Panel, a toxin concentration that is 25 times the concentration required to kill susceptible insects would be sufficient to kill heterozygotes [15]. Growers in the U.S. are required to plant non-Bt corn refuge over at least 20% of their total field acreage [8]. This ratio has been shown to be the most "economically superior" considering all costs involved for farmers [16]. The minimum refuge area of 20% is supported by data on regional genetic structure of *O. nubilalis* along with theoretical models [16]. The minimum recommended portion of normal corn on a field is increased to 40% if the land is to be sprayed, in order to increase the chance of susceptibles surviving [16].

The *high dose/structured refuge strategy* is based on three major assumptions which are outlined by the 1998 regional research committee NC-205 [16]. The first is that the frequency of major resistance genes must be sufficiently low to ensure that nearly all resistant genes are found in heterozygous (RS) individuals. The second is that heterozygote survival rates must be very low on Bt plants. This occurs when the resistance gene is nearly recessive (the codominance level is low). The third assumption is that random mating occurs within the typical dispersal distances of the adults, so that resistant insects have enough susceptible individuals within their range of travel to make it likely that their offspring will be heterozygous rather than homozygous for the resistant allele. These assumptions were considered when parameters were designated for our simulation.

The ultimate goal of this project is to determine possible refuge configurations which may reduce the rate of evolution of a resistant population of *O. nubilalis*, based on a 20% refuge plan. We begin by analyzing a deterministic model representing the basic population dynamics of *O. nubilalis*. We then analyze a deterministic model with selection to evaluate the population dynamics of the insect in an environment with selective pressure. This is followed by a discussion of the operation of the computer simulation and the application of the derived models to this simulation. Finally, the results and conclusions obtained from the performed simulations are given.

2 Deterministic Model

We begin with the introduction and analysis of a deterministic model which represents the basic vital dynamics of *Ostrinia nubilalis*. This model generates the number of larvae in a population based on the number of adults in the previous generation, and we assume there is no overlap of generations. The larval stage is vital to consider, as it is at this stage that the insect damages the corn plant through herbivory. This model will be used as a reproductive mechanism in our computer simulations.

2.1 Model

To build a model of the population dynamics of *O. nubilalis*, we assume that the initial larval population at any generation is equivalent to the number of eggs that hatch. We define μ_1 to be the fraction of eggs that are not viable, so $1 - \mu_1$ is the fraction of eggs laid by the previous generation that survive to the larval stage. The number of initial larvae, then, is $1 - \mu_1$ multiplied by the total number of eggs laid. We define γ to be the fraction of females in the adult population and a_n to be the total number of adults, so γa_n becomes the total number of females in the adult population of the n^{th} generation. The average number of eggs laid by an adult female is defined as β, so $\gamma\beta a_n$ is the total number of eggs laid by generation n. This leads to the equation:

$$l_{n+1} = \gamma\beta(1 - \mu_1)a_n \tag{1}$$

The adult population of the next generation depends on the larvae of the present generation. We use the *Verhulst Model* to provide a basis for the derivation of our model. The *Verhulst Model* is defined as follows [2]:

$$x_{n+1} = \frac{rx_n}{1 + ax_n}$$

This model was chosen because it represents a population in which x_{n+1} is bounded above by a carrying capacity, $k = \frac{r}{a}$, and x_{n+1} is close to k whenever the x_n population is large.

It is assumed that generations do not overlap and that each corn plant can only hold a limited number of larvae. The number of adults of the next generation cannot exceed the number of larvae of the present generation. From this information the following difference equation is derived:

$$a_{n+1} = \frac{\alpha l_n}{1 + \mu_2 l_n} \tag{2}$$

where:

1. α is a constant that must be less than or equal to 1 for a_{n+1} not to exceed l_n. For simplification, let $\alpha = 1$.

2. μ_2 is the larval death rate.

3. $k_1 = \frac{1}{\mu_2}$ is the adult carrying capacity.

Substituting equation (2) into equation (1) yields the following difference equation:

$$l_{n+1} = \frac{\gamma\beta(1-\mu_1)\ l_{n-1}}{1+\mu_2\ l_{n-1}} \tag{3}$$

Equation (3) can be expanded as a system of equations. First, we introduce the following definition:

$$j_n = l_{n-1}$$

Substituting this equation into equation (3), we define the system of equations as:

$$l_{n+1} = \frac{\gamma\beta(1-\mu_1)\ j_n}{1+\mu_2\ j_n} \tag{4}$$

$$j_{n+1} = l_n \tag{5}$$

The carrying capacity, or maximum survival rate of the population is:

$$k_2 = \frac{\beta\gamma(1-\mu_1)}{\mu_2}$$

2.2 Analysis of the Model

We will now analyze the system of equations (4) and (5) for equilibria and their respective stabilities. This process allows us to see the long term dynamics of the model, and determine the eventual fate of the insect population.

2.2.1 Equilibrium Points

The equilibrium points are derived by defining the reproduction functions $f(l,j)$ and $g(l,j)$, and setting them equal to l and j respectively.

Definition 1 *A reproduction function is a function* $f : I \to I$, $I \subset \mathbb{R}^n$, $n = 1, 2, 3...$ *such that if* $\mathbf{x}_m = (x_{1,m}, x_{2,m}, ..., x_{n,m}) \in I, m = 0, 1, 2...$, *then* $\mathbf{x}_m = f^m(\mathbf{x}_0)$, *where* f^m *denotes* $\underbrace{f \circ f \circ ... \circ f}_{m \text{ times}}$, *and* $f^0(\mathbf{x}_0) := \mathbf{x}_0$.

The reproduction functions of this model are:

$$f(l,j) = \frac{\gamma\beta(1-\mu_1)\alpha\ j}{1+\mu_2\ j}$$

$$g(l,j) = l$$

Definition 2 *If $f_1 : I \to I, f_2 : I \to I, ...f_n : I \to I$, $I \subset \mathbb{R}^n$, $n = 1, 2, 3...$ are reproduction functions of a system of difference equations, then, $\mathbf{x}^* = (x_1^*, x_2^*, ..., x_n^*) \in I$, is said to be an equilibrium point of $f_1, f_2, ..., f_n$ if and only if $f_1(\mathbf{x}^*) = x_1^*, f_2(\mathbf{x}^*) = x_2^*, ..., f_n(\mathbf{x}^*) = x_n^*$ are true.*

In order to find the equilibrium points we set $f(l^*, j^*) = l^*$ and $g(l^*, j^*) = j^*$. The equilibrium points are:

$$l_1^* = 0$$
$$l_2^* = \frac{(\gamma\beta(1-\mu_1)-1)}{\mu_2}$$

Where $j_1^* = l_1^*$, and $j_2^* = l_2^*$.

2.3 Stability of Equilibria

Definition 3 *An equilibrium point $\mathbf{x}^* = (x_1^*, x_2^*, ..., x_n^*) \in I$, $n = 1, 2, 3...$ of a system of reproduction functions $f_1 : I \to I, f_2 : I \to I, ...f_n : I \to I$, $I \subset \mathbb{R}^n$ is stable if and only if $\forall \epsilon_m > 0$ $\exists \delta_m > 0$, $m = 1, 2, ..., n$, such that $|x_{m,p} - x_m^*| < \epsilon_m$, $p = 1, 2, 3...$ whenever $|x_{m,0} - x_m^*| < \delta_m$.*

Theorem 1 *If A is the Jacobian matrix of the system of reproduction functions $f(x^*, y^*)$ and $g(x^*, y^*)$, and if all roots of the characteristic equation $det(A(x^*, y^*) - \lambda I)$ satisfy $|\lambda| < 1$, then all solutions of the system with initial values sufficiently close to an equilibrium approch the equilibrium [4].*

The characteristic equations for $l_1^* = 0$ and $l_2^* = \frac{(\gamma\beta(1-\mu_1)-1)}{\mu_2}$ derived from the Jacobian matrix of the reproduction functions are:

$$\lambda_1^2 - \gamma\beta(1-\mu_1) = 0,$$

and

$$\lambda_2^2 - \frac{1}{\gamma\beta(1-\mu_1)} = 0$$

respectively.

From the characteristic equations we determine the stability of the fixed points by using Theorem 1.

For $l_1^* = 0$, λ is:

$$|\lambda_1| = \sqrt{\gamma\beta(1-\mu_1)}$$

For $l_2^* = \frac{(\gamma\beta(1-\mu_1)-1)}{\mu_2}$, λ is:

$$|\lambda_2| = \sqrt{\frac{1}{\gamma\beta(1-\mu_1)}}$$

Whenever $\frac{1}{\gamma(1-\mu_1)} < \beta$, then $|\lambda_1| > 1$, and $|\lambda_2| < 1$. Here $l_1^* = 0$ is unstable, and $l_2^* = \frac{(\gamma\beta(1-\mu_1)-1)}{\mu_2}$ is locally asymptotically stable. If $\frac{1}{\gamma(1-\mu_1)} > \beta$, then $|\lambda_1| < 1$, and l_2^* is locally asymptotically stable. In this instance the equilibrium point l_2^* does not make sense biologically, since whenever $\frac{1}{\gamma(1-\mu_1)} \leq \beta$ is true, l_2^* is not a positive value.

If $\frac{1}{\gamma\beta(1-\mu_1)}$ then $l_1^* = l_2^* = 0$, and the point $l_1^* = l_2^* = 0$ is an equilibrium with $|\lambda| = 1$. In this case $|\lambda| = 1$ tells us that the equilibrium point $l_1^* = l_2^* = 0$ is an attractor whenever $l_n > 0$ and a repeller whenever $l_n < 0$. According to Theorem 1, $l_1^* = l_2^* = 0$ would not be considered stable if it were not an endpoint of the interval on which $f(l, j)$ and $g(l, j)$ are defined; however, since $l_n < 0$ does not have biological significance ($f(l, j)$ and $g(l, j)$ are not defined when $l < 0$) we take the point $l_1^* = l_2^* = 0$ to be locally asymptotically stable.

β	l_1^*	l_2^*
$\frac{1}{\gamma(1-\mu_1)} < \beta$	Unstable	Asymptotically Stable
$\frac{1}{\gamma(1-\mu_1)} = \beta$	Asymptotically Stable	–
$\frac{1}{\gamma(1-\mu_1)} > \beta$	Asymptotically Stable	–

Table 2: Stability Chart for Equilibria

Note: If $\frac{1}{\gamma(1-\mu_1)} = \beta$ then $l_2^* = l_1^* = 0$. If $\frac{1}{\gamma(1-\mu_1)} > \beta$ then $l_2^* < 0$, and does not make sense biologically.

In summary, the characteristics of the equilibria are determined by the way the value of β, the number of eggs laid by each female, relates to the value of $\frac{1}{\gamma(1-\mu_1)}$, the reciprocal of the fraction of the population that is female, multiplied by the fraction of eggs that hatch. If $\beta > \frac{1}{\gamma(1-\mu_1)}$, there are two eqilibria, $l_1^* = 0$, which is unstable, and $l_2^* > 0$, which is asymptotically stable. In this case the population of *O. nubilalis* reaches a stable positive population size. If $\beta \leq \frac{1}{\gamma(1-\mu_1)}$, there is only one non-negative equilibrium, $l_1^* = 0$ which is asymptotically stable. In this case the population eventually goes extinct.

3 Deterministic Model with Selection

In this section, we introduce and analyze a deterministic model representing the selection of resistance in *Ostrinia nubilalis*. This model will be used to calculate succeeding generations based on the selective pressures of the environment. The frequencies of two alleles are analyzed. These include the resistance allele **R**, which represents the ability to survive on Bt corn, and the susceptibility allele **S**, which represents no resistance to the Bt toxin. The three possible genotypes of these alleles are: RR, RS, and SS.

Again, we assume that the population changes in a discrete-time manner so there exists no overlapping of generations. The *fitness* of an individual (relative probability of survival and reproduction) is independent of its genotype frequency. Since this model assumes random mating, it is related to the *Hardy-Weinberg Principle* for establishing proportions of initial genotypes [12].

3.1 Hardy-Weinberg Principle

The *Hardy-Weinberg Principle* states that random mating among genotypes is equivalent to random combination of gametes and assumes the following conditions:

1. Fitness is independent of genotype frequency.
2. There is no movement into or out of the system.
3. Population size is large enough to ignore stochastic effects.
4. No mutations occur at the locus of the allele of interest.
5. Mate selection is independent of genotype.

The alleles R and S are defined as having frequencies p and q respectively, where $p + q = 1$. According to the *Hardy-Weinberg Principle*, in a population without overlapping generations, only a single generation of random mating is needed for the frequencies of the genotypes to be established as follows [12]:

$$\begin{aligned} p_{RR} &= p^2 \\ p_{RS} &= 2pq \\ p_{SS} &= q^2 \end{aligned}$$

This is commonly referred to as *Hardy-Weinberg equilibrium* [12].

3.2 Model

We now introduce the notion of fitness (w), which represents the relative survival rates of individuals. The following table summarizes the information required to find the respective frequencies of the adult population given an initial population of larvae assumed to be in *Hardy-Weinberg equilibrium.*

	RR	RS	SS
Larvae Freq.	p^2	2pq	q^2
Relative Survival Rates	w_{RR}	w_{RS}	w_{SS}
Relative Adult Freq.	$p^2 w_{RR}$	$2pqw_{RS}$	$q^2 w_{SS}$
Adult Freq.	$p^2 w_{RR}/\overline{w}$	$2pqw_{RS}/\overline{w}$	$q^2 w_{SS}/\overline{w}$

Table 1: Selection Model for Two Alleles at a Single Locus [12]

The mean fitness of the population at generation n is given by:

$$\overline{w}(n) = p_n^2 w_{RR} + 2p_n q_n w_{RS} + q_n^2 w_{SS}$$

and the average fitnesses of the alleles at generation n are defined as:

$$w_R(n) = p_n w_{RR} + q_n w_{RS} \tag{6}$$
$$w_S(n) = p_n w_{RS} + q_n w_{SS} \tag{7}$$

The frequencies of the alleles in generation n are given by the following system of difference equations [12]:

$$p_n = p^2 + pq \tag{8}$$
$$q_n = q^2 + pq \tag{9}$$

This system can be reduced to a single formula. Since $p + q = 1$ for all generations, it is obvious that:

$$q_n = 1 - p_n \tag{10}$$

Substituting the values of the adult frequencies of R from *Table 1* into (8), the difference equation for the frequency of the allele R in generation $n + 1$ becomes:

$$p_{n+1} = \frac{p_n w_R(n)}{\overline{w}(n)} \tag{11}$$

Now we focus our attention on deriving equations that represent the fitness of the three different genotypes: RR, RS, and SS. These fitnesses will help us clearly define the values of the fitnesses of each allele. The fitness of the genotype depends on the environment in which it is found: Bt corn or normal corn.

$$w_{RR} = \text{Prob[RS found in Bt]}w_{RS}[Bt] + \text{Prob[RS found in normal]}w_{RS}[normal]$$
$$w_{RS} = \text{Prob[RS found in Bt]}w_{RS}[Bt] + \text{Prob[RS found in normal]}w_{RS}[normal]$$
$$w_{SS} = \text{Prob[SS found in Bt]}w_{SS}[Bt] + \text{Prob[SS found in normal]}w_{SS}[normal]$$

The following simplifying assumptions are vital to the deterministic model:

1. An individual with both resistant alleles is unaffected by Bt corn. Hence, $w_{RR}[Bt] = 1$.

2. An individual with genotype RS is not completely resistant to Bt corn. Hence, $0 \leq w_{RS}[Bt] \leq 0.025$ [16]. Here we denote w_{RS} as δ.

3. An individual with both non-resistant alleles has no resistance to Bt corn. Hence, $w_{SS}[Bt] = 0$ [16].

4. Individuals of all genotypes persist on normal corn. Hence, $w_{RR}[normal] = w_{RS}[normal] = w_{SS}[normal] = 1$.

Therefore, if we define f_{Bt} to be the fraction of Bt corn, it is easy to see that

$$w_{RR} = 1$$
$$w_{RS} = f_{Bt}\delta + (1 - f_{Bt}) = 1 - (1 - \delta)f_{Bt}$$
$$w_{SS} = 1 - f_{Bt}$$

From this information and equation (11) we obtain a simplified equation for p_{n+1}, and since $q_n = 1 - p_n$ we consider only p_n.

$$p_{n+1} = \frac{p_n - p_n q_n f_{Bt}(1 - \delta)}{1 - q_n^2 f_{Bt} + 2p_n q_n f_{Bt}(1 - \delta)} \tag{12}$$

3.3 Analysis of the Model

We will now analyze the nonlinear difference equation (12) for equilibria and their respective stabilities. This process allows us to see the long term dynamics of the model, and determine the eventual fate of the insect population.

3.3.1 Equilibrium Points

The reproduction function for equation (12) is:

$$f(p) = \frac{p - pqf_{Bt}(1-\delta)}{1 - q^2 f_{Bt} + 2pqf_{Bt}(1-\delta)}$$

The equilibrium points of this model are:

$$p_1^* = 0,\ q_1^* = 1$$
$$p_2^* = 1,\ q_2^* = 0$$
$$p_3^* = \frac{\delta}{(3-2\delta)},\ q_3^* = \frac{3(1-\delta)}{(3-2\delta)}$$

If $\delta = 0$ then $p_3^* = p_1^* = 0$ and if $\delta = 1$ then $p_3^* = p_2^* = 1$.

3.3.2 Stability of Equilibria

Theorem 2 *Let x^* be a hyperbolic fixed point (an equilibrium point where $|f'(x^*)| \neq 1$) of a smooth function $f : I \to I$ where I is an open interval in $\mathbb{R}$ [5].*

1. *If $|f'(x^*)| < 1$, then x^* is locally asymptotically stable.*
2. *If $|f'(x^*)| > 1$, then x^* is unstable.*

From the reproduction function we determine the stability of the fixed points by using Theorem 2.

For $p_1^* = 0$, $f'(p)$ is:

$$|f'(p_1^*)| = \frac{1 - f_{Bt}(1-\delta)}{(1 - f_{Bt})} \geq 1, \forall \delta \text{ and } f_{Bt}$$

For $p_2^* = 1$, $f'(p)$ is:

$$|f'(p_2^*)| = 1 + 3f_{Bt}(1-\delta) \geq 1, \forall \delta \text{ and } f_{Bt}$$

For $p_3^* = \frac{\delta}{(3-2\delta)}$, $f'(p)$ is:

$$|f'(p_3^*)| = \frac{3f_{Bt}(1-\delta) + |2\delta - 3|}{3f_{Bt}(1-\delta)^2 + |2\delta - 3|} \leq 1, \forall \delta \text{ and } f_{Bt}$$

For $0 < \delta < 1$ and $f_{bt} \neq 0$, the less than or equal to and greater than or equal to signs become strictly less than and greater than, respectively. Thus for $0 < \delta < 1$, p_3^* is locally asymptotically stable ($|f'(p_3^*)| < 1$), and p_1^* and p_2^* are unstable ($|f'(p_1^*)| > 1$ and $|f'(p_3^*)| > 1$). The special cases: $\delta = 0$, $\delta = 1$ and $f_{Bt} = 0$ are considered further in the following section.

Special Cases

When $f_{Bt} = 0$, then $w_{RR} = w_{RR}[normal] = w_{RS} = w_{RS}[normal] = w_{SS} = w_{SS}[normal] = 1$, and there is no selection occuring. Thus the population is in *Hardy-Weinberg Equilibrium*, and $p = p^*, \forall p \in [0, 1]$. In this case p^* is stable, but is not asymptotically stable.

The following summarizes the special cases of $\delta = 0$ and $\delta = 1$:

1. If $\delta = 1$, $w_{RR}[Bt] = 1$ and $w_{SS}[Bt] = 0$. **R** is completely dominant.
2. If $\delta = 0$, $w_{RR}[Bt] = 1$ and $w_{SS}[Bt] = 0$. **R** is completely recessive.

Note that for $\delta = 0$ the first derivatives of the reproduction function are:
For $p_1^* = 0$, $f'(p)$ is:

$$|f'(p_1^*)| = 1$$

For $p_2^* = 1$, $f'(p)$ is:

$$|f'(p_2^*)| = 1 + 3f_{Bt}$$

For $p_3^* = \frac{\delta}{(3-2\delta)}$, $f'(p)$ is:

$$|f'(p_3^*)| = 1$$

Also note that for $\delta = 1$ the first derivatives of the reproduction function are given as follows.
For $p_1^* = 1$, $f'(p)$ is:

$$|f'(p_1^*)| = \frac{1}{(1 - f_{Bt})}$$

For $p_2^* = 1$, $f'(p)$ is:

$$|f'(p_2^*)| = 1$$

For $p_3^* = \frac{\delta}{(3-2\delta)}$, $f'(p)$ is:

$$|f'(p_3^*)| = 1$$

To determine the stability of the nonhyperbolic equilibrium points that exist when $\delta = 0$, and when $\delta = 1$, an additional technique is required.

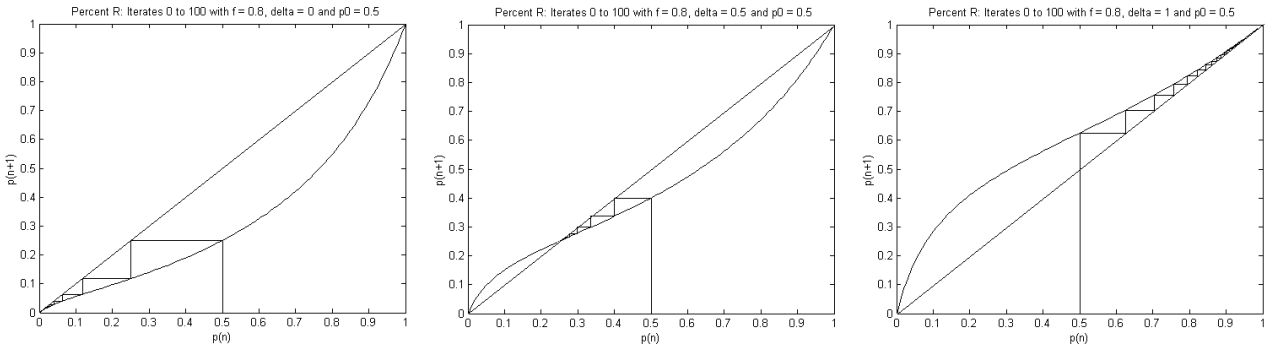

Figure 1: *Cobwebbing Graphs: Deterministic Model with Selection*

Cobwebbing Graphs

Cobbwebbing is used to graphically verify the stability of the equilibria. According to the graphs, for $\delta = 0$, $p_1^* = p_3^* = 0$ is locally asymptotically stable, and $p_2^* = 1$ is unstable. For $\delta = 1$, $p_2^* = p_3^* = 1$ is locally asymptotically stable, and $p_1^* = 0$ is unstable.

The following chart summarizes the stability of the equilibria for different values of δ.

δ	p_1^*	p_2^*	p_3^*
$\delta = 0$	Asymptotically Stable	Unstable	–
$0 < \delta < 1$	Unstable	Unstable	Asymptotically Stable
$\delta = 1$	Unstable	Asymptotically Stable	–

Table 3: Stability Chart for Equilibria

Note: If $\delta = 0$ *then* $p_3^* = p_1^* = 0$ *and if* $\delta = 1$ *then* $p_3^* = p_2^* = 1$.

In summary, the equilibria and their characteristics are determined by the value of δ. If $\delta = 0$, the resistance allele (R) is essentially fully recessive, so all heterozygotes perish on Bt corn. In this case, there is only one stable equilibrium ($p^* = 0$), which means that the resistance allele (R) goes extinct for any initial frequency of this allele. If $0 < \delta < 1$, then the resistance allele will not go extinct for any initial frequency. Instead, the frequency approaches the equilibrium point: $p_3^* = \frac{\delta}{(3-2\delta)}$. If $\delta = 1$, the resistance allele is fully dominant, so all heterozygotes survive on Bt corn. In this case, the stable equilibrium point is $p^* = 1$, which means that the frequency of resistance will approach 100% for any initial resistance frequency.

4 Stochastic Simulation

We created a computer simulation using JAVA to analyze the evolution of resistance corresponding to various arrangements of refuge within a Bt corn field. The relative widths of stripes in a field were varied to test the effects of the heterogeneity of the environment on the evolution of resistance. Each simulation was run 30 times over

100 generations. The changes in the frequencies of the resistant (R) and susceptible (S) alleles were determined after each larval generation, and the ultimate dynamics of the allele frequencies occurring in each generated field were investigated.

Knowledge of the biology of the insect is essential for constructing the simulations to yield the most realistic results possible. To make biological processes simpler for the efficiency of the simulation, however, individual insects are disregarded and the processes they undergo are applied to alleles. The equations (1) and (2) are used to generate succeeding generations of larval and adult alleles. An adult population consisting of only females is considered, which means that new alleles are generated from every existing allele. The reproduction habits of *O. nubilalis* are incorporated in the simulation as well, represented by the creation and distribution of new alleles.

Insect populations are affected by a variety of naturally occurring events. The survival rates of larvae are different for those that hatch in the spring and those that hatch during mid-summer [3]. The toxicity of the environment in which the larvae is born also influences survival rates according to alleles involved. We assume that that the resistant allele (R) does not adversely affect the ability of the insect to reproduce. We also assume that no homozygous susceptible individuals survive on Bt corn [16].

Field Arrangement

The simulations were performed for five different arrangements of refuge within a Bt corn field. One spatial arrangement contains 100% Bt corn and one contains 100% normal corn. These arrangements serve as controls to test the accuracy of the simulations, as the general trend of the results can be predicted. The remaining three fields consist of 80% Bt corn and 20% non-Bt corn with varying degrees of uniformity of integration in stripe patterns. The simulated field is composed of 200x50 square patches. Each stripe is one patch wide and runs the entire length of the field. The patch dimensions were established to be 215 ft^2 for the efficiency of insect distribution. Therefore, the simulated field size is approximately 8 miles wide by 2 miles long and is composed of 200 stripes. The specific dimensions, however, are not as significant as the degree of uniformity because the field is programmed as being torus shaped. The uniformity of the three heterogeneous fields varies as follows:

1. Normal corn and Bt corn are highly integrated with every 4 stripes of Bt corn alternating with 1 stripe of normal corn.

2. Normal corn and Bt corn are less integrated with every 80 stripes of Bt corn alternating with 20 stripes of normal corn.

3. Normal corn and Bt corn are not integrated, but placed in blocks with 160 stripes of Bt corn adjacent to 40 stripes of normal corn.

The figure below shows a pictorial representation of the five arrangements.

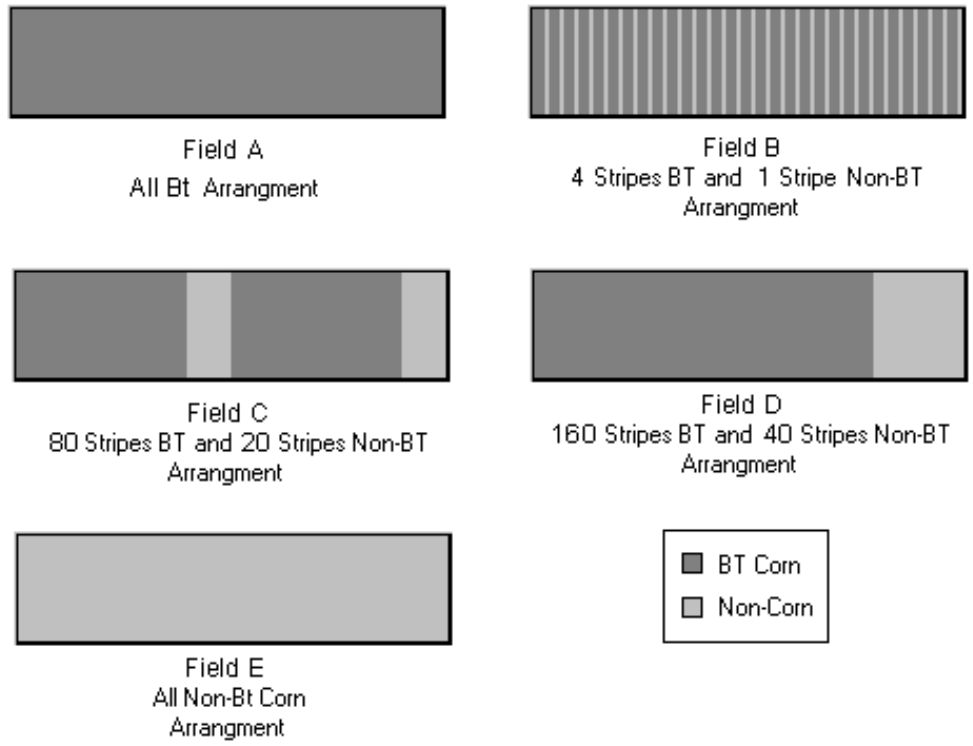

Figure 2: *Field Arrangements*

Numerical Parameters

Many of the numerical parameters which determine the dynamics of the simulations were collected from a variety of literary sources. The remaining were deduced relative to each other considering the efficiency of the simulation. The parameters used in the simulation are:

1. The initial frequency of the resistance allele (R) is: p_0 =8.39x10^{-4} [16].
2. The fitness of the genotype RS on Bt corn is: $w_{RS} = \delta = 0.025$ [16].
3. The fitness of the genotype SS on Bt corn is: $w_{SS} = 0$ [16].
4. The fitness of the genotype RR on Bt corn is: $w_{RR} = 1$.
5. Only females were considered, thus $\gamma = 1$.
6. The number of egg packets laid by each female insect ranges from 15 to 25 [18][9].
7. The average number of eggs per packet laid by each female insect is 23 [9].

8. The fraction of eggs laid which are not viable, μ_1, is 0.65.

9. The larval death rates are: $\mu_{2,1} = 0.02$ and $\mu_{2,2} = 0.09$. These values were estimated to be as realistic as possible considering a low enough carrying capacity to allow the simulations run efficiently.

10. The initial capacity of alleles per patch is: 300.

11. The maximum egg laying range of the female is 1500 ft [3].

12. The size of the field is 8 miles by 2 miles.

The Simulation

The simulation begins with a field composed of square patches. A number of alleles are randomly distributed over an empty field with a limit on the initial number of alleles placed in each patch. This initial distribution of alleles is used for all simulations on all fields so that the dynamics can be statistically compared. The specific composition of the heterogeneity of the field is then set, and in each patch, processes are set to occur over each generation. These processes are mortality, reproduction, and distribution.

Mortality

The first vital process to occur within each patch is death. There are two types of mortality which occur here: death due to natural causes such as predation or competition (*natural death*), and death due to susceptibility to Bt (*Bt death*). *Natural death* occurs at the same rate for both alleles. The number of larval alleles surviving to adulthood are generated using equation (2), where $\alpha = 1$, and μ_2 varies between two values which alternate with successive generations. These are: $\mu_{2,1} = 0.02$ and $\mu_{2,2} = 0.09$. *Bt death* is variable depending on whether or not the patch is composed of Bt corn and depending on the fitness of each allele with respect to the environment. If the patch is composed of normal corn, *Bt death* does not occur for either allele. If the patch is composed of Bt corn, the number of surviving resistant (R) and susceptible (S) alleles are represented by the following equations:

$$R_{n+1} = w_R R_n$$
$$S_{n+1} = w_S S_n$$

The fitnesses w_R and w_S are defined by equations (6) and (7). With the defined parameters, these equations become:

$$w_R(n) = p_n + q_n(0.025)$$
$$w_S(n) = p_n(0.025)$$

The frequencies p_n and q_n are recalculated with each generation.

Reproduction

During the reproduction process, a number of new alleles are created in each patch, simulating the number of eggs laid by the female adults. This process comes from equation (1). For the simulation, $\beta = (\#\text{eggs per packet})(\#\text{of packets})$, where the number of eggs per packet is 23, and the number of packets laid is a randomly generated number between 15 and 25. The number of new alleles produced, then, is: $23(1 - .65)$ and these will be distributed between 15 and 25 times.

Distribution

During the stochastic distribution process, alleles are distributed as the female adult would distribute her eggs. We assume that the female is more likely to lay eggs near her place of birth. We represent this with a two-dimensional approximation to a normal distribution of alleles, centered over the patch in which the allele was created. The maximum range in which a female lays eggs is set to be 1500 ft, so for the efficiency of placing the normal distribution by patch, this number is divided by seven, making each patch 215 ft^2. Each surrounding patch within the range is assigned a probability of having an allele placed in it according to a normal distribution chart [11].

5 Results

The results of 30 simulations of the frequency of the resistance allele (R) in the overall population are plotted over 100 generations. The means of the fields are graphed against each other for comparison. Graphs of the standard deviations for fields B, C, D, and E are also included in this section. The simulations and standard deviations of Field A are not included because the populations of both alleles (R and S) go extinct within very few generations.

Generation Plots

The following graphs show the results of 30 simulations over a 100 generation time period.

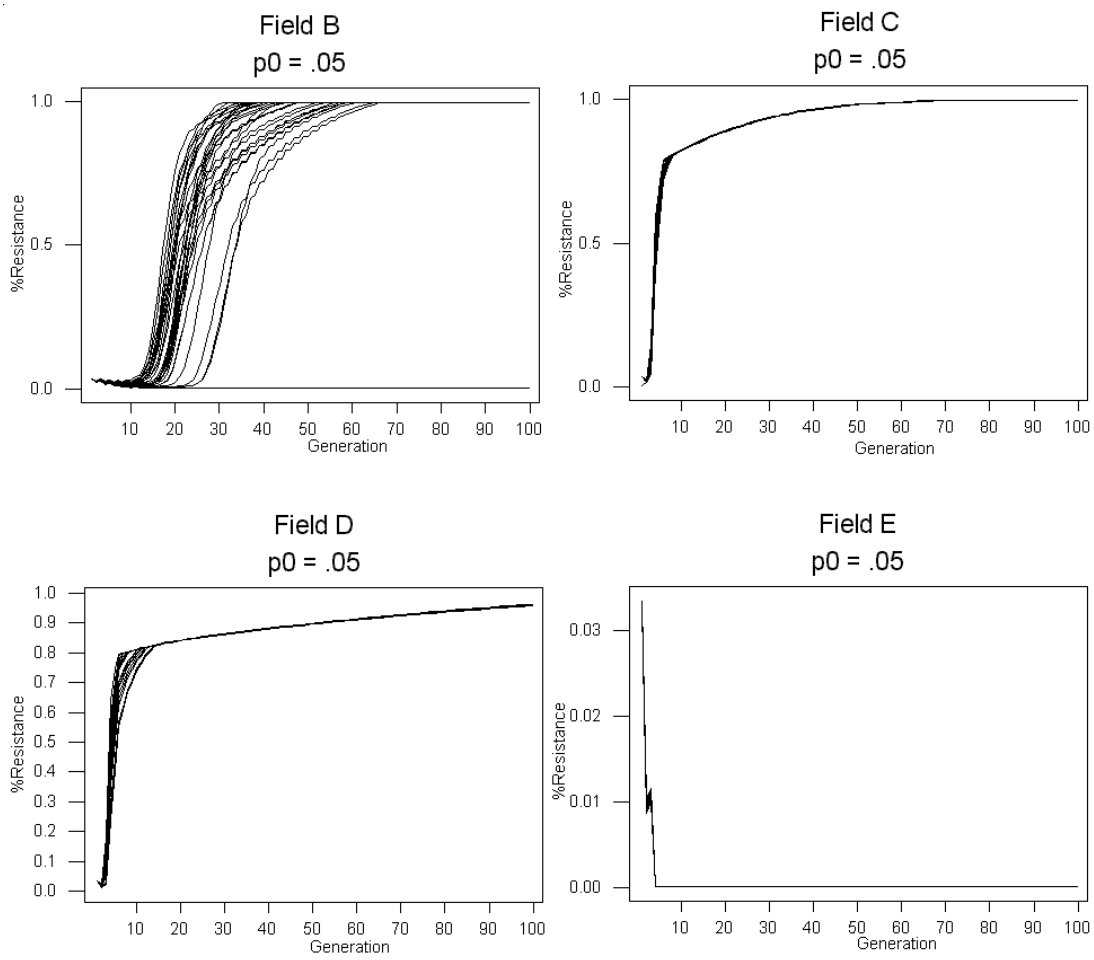

Figure 3: *Stochastic Simulation Graphs*

Means

The following graph incorporates the plot of the means for fields B, C, D, and E. The mean converges the overall behavior of the simulations for every field. Field A is not shown since allele R and allele S go extinct within the first generation.

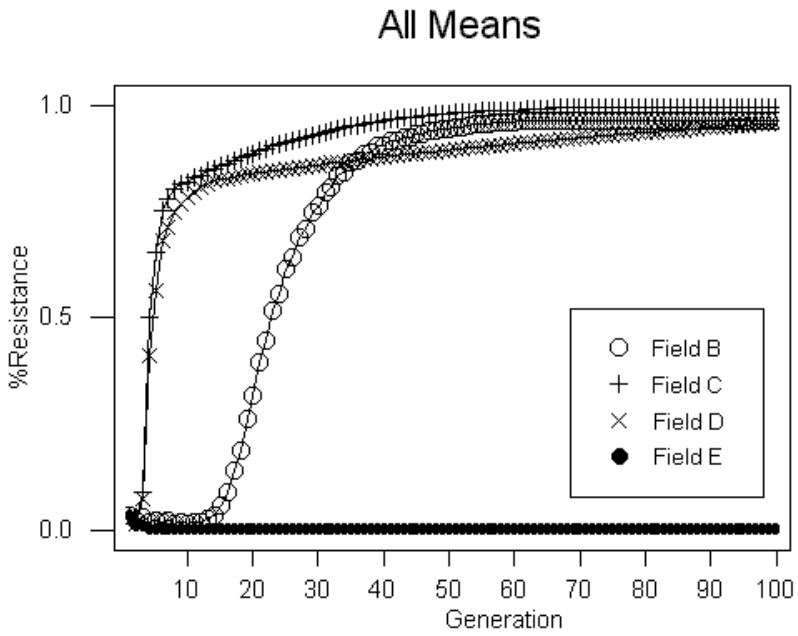

Figure 4: *Means*

Standard Deviation

The following graphs show the standard deviation of fields B, C, D, and E for each generation. On all fields, except field B, the standard deviations eventually go to zero. This occurs when all simulations have reached equilibrium. Field B does not show this behavior because of the single simulation in which the resistant allele (R) goes extinct. Field A is not shown since both alleles go extinct within the first generation.

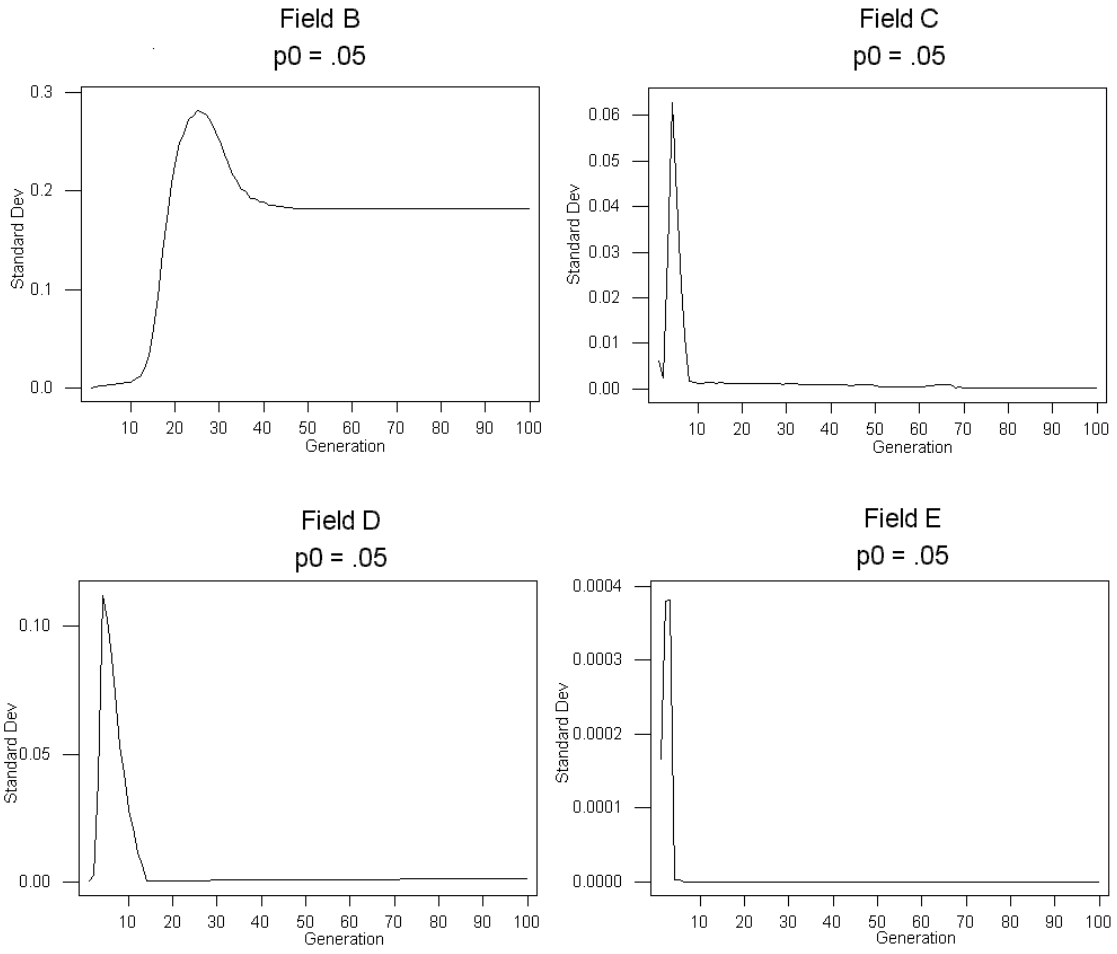

Figure 5: *Standard Deviation Graphs*

Deterministic Model

The graphs of the deterministic model are included to provide a visual reference for comparison with the graphs of the stochastic simulations. The evolution of resistance in a field with 80% Bt corn and $\delta = 0.025$ for two different initial frequencies of the resistant allele (R) is graphed. In addition, the evolution of resistance after 200 generations is included for fields with varying percentages of normal corn. The resistance

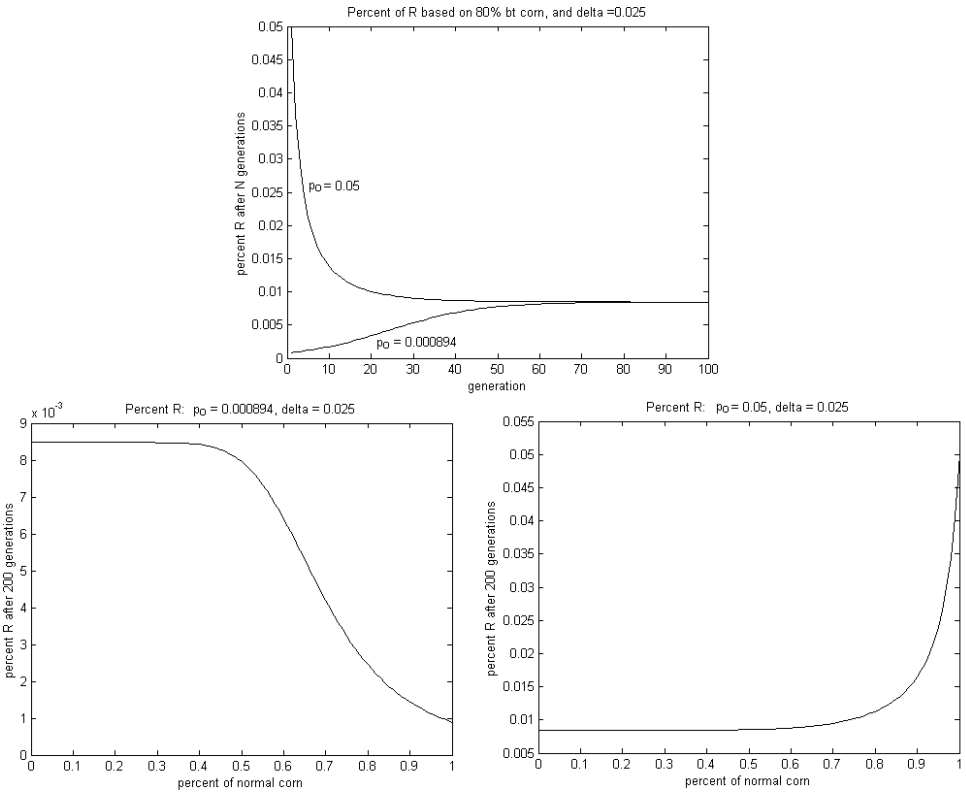

Figure 6: *Deterministic Model Graphs*

in the deterministic model does not approach 100% as it does in the simulation. This is because this model represents an infinitely large population in which an allele with a low frequency does not go extinct from natural causes.

6 Discussion

We observed through our research that the evolution of resistance in the stochastic simulations followed the general trend of evolution depicted by the deterministic models. The differences between the mean trends of the simulations are a result of the varying degrees of heterogeneity.

Spatial arrangement is not a factor of evolution in Field A because it is comprised entirely of Bt corn. The deterministic model shows that the resistance should immediately increase under these conditions. However, in our simulations, the initial

frequency of the resistance allele (R) is very low relative to the carrying capacities of the patches. The result is the extinction of the resistance allele within the first few generations due to natural death. The susceptible alleles are all eliminated by Bt exposure. Thus, in our computer simulations, the entire population goes extinct on Bt corn with a low initial frequency of resistance.

Field B is the most thoroughly mixed field. Bt and normal corn are integrated on this field by alternating four stripes of Bt corn with one stripe of normal corn. The graph of Field B in Figure 3 depicts the behavior of resistance evolution for 30 simulations. In 29 of the simulations, the population reaches 100% resistance after various lengths of time, but the resistance allele goes extinct in one simulation. The stochastic process of death by natural selection forces the resistance allele in one simulation to be eliminated. This result does not present a problem though, as it is a justified anomaly. The graphs of the means and the standard deviations were included for statistical analysis. The mean of each field summarizes the overall behavior of that field. The mean of Field B is not altered significantly by the simulation in which the resistance goes extinct. The standard deviation does not decrease to zero after its peak value as it does in each of the other fields. This is the effect of the simulation in which resistance is eliminated.

The two types of corn are mixed much less on Field C than on Field B. Bt and normal corn are integrated on this field by alternating 80 stripes of Bt corn with 20 stripes of normal corn. The interval in which all simulations reach 100% resistance is relatively small. Resistance increases rapidly within the first ten generations; however, after the tenth generation, the rate of evolution of resistance appears to decrease.

The two types of corn are not mixed on Field D. All Bt corn (160 stripes) is placed in a block adjacent to the entire portion of normal corn (40 stripes). The simulations all appear to reach 80% rapidly, then the rate of increase of resistance sharply decreases.

Spatial arrangement is not a factor of evolution in Field E because it is comprised entirely of normal corn. Our deterministic model shows that the allele frequencies under these conditions would approach a positive equilibrium in a natural system with a very large initial population of insects. However, since the initial frequency of the resistance allele (R), is very low relative to the carrying capacities of the patches, the resistance allele goes extinct within the first few generations due to natural death. When the resistance drops to approximately 0.01%, it appears to show a brief increase before going extinct due to the higher natural death rate in our simulations. This event may be related to the equilibrium which would be reached in a natural system, as demonstrated by the deterministic model (Figure 6).

The initial frequency of the resistance allele is significant in our simulations. The *high dose/structured refuge strategy* proposes that the inital frequency of the resistance allele must be sufficiently low to ensure that nearly all resistant genes in the population are in heterozygous individuals. One literary source gave an initial frequency value of $8.39\text{x}10^{-4}$ and a 95% confidence interval for the frequency of [0, $4.38\text{x}10^{-3}$] for very large populations [16]. However, for our stochastic simulations, these tested values proved to be too low. The combination of death by Bt toxin in

the heterozygous individuals along with natural death rates pushed the resistance allele to extinction because the carrying capacity of each patch was very low relative to the initial frequency. Therefore, to obtain better results, a higher initial frequency ($p_0 = 0.05$) was used. This initial frequency of the resistant allele was high enough result in evolution of 100% resistance over time, which is the main concern of our project.

7 Conclusions

The simulations performed showed that normal corn more intricately interspersed with Bt corn results in the slower increase of resistance frequency. The general behavior pattern of the evolution of resistance observed in the deterministic models is the same as that observed in the stochastic simulations, which leads us to conclude that the results of the simulation are reasonable bases for predicting long term behavior of resistance frequency. The only inconsistancies occurred in the extreme cases of the pure Bt and pure normal corn fields. These were results of the low carrying capacity relative to the low initial frequency of the resistance allele and the effects of natural death.

Although less distinction can be observed between the two fields with less integration, the distinction between the least integrated and most integrated fields can be clearly observed in the results of the simulations. The resistance frequency approached 100% in each case; however, the rates at which the frequency increased appeared to depend on the level of refuge integration. This phenomenon is most likely related to the distribution of resistance alleles. In a more thorougly mixed environment, resistant insects are more likely to be in close vicinity with susceptible insects. This increases the chances of maintaining a significant number of heterozygotes in the population, which are less likely to survive than insects homozygous for the resistance gene. Thus, with a sufficiently diluted frequency of resistance in a naturally large population achieved by increased intricacy of field refuge integration, evolution can be slowed.

8 Acknowledgements

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References

[1] Bourguet D., A. Genissel, and M. Raymond. "Insecticide Resistance and Dominance Levels." *Journal of Economical Entomology*. 93(6): 1588-1595, 2000.

[2] Brauer, Fred and Carlos Castillo-Chavez. *Mathematical Models in Population Biology and Epidemiology*. New York: Springer, 2000.

[3] Calvin, Dennis. Pennsylvania State University. E-mail. 25 July 2001.

[4] Castillo-Chavez, Carlos, Fred Gould, and Simon A. Levin. "Physiological and Behavioral Adaptation to Varying Environments: A Mathematical Model." *Evolution*. 42(5): 986-994, 1988.

[5] Castillo-Chavez, Carlos, and Abdul-Aziz Yakubu. Preprinted. 2001.

[6] Crow, James F. and Motoo Kimura. *An Introduction to Population Genetics Theory*. New York: Harper & Row, 1970.

[7] Edelstein-Keshet, Leah. *Mathematical Models in Biology*. 1st ed. McGraw-Hill, Inc., 1988.

[8] *EPA and USDA Position Paper on Insect Resistance Management in Bt Crops*. EPA Office of Pesticide Programs, 12 July 1999. 19 July 2001. Internet Web Site. http://www.epa.gov/pesticides/biopesticides/otherdocs/bt_position_paper_618.htm.

[9] "European Corn Borer." *Ag Connection*: vol.4, no.6, June 1998. 22 July 2001. Internet Web Site. http://outreach.missouri.edu/agconnection/newsletters/is-98-06.htm.

[10] *European Corn Borer*, March, 2001. 22 July 2001. Internet Web Site. http://www.gov.mb.ca/agriculture/crops/insects/fad08s01/fad08s01.html.

[11] Freund, John E., and Gary A. Simon. *Statistics, A First Course*. 5th ed. New Jersey: Prentice-Hall, Inc. Back cover, 1991.

[12] Hastings, Alan. *Population Biology: Concepts and Models*. New York: Springer, 1997.

[13] Hellmich, R.L., W.D. Hutchison, and K.R. Ostlie: eds. *Bt Corn & European Corn Borer: Long Term Success Through Resistance Management*. Regents of the University of Minnesota, 1997. 18 July 2001. Internet Web Site. http://www.extension.umn.edu/distribution/cropsystems/DC7055.html.

[14] Schaafsma, Art and Mark Sears. *Responsible Deployment of Bt Corn Technology in Ontario*, 23 December 1998. 20 July 2001. Internet Web Site. http://www.agcare.org/farmers2.html.

[15] *Sets of Scientific Issues Being Considered by the Environmental Protection Agency Regarding: Bt Plant-Pesticides Risk and Benefit Assessments.* FIFRA Scientific Advisory Panel Meeting, SAP Report No. 2000-07, 12 March 2001. 25 July 2001. Internet Web Site. http://www.biotech-info.net/bt-transgenics.html#ECB.

[16] *Supplement to: Bt Corn & European Corn Borer: Long Term Success Through Resistance Management, NCR-602.* Regional Research Committee, NC 205, 1998. 19 July 2001. Internet Web Site. http://ent.agri.umn.edu/ecb/nc205doc.htm.

[17] Tuckwell, Henry C. *Elementary Applications of Probability Theory* 2nd ed. London: Chapman & Hall, 1995.

[18] Witkowski, John and Robert Wright. *The European Corn Borer:Biology and Management*, 11 July 1997. 22 July 2001. Internet Web Site. http://entomology.unl.edu/ecb/ecb1.htm.