Sensitivity Analysis of a Spatially-Explicit Stochastic Simulation Model of the Evolution of Resistance in *Helicoverpa zea* (Lepidoptera: Noctuidae) to Bt Transgenic Corn and Cotton

NICHOLAS P. STORER,[1] STEVEN L. PECK,[2] FRED GOULD, JOHN W. VAN DUYN, AND GEORGE G. KENNEDY

Department of Entomology, College of Agriculture and Life Sciences, North Carolina State University, Raleigh, NC 27695

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ABSTRACT The sensitivities of a model simulating the evolution of resistance in *Helicoverpa zea* to Bt toxins in transgenic crops were investigated by examining effects of each of the model parameters on the frequency of resistance alleles after 8 yr. The functional dominance of resistance alleles and the initial frequency of those alleles had a major impact on resistance evolution. The survival of susceptible insects on the transgenic crops and the population dynamics of the insect, driven by winter survival and reproductive rates, were also important. In addition, agricultural practices including the proportion of the acreage planted to corn, and the larval threshold for spraying cotton fields affected the R-allele frequency. Many of these important parameters are inherently variable or cannot be measured with accuracy, so model output cannot be interpreted as being a forecast. However, this analysis is useful in focusing empirical research on those aspects of the insects' life system that have the largest effects on resistance development, and indicates ways in which to improve products and agricultural practices to increase the expected time to resistance. The model can thus be used as a scientific basis for devising a robust resistance management strategy for Bt crops.

KEY WORDS *Bacillus thuringiensis*, transgenic crops, computer simulation, resistance management

IN RECENT YEARS, AGRICULTURE has seen the commercialization and widespread adoption of transgenic insecticidal crops based on the δ-endotoxins from the bacterium *Bacillus thuringiensis* (Berliner). These 'Bt' crops can be valuable pest management tools, improving the efficiency and safety of agricultural production. They can reduce the amount of chemical inputs required and improve agricultural sustainability. Preserving the efficacy of transgenic insecticidal crops has become a high priority of entomologists, the biotechnology industry, and regulatory authorities.

If this new technology is to be widely deployed and durable, measures are required that slow the evolution of resistant insect populations. Without the implementation of effective insect resistance management (IRM) practices, agricultural biotechnology is likely to follow the same pattern of control failures experienced with conventional insecticides, and new insecticidal genes will then be needed to replace the Bt toxins (Gould and Tabashnik 1998). The development costs for new transgenic traits are high, and farmers and consumers ultimately will bear the costs of this new "pesticide treadmill" (Knight and Norton 1989). New traits may not have such a well-targeted spectrum of activity, such a safe toxicity profile, or be as safe to the environment and the consumer as the Bt δ-endotoxins. If an insecticidal trait loses efficacy after just a few years, as predicted by some scientists (Mellon 1998), there may be no viable alternative trait ready to replace it. However, IRM policies that seek to preserve susceptible populations indefinitely would restrict the use of transgenic crops to such an extent that their benefits to the farmer, the environment, and the consumer would be lost. Resistance management strategies that allow flexibility in the tactics used, and that are likely to keep resistance alleles at low frequencies for 10–15 yr or longer constitute a rational compromise favored by regulatory authorities. As we become more experienced with resistance management we shall learn more about the system-specific factors involved in determining the time course of resistance evolution. As a first step, by understanding the roles of agronomic operations, pest biology, pest population dynamics, and genetics in resistance evolution, our ability to manage resistance is likely to improve.

The theoretical principles of the high dose + refuge strategy have been well-established (see Gould 1998).

[1] Current address: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268–1054 (E-mail: nstorer@dow.com).

[2] Department of Biomathematics, North Carolina State University, Raleigh, NC 27695.

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There are two fundamental foundations upon which the strategy is built: 1) the reliable delivery of a toxin dose high enough to reduce the fitness of insects heterozygous for a resistance allele to near zero making resistance functionally recessive; 2) the assurance of a high degree of random mating between insects from the refuge and those from the transgenic crop.

Most currently available Bt cotton (*Gossypium hirsutum* L.) and corn (*Zea mays* L.) cultivars are thought to achieve high doses for their respective target pests *Ostrinia nubilalis* (Hübner) and *Heliothis virescens* (F.). However, it has been repeatedly shown that for *Helicoverpa zea* (Boddie), currently available Bt corn and Bt cotton varieties do not present a high dose (Mahaffey et al. 1995, Lambert et al. 1997, Pilcher et al. 1997, Storer et al. 2001). Because ≈10–25% of *H. zea* larvae bearing no resistance alleles can survive on Bt corn and cotton, larvae bearing one resistance allele are expected to have an even higher rate of survival. That is, resistance will likely not be recessive. Tang et al. (1997) showed that the Bt dose that kills 80% of susceptible *Plutella xylostella* (L.) (diamondback moth) kills only 50% of heterozygous larvae (a functional dominance value of 0.62), while the dose that kills 95% of susceptible larvae kills only 70% of heterozygous larvae (functional dominance = 0.31). Similarly, two experiments reported by Gould et al. (1995) found that Bt concentrations that killed 75% of a susceptible strain of *H. virescens* caused 25% and 50% mortality of larvae heterozygous for resistance genes. These mortality figures translate to functional dominance of 0.67 and 0.33, respectively. Thus, for Bt corn and Bt cotton with *H. zea*, the requirements of the high dose + refuge strategy are not met. Though selection pressure for resistance is weaker because of the lower toxicity, response to selection is expected to be greater because of the higher survival of heterozygotes relative to survival of homozygous susceptible insects.

Resistance evolution is an ecological phenomenon and must be studied in an ecological context. The rate of insecticide resistance evolution depends on genetic, biological and operational factors (Georghiou and Taylor 1977a, b; Tabashnik and Croft 1982; Tabashnik 1994; McGaughey and Whalon 1992). The refuge requirements are very dependent on the pest behavior (Onstad and Gould 1998) and biology, and on agricultural practices (Alstad and Andow 1995, Peck et al. 1999). Because insect resistance management through the deployment of refugia requires movement of individuals between areas of transgenic crops and untransformed hosts, the spatial distribution of Bt and non-Bt host plants is important for the success of an IRM plan. The amount of movement also affects the rate at which local populations evolve resistance and the rate at which resistant populations spread through a region (Mallet and Porter 1992). Additionally, population subdivision can create locally high initial frequencies of resistance alleles through random genetic drift. Selection may act more rapidly on such a sub-population and create local resistance (Caprio and Tabashnik 1992). Peck et al. (1999) showed how the spatial dynamics in a complex environment could affect the rate of local and regional resistance evolution. Consequently, IRM plans need to consider the specifics of the host, insect, and even geography.

Computer simulations can provide a test of an entire regional IRM plan, taking into account all the factors involved in resistance evolution, (Tabashnik 1986, Kennedy et al. 1987). They can integrate all that is known about the genetics, biology, population dynamics, and agronomy relevant to resistance evolution. They can be used to compare the validity of different IRM options, and suggest improvements to those plans. They can provide strong indications of the sustainability of any particular plan over extended periods. Finally, they can demonstrate where our knowledge of the system is weakest, and what empirical data are required to improve our ability to manage resistance.

Because resistance alleles, as genetic mutations of wild-type alleles, are likely to be initially rare, stochastic processes, such as genetic drift, local extinctions, and deviations from Hardy-Weinberg genetics, are likely to play significant roles in the evolution of resistant populations. Modeling of these stochastic processes requires that numbers of insects of each genotype in each field should be integers, and events should be probabilistic rather than deterministic.

In our companion paper (Storer et al. 2003), we describe a computer model (modified from that of Peck et al. [1999]) that integrates agronomy, the biology of *H. zea*, insect resistance genetics, and the effects of currently available Bt corn and cotton varieties on the insect. The model simulates resistance evolution over a wide area of mixed crops and weed hosts that occur in eastern North Carolina, and describes the role of spatial processes in the spatial and temporal pattern of resistance evolution. Here, each of the model parameters is varied to investigate its role in resistance evolution. Through this process, we gain a greater understanding of which factors in resistance evolution are likely to be most important in the field. Through examining intermediate model output, we also gain insight into how each of the factors acts to affect resistance evolution.

The output from such a model cannot be regarded as predictive because many parameters have values that are unmeasured in the real world, that are not measurable, or that are inherently variable. Sensitivity analyses are used to identify those parameters whose values have the largest effects on the model output. The results of these analyses can be used to guide further empirical research to measure the true values and are needed to correctly interpret the model output when formulating resistance management strategies that will be robust in the real world. This paper addresses the model sensitivities to several key parameters.

Materials and Methods

The model design is described in a companion paper (Storer et al. 2003). Default values for the parameters discussed here are given in Table 1. The sensitivity of the model to the parameter values shown in Table 1 was tested. For these runs, two levels of Bt corn (*BtCR* = 25% and 75%) and two levels of Bt cotton deployment (*BtCT* = 25% and 75%) were used. Crops were assigned randomly to fields each year. All other parameters, except for the one under investigation, were held constant at their default values. The model was run using the range of values for each of the parameters shown in Table 1 for each Bt corn/Bt cotton combination.

A variety of criteria were used in setting the ranges tested, and the relative ranking of parameters by importance will depend on the range tested. Uncertainty in field measures of parameter values comes from two sources—natural variation and inexact measurement. The relative importance of these sources differs among parameters in the model. In each case, the range tested was designed to capture that which seemed to the authors plausible for eastern North Carolina over the next 10 yr. While this approach relies on subjectivity and is not ideal for analyzing the full behavior of the model, it enables us to identify to which parameters the model is most sensitive, and is useful for setting field research goals to define better the values of the most important parameters. The model was run to simulate eight years (after a 3-yr null run), sufficient to determine the effects of the parameters. Three repetitions at each model setting were run. Analysis of variance (ANOVA) for the log-transformed R-allele frequency after 8 yr of Bt crop deployment (q_8) was conducted as a completely randomized 3-way factorial design with *BtCR* and *BtCT* as the other two factors.

Environmental and Operational Factors

T—Length of Season. This parameter is related to the timing of the start of *H. zea* adult eclosion in the spring, and on the timing of crop destruction in the fall. The default value of 123 d allows completion of three full generations and partial completion of a fourth. Most insects have the opportunity to enter diapause. A range of three weeks was tested in this analysis, which affects the proportion of the fourth generation that can be completed before winter or crop termination. This range covers the period during which the final 20% of cotton bolls open in North Carolina, and the period during which defoliants may be applied, and therefore captures what is biologically plausible.

WS—Overwinter Survival. In the field, winter survival of diapaused pupae is highly variable with survival values of <1% and >30% reported under different conditions (Neunzig 1969). Winter temperatures and rainfall, soil disturbance (through tillage), and predation in the soil all contribute to mortality of diapaused pupae (Neunzig 1969, Roach 1981, Kring et al. 1993). Within a region, these factors vary from field

Table 1. Parameters in the model, their default values, and the range tested in the sensitivity analysis

Parameter name	Description	Default Value	Justification	Range tested
T	Number of days per pest season	123 d	no data	116–137
WS	Survival of overwintering diapaused pupae	3.4%	Caron et al. (1978)	1.7%–6.8%
μ_{DP}	Mean date of diapause induction	Day 86 (≈August 27)	Stinner et al. (1977)	Day 78–Day 94
Pcorn	Proportion of fields in region planted to corn	55%	NCDA&CS (1999)	35%–75%
K_{egg}	Mean egg threshold in one acre of non-Bt cotton (per acre)	60,000	Bacheler (1996), Farrar and Bradley (1995)	40,000–80,000
K_{1ar}	Mean larval threshold in one acre of cotton (per acre)	6,500	Bacheler (1996)	5,000–8,000
wp	Percentage of insect population using weed hosts in spring	10%	Neunzig (1963)	5%–40%
S_{ew}	Percentage of SS larvae surviving the Bt in whorl-stage Bt corn	0%	Storer (1999)	0%–1%
S_{er}	Percentage of SS larvae surviving the Bt in ear-stage Bt corn	25%	Storer et al. (2001), Pilcher et al. (1997)	10%–35%
S_{ct}	Percentage of SS larvae surviving the Bt in Bt cotton	25%	Lambert et al. (1996), Mahaffey et al. (1995)	5%–35%
R_1	Replacement rate for first generation	1.5	Storer (1999); J. S. Bacheler, personal communication	1–4
R_2	Replacement rate for second generation	75	Storer (1999); J. S. Bacheler, personal communication	55–100
R_3	Replacement rate for third and subsequent generations	10	Storer (1999); J. S. Bacheler, personal communication	8–12
EM	Probability of emigrating from a suitable field	0.1	No data	0.05–0.40
ϕ_d^2	Variance for flight distance kernel	4.3 fields	No data	2–8 fields
q_0	Initial region-wide frequency of resistance alleles	10^{-4}	Burd et al. (2001)	10^{-6}–10^{-2}
h	functional dominance of resistance allele	0.5	Gould et al. (1995), Burd et al. (2000, 2001)	0.1–0.8

to field and from year to year. The default value used, 3.4%, was that measured by Caron et al. (1978) in eastern North Carolina. The range analyzed was from half the default to double the default, to capture a wide range that allows the population dynamics to function as observed in eastern North Carolina. The published literature does not suggest that higher survival rates are commonplace, and the model is limited to using the values that are plausible for all years over a wide area. More extreme values for *WS* were investigated (up to 20%) but these caused the population to rise far higher than is observed in North Carolina. Output from the model run with implausible population dynamics is not meaningful, and so is not reported here. The winter survival of diapaused pupae is applied equally to insects of all genotypes each year in the model.

μ_{DP}—*Mean Day of Diapause Induction.* Because diapause induction in *H. zea* is largely a function of photoperiod during larval development (Rabb et al. 1975), the mean date pupae enter diapause occurs at the same calendar date each year. The number of days from initial spring emergence (model day 0) to the mean diapause date (model day μ_{DP}) is therefore, determined by the date of initial spring emergence. An earlier start is reflected in a later mean diapause model day. The timing of spring emergence in turn depends primarily on soil temperatures in early spring (Logan et al. 1979), which are inherently variable. The default value (day 86, ≈27 August) was taken from the data presented by Stinner et al. (1977), and a range of 16 d was tested to reflect the typical range in timing of peak trap catches for the region (J. S. Bacheler, personal communication).

Pcorn—Proportion of a Region that is Planted to Corn Rather Than Cotton. This is not constant across years or across regions. Kennedy and Storer (2000) showed there have been large changes in the proportion of agricultural land planted to various crops in short time scales, depending on the economics of growing each crop from year to year. Cotton production in eastern North Carolina has greatly increased after boll weevil eradication (Carlson et al. 1989), mostly at the expense of corn. The introduction of transgenic crops may again drastically alter the relative economics of growing different crops and thus alter cropping patterns. Furthermore, there are regional differences in relative acreage of corn and cotton—in Georgia, for example, 35% of corn + cotton acres are corn, whereas in southeastern Virginia, 65% are corn (USDA National Agricultural Statistics Service, 2000). On smaller geographic scales, the distribution of corn and cotton fields is not even, depending on local conditions and history, although the area modeled is a part of a larger identical region (insects that leave one side are replaced by identical insects entering the opposite side). The default value of 55% is the proportion for North Carolina in 1998 (NCDA&CS 1999), while the range tested captured the variation from Virginia to Georgia, as well as what may be plausible in parts of North Carolina based on previous years.

K_{egg} *and* K_{lar}—*Egg and Larval Thresholds for Spraying Cotton Fields.* The default values used follow the recommendation by North Carolina Cooperative Extension (Bacheler 1996). However, scouting efficiency and accuracy, farmer response time, and local variations can change the actual level of eggs present at the time of spraying. As farmers and extension entomologists have become more experienced with Bt crops, larval spray thresholds have likewise changed. Furthermore, other cotton pests such as *Lygus lineolaris* (Palisot de Beauvois) (tarnished plant bug) and a complex of stink bugs are becoming key pests in Bt cotton (Hardee and Bryan 1997). Sprays to control these will affect the relative production of *H. zea* from Bt and non-Bt cotton fields. To reflect this uncertainty, a range was tested from 33% below to 33% above the default for egg threshold, and 25% below to 25% above the default for the larval threshold. These are based on opinions of extension personnel working in the field, and are sufficiently broad to understand the effects of the parameter on the model output.

wp—Proportion of H. zea Larvae in Early Season Weeds. This is hard to estimate in the field and certainly varies among regions (Neunzig 1963, Sudbrink and Grant 1995). There are no available data on the production of adults from these hosts. Accordingly, a wide range of values, from 5% to 40% of the population, was deemed plausible for testing.

Transgenic Crop Parameters

S_{cw}—*Survival of Susceptibles on Whorl-stage Bt Corn.* The concentration of Bt toxin in whorl-stage corn causes very large mortality of susceptible larvae (Pilcher et al. 1997; Storer 1999; J.W.V.D., unpublished data). The plausible survival range tested was from 0 to 1%. Bt doses that cause such low survival of SS larvae are likely to also make the survival of RS larvae very low (this is the basis of the high-dose approach to resistance management), and thus likely to make the resistance allele recessive. To reflect this, survival of RS larvae was set at $2 \times S_{cw}$. Within the range of values tested, this translates to a functional dominance of between 0.0 and 0.0101.

S_{cr}—*Survival of Susceptibles on Ear-stage Bt Corn.* Storer et al. (2001) showed that larval survival in the field on Bt corn relative to non-Bt corn averaged around 25% but varied between 10% and 35%. Survival depends on the Bt hybrids involved and on local environmental conditions, and it varies among *H. zea* populations (Stone and Sims 1993, Luttrell et al. 1999). The range tested reflects that measured in Storer et al. (2001).

S_{ct}—*Survival of Susceptibles on Bt Cotton.* The percent survival of *H. zea* larvae to adult on commercial Bt cotton has not been formally reported. However, indications are that larval mortality is similar to, and as variable as, that on Bt corn varieties (Mahaffey et al. 1995, Lambert et al. 1996, Jackson et al. 2001). The range of values tested reflects the likely variability for this parameter.

For all crops, survival values for RS and RR genotypes on Bt and non-Bt crops were calculated based on the SS survival values, functional dominance of the R-allele (which is very low on whorl-stage Bt corn), and fitness costs as described in a companion paper (unpublished data).

Insect Biological Parameters

R_1—Replacement Rate for First Generation. Light trap data generally support the assumption that the first spring generation in eastern North Carolina is a replacement generation, producing approximately the same number of adults as emerged from diapause (J. S. Bacheler, personal communication). However, there are no accurate measures of this variable published. We tested a wide range of values to reflect this uncertainty (Table 1). However, this parameter is a major driver of the population dynamics. Results only from those parameter values that allowed the population density to remain within the range that is usual for eastern North Carolina were deemed plausible and are presented.

R_2 – Replacement Rate for Second Generation. The observed population dynamics of *H. zea* in eastern North Carolina require a high, but variable, replacement rate for the second generation emerging for ear-stage corn (J. S. Bacheler, personal communication). Again, we tested a wide range of values to reflect this variability (Table 1). Results only from those parameter values that allowed the population density to remain within the range that is usual for eastern North Carolina were deemed plausible and are presented.

R_3—Replacement Rate for Third Generation. Again, the values tested for the replacement rate in the cotton generations reflect the observed population dynamics from light trap data rather than any direct field measurements (J. S. Bacheler, personal communication). Again, results only from those parameter values that allowed the population density to remain within the range that is usual for eastern North Carolina are presented.

EM and Φ_d^2—Probability of an Insect Leaving a Suitable Field and Variance in the Adult Dispersal Kernel. Movement of *H. zea* moths in North Carolina has not been tracked. Indications from studies elsewhere are that moths are capable of moving over vast distances on weather fronts (Pair et al. 1995), but that normal dispersal is local if there are suitable hosts (Culin 1994). Because *H. zea* is a polyphagous pest that moves among different crops according to their relative phenology, a high level of population mixing is guaranteed. As these two parameters together determine how the adults disperse through the model region, the effect of varying both together was also examined to obtain more extremes in movement than by varying them individually. Because the amount of movement is likely to be very variable, the probability of dispersing was varied from 50% of the default to four times the default, while the mean dispersal distance was varied from one half to twice the default. The ranges tested seem plausible and are broad enough to capture the effect of the parameter values on model output.

Genetic factors

q_0—Initial Frequency of Resistance Alleles. There is currently one estimate of the R-allele frequency in a *H. zea* population in eastern North Carolina of 0.00043 (Burd et al. 2001). As this is a single estimate, with wide confidence limits, values tested cover a wide range that seems plausible.

h—Functional Dominance of Resistance Alleles. Because different Bt varieties in different environmental conditions have different effects on *H. zea* larvae, it is likely that dominance is system-specific. Finally, there may be several different genes with resistance alleles, or several different R-alleles for one gene. Each may have a different dominance. Indications from Burd et al. (2000, 2001) are that laboratory-selected resistance alleles can be dominant or incompletely dominant. The values tested covered a wide range from incompletely dominant to incompletely recessive. Based on these data for *H. zea*, and on published dose–response data for Bt-resistance *H. virescens* (Gould et al. 1995), this range seems plausible.

The above sensitivity analyses were performed by altering one parameter at a time. This method does not permit examination of interactions among parameters. An exhaustive test of all interactions is not possible (to test all the two-way interactions among the 16 parameters would take ≈2.5 yr of computer time running the model on the computer system used for this paper), but here we describe the results of two key tests for parameter interactions.

q_0 X h Interaction. The two genetic factors have powerful effects on resistance evolution, representing both the starting point and the heterozygote advantage. Runs were made to investigate any interaction between these by running the model at each pair of parameter values for each level of Bt corn and Bt cotton deployment. The output was analyzed as a four-way factorial design.

S_{cr} and S_{ct} X h Interaction. If the survival of larvae on Bt crops is reduced, it is expected that the functional dominance would also change. The two experiments presented in Gould et al. (1995) on the survival of susceptible and heterozygous resistant *H. virescens* larvae on Bt diet, indicated that at SS survival of 0.1, functional dominance would be reduced from 0.67 to 0.48 in the first experiment and from 0.33 to 0.31 in the second experiment. It is important to understand how changes to these parameters can affect resistance evolution. To simplify the interpretation of the results of these runs, parameters for SS survival on both ear-stage Bt corn and Bt cotton were set to the same value, which ranged from 0.1 to 0.3. R-allele dominance ranged from 0.3 to 0.7, a range that fits the *H. virescens* resistance data (Gould et al. 1995). Again, a four-way factorial design was used.

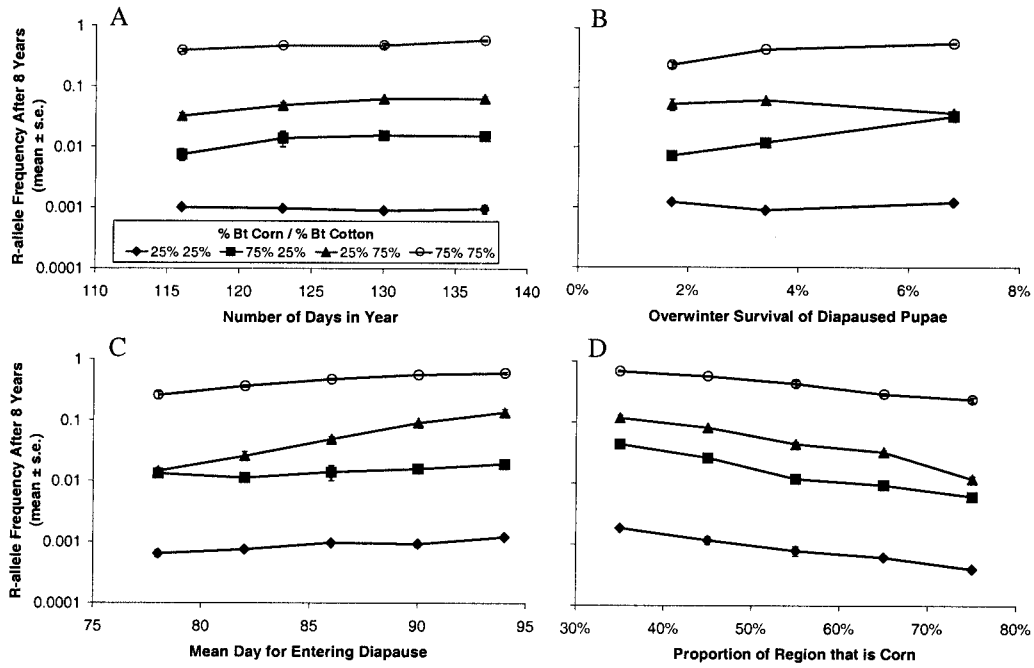

Fig. 1. Effect on R-allele frequency, after 8 yr of deployment of two levels of Bt corn and two levels of Bt cotton, of (A) season length, *T*; (B) overwinter survival of diapaused pupae, *WS*; (C) mean date for entering diapause, μ_{DP}; and (D) the proportion of corn + cotton acreage planted to corn, *Pcorn*. Values for all other parameters are the default values shown in Table 1. Each point represents the average of three runs.

Results and Discussion

Environmental and Operational Factors

T. Longer seasons increased the rate of resistance evolution (Fig. 1A). The effect was significant but very small, and depended on the levels of Bt corn and Bt cotton deployment (significant 3-way interaction in ANOVA shown in Table 2). Susceptible phenotypes surviving on Bt crops were delayed in their development compared with resistant phenotypes. More of the susceptible insects than the resistant ones were induced to diapause after three generations and did not attempt a fourth because of the developmental delay on Bt crops; most of the fourth generation died as the season ended before they completed development. Consequently, shorter seasons caused higher mortality of resistant larvae than of susceptible larvae and slowed resistance evolution. Conversely, longer seasons allowed higher survival of fourth generation larvae, whose R-allele frequency was slightly higher than the population average, and hastened resistance evolution. Because the timing of diapause induction is independent of the season length, very few susceptible insects on Bt crops attempted a fourth generation because of their developmental delay. Therefore, their survival was not affected by season length, and there was no decline in the rate of resistance evolution at longest season length.

The biological significance of this effect is probably very small, not much altering the rate at which the pest population adapts to Bt crops. The parent model (Peck et al. 1999) was far more sensitive to this parameter, because in that version only insects that were pupae at the end of the season entered diapause. Hence small changes in the timing of winter when coupled with developmental delays on Bt crops, had considerable effects on differential survival of insect genotypes. Diapause, as modeled in the current paper, more accurately reflects the biology of *H. zea* in North Carolina and greatly dampens the effect of season length because all insect genotypes have a high probability of entering diapause after three generations, long before the season ends.

WS. Winter survival affected resistance evolution (Fig. 1B) and ANOVA (Table 2) indicated that in doing so it interacted with both *BtCR* and *BtCT.* At high Bt corn deployment, higher winter survival hastened resistance; at low Bt corn deployment, winter survival had little effect. Higher winter survival increased the population size the following spring. As the first two generations were not controlled by insecticides, populations in corn ears were higher, which caused cannibalism to be more intense. For example, after 3 yr with $BtCR = 0.75$ and $BtCT = 0.25$, at $WS = 6.8$, the mean neonate density in corn was 1.099 per ear (SD = 0.112), whereas at $WS = 1.7$, the mean neonate density was 0.244 (SD = 0.014). As shown in Appendix A of a companion paper (Storer et al. 2003) higher neonate densities led to a larger survival differential between resistant and susceptible phenotypes. Thus where Bt corn deployment was high, and winter survival was high, resistance evolved

Table 2. ANOVA for the R-allele frequency (log transformed) after 8 yr of Bt deployment for parameter sensitivity tests with significant effects. Type III *F*-values followed by an asterisk are significant at the 1% level. See Table 1 for interpretation of parameters and for the ranges tested

Parameter Name	Parameter	*BtCT*	Parameter **BtCT*	*BtCR*	Parameter **BtCR*	*BtCT* BtCR*	3-way interaction
T							
df	3, 32	1, 32	3, 32	1, 32	3, 32	1, 32	3, 32
F	12.3*	4754.3*	0.63	1897.8*	1.06	6.65	7.1*
WS							
df	2, 24	1, 24	2, 24	1, 24	2, 24	1, 24	2, 24
F	25.5*	3736.4*	15.6*	1579.4*	43.9*	16.0*	1.33
μ_{DP}							
df	4, 40	1, 40	4, 40	1, 40	4, 40	1, 40	4, 40
F	57.9*	5714.1*	16.2*	2642.3*	8.3*	32.7*	6.6*
Pcorn							
df	4, 40	1, 40	4, 40	1, 40	4, 40	1, 40	4, 40
F	183.5*	6654.1*	2.12	3300.5*	2.7	53.77*	11.51*
K_{lar}							
df	4, 40	1, 40	4, 40	1, 40	4, 40	1, 40	4, 40
F	10.7*	2069.9*	1.42	1052.6*	1.69	46.5*	0.47
S_{cr}							
df	4, 40	1, 40	4, 40	1, 40	4, 40	1, 40	4, 40
F	43.0*	6666.5*	0.72	3554.5*	10.7*	62.1*	2.49
S_{ct}							
df	3, 32	1, 32	3, 32	1, 32	3, 32	1, 32	3, 32
F	896.5*	5078.1*	61.2*	1859.8*	61.7*	114.4*	18.5*
R_1							
df	3, 32	1, 32	3, 32	1, 32	3, 32	1, 32	3, 32
F	35.3*	4620.3*	17.6*	2212.4*	18.2*	14.0*	0.57
h							
df	6, 56	1, 56	6, 56	1, 56	6, 56	1, 56	6, 56
F	1647.4*	5668.9*	89.8*	2428.5*	73.7*	109.2*	32.9*
q_0[a]							
df	4, 40	1, 40	4, 40	1, 40	4, 40	1, 40	4, 40
F	11.2*	79.8*	3.6	49.3*	4.1*	2.53	6.79*

[a] Dependent variable tested is $\ln(q_8/q_0)$, where q_8 = R-allele frequency after 8 yr, and q_0 = initial R-allele frequency.

more rapidly. The effect was somewhat dampened if Bt cotton deployment was high, because selection in Bt cotton played a larger role in resistance evolution. The effect of this parameter was generally small and not likely to have a biological significance. However, it is apparent that a series of mild, dry winters, or widespread adoption of conservation tillage practices that reduce pupal mortality, could speed resistance evolution.

Additional model runs were made where the winter survival each year was a random number drawn from a normal distribution with a mean of 1.7% and a standard deviation of 3.1%. Values of <0.5% were rejected and redrawn. This produced values for winter survival with a mean of 3.5% (close to the default value) and standard deviation of 2.1%, a minimum of 0.5%, and an effective maximum of 15.3%, approximately the range reported by Caron et al. (1978). These runs showed the same mean rate of resistance evolution, though with a larger among-run variance.

μ_{DP}. The effect of varying the average time step at which pupae enter diapause on resistance evolution is shown in Fig. 1C. The effect of this parameter on the R-allele frequency after 8 yr depended on the levels of Bt corn and Bt cotton deployment (i.e., a significant 3-way interaction in Table 2).

The largest effect was when Bt cotton deployment was high and Bt corn deployment was low. In this situation, the diapause date would probably have a very significant biological effect, changing the R-allele frequency by an order of magnitude after 8 yr within the parameter range tested. When the mean diapause date was later, a higher proportion of third generation insects did not enter diapause but began a fourth generation. Most of this generation failed to complete development because of crops becoming unsuitable as hosts. This was especially true for SS larvae from Bt cotton, whose development is delayed. The effect was somewhat compensated for by a smaller overall population the following year (resulting from the low ability of the fourth generation to complete development to pupation in time for the onset of winter) decreasing cannibalism and decreasing selection for resistance in Bt corn ears. At high Bt corn deployment, decreased cannibalism compensated better for the increased loss of SS genotypes from the diapaused pupal population, and thus the effect of varying the mean diapause date was much smaller than at low Bt corn deployment.

Pcorn. Increasing the proportion of the region that was planted to corn rather than cotton decreased the rate of evolution (Fig. 1D). The decrease was dependent on both the proportion of corn planted to Bt and the proportion of cotton planted to Bt (3-way interaction significant in Table 2). Selection for resistance was greater in the cotton generations than in the corn generations, because of spraying of refuge cotton. Thus, if the amount of cotton increased, the amount of

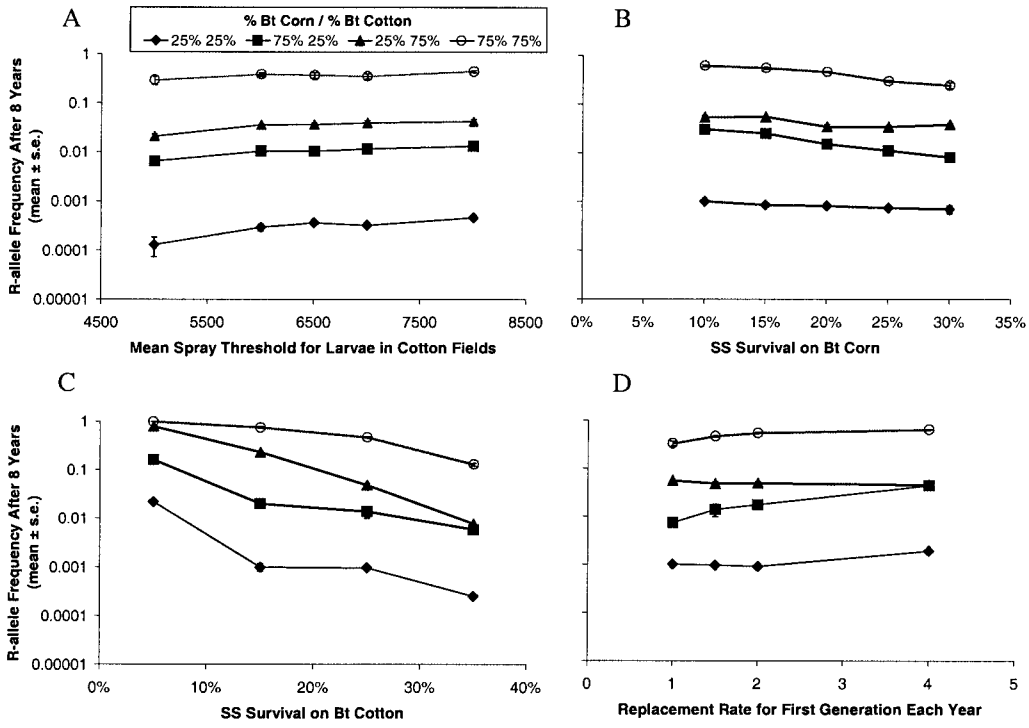

Fig. 2. Effect on R-allele frequency after 8 yr of deployment of two levels of Bt corn and two levels of Bt cotton, of (A) larval spray threshold in cotton fields K_{lar}; (B) susceptible survival on Bt corn ears, S_{cr}; (C) susceptible survival on Bt cotton, S_{ct}; and (D) first-generation replacement rate, R_1. Values for all other parameters are the default values shown in Table 1. Each point represents the average of three runs.

selection for Bt resistance increased. Additionally, as the amount of corn decreased, the density of the population in corn ears increased. For example, after 3 yr with *BtCR* = 0.75 and *BtCT* = 0.25, at *Pcorn* = 0.35 mean neonate density was 1.377 per ear (SD = 0.04), whereas at *Pcorn* = 0.65 mean neonate density was 0.304 per ear (SD = 0.02). Thus, selection resulting from the cannibalism in Bt corn ears increased, and resistance evolved faster. It should be noted that in the scenario where Bt corn was planted on 75% of corn acres and Bt cotton was planted on only 25% of cotton acres, the effects of the increased cotton acreage and the increased cannibalism in corn, combined, were strong enough to outweigh reductions in total Bt acreage caused by replacing corn with cotton (see the 75%/25% line in Fig. 1D). Under this Bt crop deployment scenario, if 65% of all fields across the region are planted to corn, then 57.5% (75% of 65% + 25% of 35%) of all fields are planted to Bt crops, whereas if 35% of all fields are planted to corn, only 42.5% (75% of 35% + 25% of 65%) of fields are planted to Bt crops.

The magnitude of these effects is biologically significant, affecting the R-allele after 8 yr by a factor of five. These results indicate that the rate of evolution will vary across regions even when using the same refuge-based IRM strategy. There may be a case for local adjustments to be made to the plans to reflect this.

K_{egg} and K_{lar}. Given that threshold spraying of refuge cotton fields reduces the effective refuge size, it might be expected that the mean threshold used would affect the effective refuge size and the evolution of resistance. Varying the egg threshold alone did not have this effect ($F = 0.69$; df = 4, 40). If the egg threshold was set high, fewer cotton fields were sprayed early on, but the larval threshold, which remained at the default value in these runs, was exceeded more quickly. Overall spray mortality in both Bt and non-Bt cotton fields remained the same. However, changing the larval spray threshold in cotton fields did affect resistance evolution (Fig. 2A). The main effect of this parameter was significant (Table 2): increasing the spray threshold increased the evolution of resistance. Because the spray threshold applied to both Bt and non-Bt cotton, a higher threshold increased the population in both, but the net effect was to increase the ratio of production from the Bt fields to production from non-Bt fields. For example, after three years with *BtCR* = 0.25 and *BtCT* = 0.25, at K_{lar} = 5000, the mean ratio was 0.372 (SD = 0.008), whereas at K_{lar} = 8000 the mean ratio was 0.452 (SD = 0.013). Thus, disproportionately more resistance alleles survived at higher threshold. Accompanying this, a higher overall population in ear-stage corn the following season caused greater cannibalism intensity. This effect was the same at all Bt deployment levels

(no significant interaction with *BtCR* or *BtCT*). However, the magnitude of the effect would likely have little biological significance, except at the lowest Bt deployment level (Fig. 2A).

wp. The proportion of first generation *H. zea* larvae living on weed hosts, as opposed to corn or cotton, had no significant effect on resistance evolution ($F = 2.89$; df = 3, 32). Weed hosts act as alternative refugia for first generation larvae, and thus reduce selection for resistance. However, the expression of Bt in whorl-stage corn plants was regarded in this model as being high enough to make the R-allele very recessive, so evolution during this generation was slow compared with the lower doses in subsequent generation.

Thus, the model indicates that the number of weed hosts, in the range tested, is not very important in determining resistance evolution to currently available Bt crops. The ephemeral presence of weeds should in fact promote insect movement and hence population mixing, but as Gould (1998) noted, random mating is less important for slowing evolution when the toxin is not at a high dose.

Transgenic Crop Parameters

S_{cw}. Varying the actual mortality of SS larvae on whorl-stage corn in the model had no effect on resistance evolution ($F = 0.37$; df = 3, 32). As indicated in the discussion of the effect of early season weed hosts, the rate of evolution was determined primarily by events occurring in subsequent generations, where the effective Bt dose was much lower and the functional dominance was much higher.

S_{cr}. Lower survival of SS on Bt corn (higher Bt dose) created more intense selection for RS and RR individuals, and hence hastened resistance (Fig. 2B). The Bt corn fields produced fewer susceptible insects to dilute those carrying R alleles. The strength of this effect was higher at high Bt corn deployment (significant $S_{cr} \times BtCR$ interaction in Table 2) because there was a greater proportion of the population under selection pressure. At these levels, the effect is likely to be biologically significant, causing a fourfold effect on the 8-yr R-allele frequency.

In the range of values tested, there was always survival of SS and RS genotypes on Bt corn, with additive inheritance of the resistance trait (functional dominance = 0.5). Only by increasing the Bt dose in corn to levels sufficiently high to make resistance functionally recessive, would such a corn variety be expected to improve resistance management.

S_{ct}. As with survival on Bt corn, lower survival of susceptibles on Bt cotton created more intense selection for RS and RR individuals, and hence hastened resistance (Fig. 2C). The strength of this effect was generally greater than for survival on Bt corn, and depended on the amount of Bt cotton deployed (significant S_{ct} x *BtCT* interaction in Table 2). At high Bt cotton, the effect was largest because in this case, most selection for resistance occurred in Bt cotton fields. The effect also depended on the level of Bt corn deployment (significant S_{cr} x *BtCR* interaction). The effect of decreasing Bt cotton survival was slightly lessened at high Bt corn deployment for two reasons. First, the relative role of Bt corn in determining the rate of resistance was greater if its deployment was higher. Second, insect populations were lower and cannibalism in Bt corn was less intense if Bt cotton survival was lower. Thus, selection for resistance in the second generation was reduced.

This parameter is extremely significant in its effect on resistance evolution, affecting the 8-yr R-allele frequency by as much as 100-fold. At the default settings for other parameters, this translates to a 20 or more year difference in time to resistance development with 75% Bt cotton and 25% Bt corn. However, as with Bt corn, only by increasing the Bt dose in cotton to levels sufficiently high that resistance is likely to be functionally recessive, would the risk of resistance evolution be reduced.

Insect Biological Parameters

R_1. The effect of varying the first generation replacement rate on resistance evolution depended on the levels of Bt cotton and Bt corn (Fig. 2D, Table 2). Only where Bt corn deployment was high, and especially where, in addition, Bt cotton deployment was low, was the effect biologically significant—a higher replacement rate caused faster evolution. This was a result of higher populations moving into ear-stage corn, where cannibalism on Bt ears enhanced the advantage of resistant larvae. If Bt cotton deployment was high, and Bt corn deployment was low, selection in cotton outweighed the altered level of selection in Bt corn: cannibalism in corn ears, being density-dependent, dampened the effect of early season population size on the population size in cotton.

R_2. As with R_1, R_2 directly affected the density of larvae in corn ears. Though the same trends occurred from varying the second generation replacement rate as for the first, these were not significant within the range of values for R_2 tested ($F = 0.48$; df = 2, 24). To maintain the population dynamics in the model within realistic bounds, the range in values tested for R_2 was proportionately far smaller (from 55 to 95) than the range tested for R_1 (from 1 to 4), so the effects on larval density were less extreme.

R_3. The replacement rate for the cotton generations also had no significant effect on the evolution of resistance ($F = 0.56$; df = 2, 24). Within the range of values tested, threshold spraying of cotton fields compensated for changes in population density. However, in the field, alterations to the natural enemy complex in Bt cotton may result from altered spraying patterns and different insecticides deployed against pests previously controlled collaterally with *H. zea* and *H. virescens.* These could change the replacement rate to values outside of the range tested and alter the population dynamics. If this were the case, further model runs would be needed to understand the consequences.

EM and Φ_d^2. Increasing the probability that an adult flies out of a field that is attractive (*EM,* moth "rest-

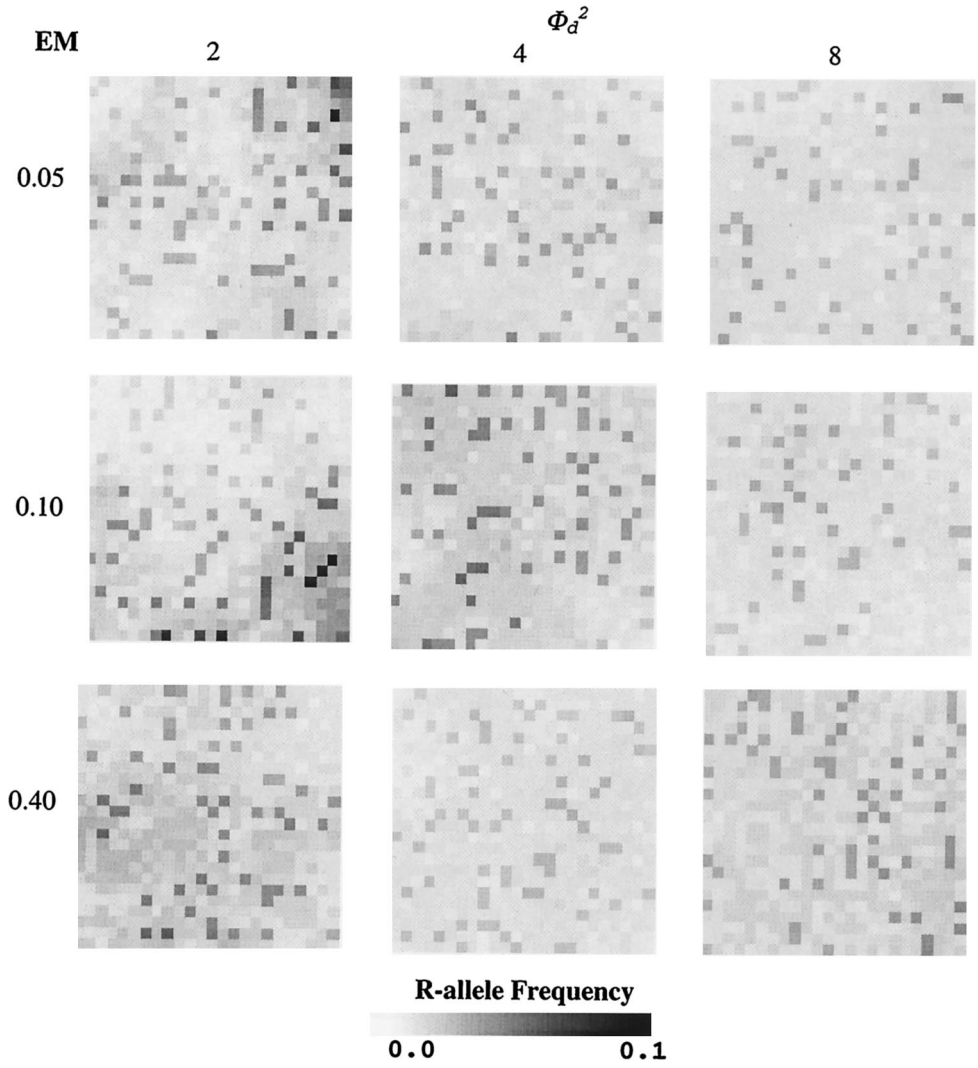

Fig. 3. Distribution of R-allele frequencies at end of 8-yr using different values for the insect movement parameters *EM* and Φ_d^2. The region-wide R-allele frequency is ≈0.01 in each region. Darker squares represent higher R-allele frequency according to the gray scale. The region is 24 × 24 fields. Seventy-five percent of corn is Bt, 25% of cotton is Bt. All other parameters are set at default values shown in Table 1.

lessness") and changing the distance over which the insects can fly (Φ_d^2) had no effect on the rate of region-wide resistance evolution (F = 1.06 and 0.71, respectively; df = 3, 32). R- and S-alleles mixed frequently each generation under all parameter settings because of high degrees of local movement among crop types caused by changing crop phenology. This movement masked the relatively minor effects of the background movement on the degree of allele mixing. Furthermore, there was no significant interaction between these two factors when varied together (F = 1.23; df = 9, 128), indicating that even when they were set to maximize or minimize movement, there was little effect on the increase in the overall R-allele frequency.

However, the two movement parameters did affect regional movement, evidenced by the variability in R-allele frequency across the region. Where movement was lowest, the variability among fields was highest because there was least intermingling of local subpopulations. This is illustrated in Fig. 3 which shows the R-allele frequency in each field after 8 yr of Bt deployment (75% of corn as Bt, 25% of cotton as Bt) at three levels of *EM* and three levels of Φ_d^2. The figure in the top left shows the results with the lowest movement settings, that in the bottom right has the most movement. The adjusted coefficient of variation, as given by equation 2 in a companion paper (Storer et al. 2003), is a measure of this variability. At low levels of movement, there was more variation across the

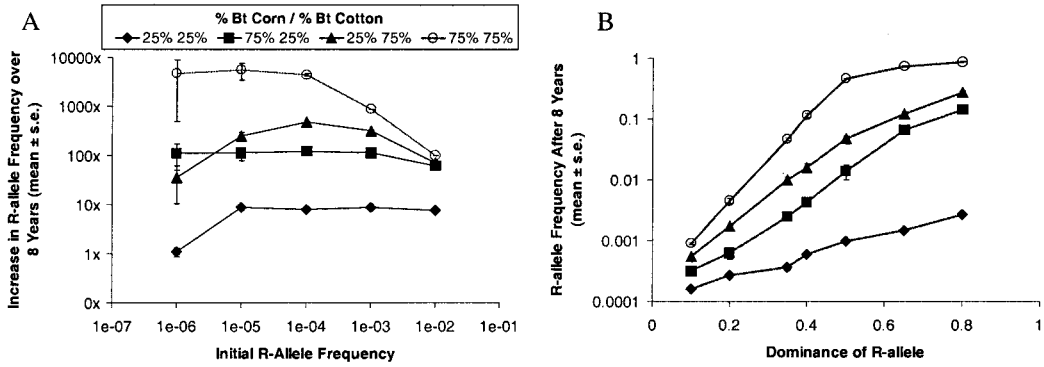

Fig. 4. Effect of (A) initial R-allele frequency, q_0, on the increase in R-allele frequency, and (B) R-allele dominance, h, on R-allele frequency after 8 yr of deployment of two levels of Bt corn and two levels of Bt cotton. Values for all other parameters are the default values shown in Table 1. Each point represents the average of three runs.

region in R-allele frequency and field-to-field variation in R-allele frequency was more extreme (adjusted coefficient of variation = 0.008, SE = 0.0007, n = 3) than at higher levels of movement (adjusted coefficient of variation = 0.002, SE = 0.0004, n = 3). These differences were not strong, but may cause observable variation in R-allele frequency at the lowest dispersal setting.

Several researchers have shown through computer models that when spatial aspects of population dynamics and genetics are taken into consideration, insect movement can have an important impact on resistance evolution (Mallet and Porter 1992, Peck et al. 1999, Caprio and Tabashnik 1992, Taylor and Georghiou 1979). Maximizing matings between RR individuals from toxic crops with SS individuals from refuge crops is vital to keeping R-alleles rare and for the high dose plus refuge strategy to be successful (Gould 1998). In such cases, a moderate level of movement that promotes such matings but keeps the spatial spread of R alleles low is often best for delaying resistance. In this model, the fitness differential between RS and SS larvae created by the moderate toxin dose made promoting RR × SS matings a less important part of an IRM strategy. Thus, the importance of movement was reduced.

Genetic Factors

q_0. The initial R-allele frequency had a strong impact on the frequency after 8 yr, simply because it was the starting point of resistance. Of greater interest is the rate of increase in R-allele frequency, here calculated as q_8/q_0 where q_8 is the R-allele frequency after 8 yr (Fig. 4A). ANOVA was conducted on the log-transformed values. The initial R-allele frequency had a significant interaction with *BtCR* and *BtCT* (Table 2). The effects were primarily due a reduced rate of increase when the initial frequency was high and selection was strong (high *BtCR* and high *BtCT*) as the population became saturated with R-alleles. The rate of increase was low when R-alleles were very rare and selection was weak (low *BtCR* and low *BtCT*). Under these conditions, stochasticity caused frequent field-level extinctions of the R-allele. This is of biological significance because it implies that if R-allele frequencies are low and Bt use is low, resistance may never gather sufficient momentum to overcome the effects of random local extinction. If neither local R-allele extinction or population saturation occurred (e.g., at moderate initial R-allele frequencies or high initial R-allele frequencies but low deployment), the R-allele frequency had no effect on the rate of evolution (Fig. 4A), indicating that there was no significant frequency-dependent selection.

h. The dominance of the R-allele affected the evolution of resistance as shown in Fig. 4B. The two-way and three-way interactions among h, *BtCR*, and *BtCT* shown in Table 2 indicate that the effect of h depended on the total amount of Bt planted and the amount of each of the crops planted to Bt. The larger the amount of Bt planted, the larger the effect. Dominance directly affected the survival of RS larvae on Bt crops, and thus, directly affected the R-allele frequency. At high dominance values and high Bt deployment levels, the R-allele frequency approached 1.0 and so the rate of increase declined. This parameter is probably the most biologically important parameter causing the greatest effect on the rate at which the population adapts to the Bt crops.

q_0 X h Interaction. ANOVA showed a significant interaction between the genetic factors in both the 8-yr R-allele frequency (log+1-transformed) (F = 366.0; df = 16, 276; $P < 0.01$) and the 8-yr increase in R-allele frequency (i.e., q_8/q_0), log-transformed (F = 4.67; df = 16, 276; $P < 0.01$). At high initial R-allele frequency, the effect of R-allele dominance was reduced because the R-allele approached saturation after 8 yr; similarly, at high R-allele dominance, the effect of initial frequency on 8-yr frequency is reduced. Importantly, as shown in Fig. 5, the increase in R-allele frequency was similar across all levels of initial R-allele frequency except at the lowest level tested (1×10^{-6}). At this low level, the rate of increase was slow for recessive resistance. In this situation, only homozygous resistant insects have an advantage on Bt

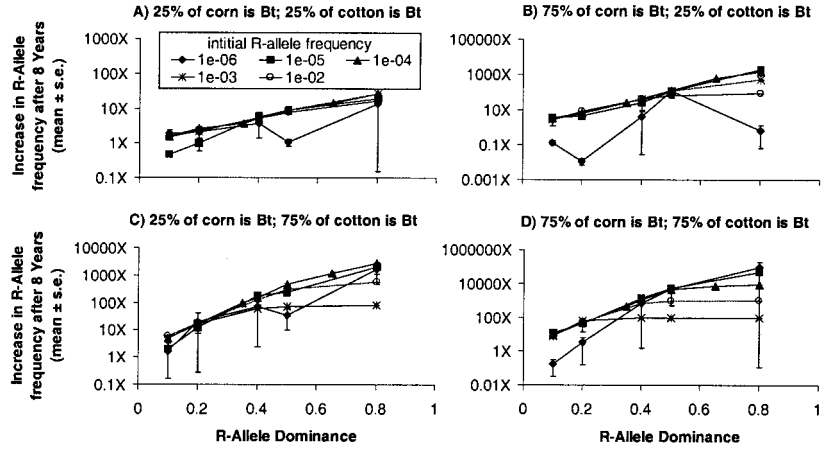

Fig. 5. Effect of initial R-allele frequency, q_0, interaction with R-allele dominance, h, on the increase in R-allele frequency over 8 yr of deployment of two levels of Bt corn and two levels of Bt cotton. Values for all other parameters are the default values shown in Table 1. Each point represents the average of three runs.

crops over susceptible insects, and these are so rare at the low gene frequency that they do not appear in most fields. Indeed genetic drift became important, and in many runs the gene frequency actually declined because of local extinctions of the allele (i.e., the rate of increase was <1). This indicates that a rare incompletely recessive R-allele may never be able to increase in frequency in a finite field population, and thus, never create a resistance problem.

S_{cr} and S_{ct} X h Interaction. The susceptible survival on Bt crops and the functional dominance of resistance interacted significantly ($F = 9.72$; df = 9, 128; $P < 0.01$), and the nature of the interaction depended upon the amount of Bt corn and Bt cotton deployed (4-way interaction $F = 4.71$; df = 9,128, $P < 0.01$).

Figures 2B and C showed that lower SS survival increased the expected R-allele frequency; Fig. 4B showed that higher dominance increased the expected R-allele frequency. Figure 6 shows that, as expected, if the R-allele dominance was high and Bt survival was low, resistance developed rapidly; conversely, if the R-allele dominance was low and the Bt survival was high, resistance developed slowly. This held true for all Bt deployment levels. However, decreased dominance would be expected to accompany decreased susceptible survival. Figure 6 indicates that the outcome of these two opposing forces depended on the level of Bt deployment. For example, at SS survival of 10% and R-allele dominance of 0.3, the R-allele frequency was higher than at SS survival of

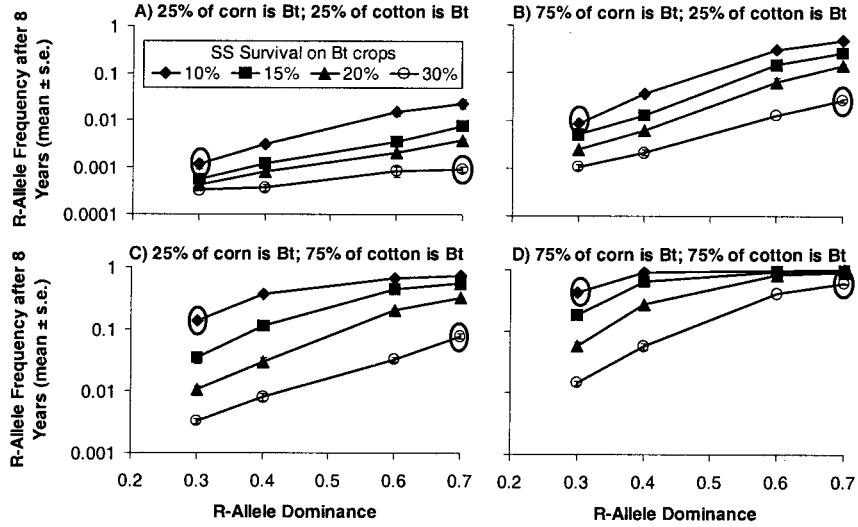

Fig. 6. Effect of SS survival on Bt crops, S_{cr} and S_{ct} interaction with R-allele dominance, h, on R-allele frequency after 8 yr of deployment of two levels of Bt corn and two levels of Bt cotton. Values for all other parameters are the default values shown in Table 1. Each point represents the average of three runs. Circled values are discussed in the text.

30% and R-allele dominance of 0.7 only if Bt corn was planted in a small proportion of corn fields (see the circled data-points in Fig. 6A and C). At high Bt corn deployment, the situation is reversed (see the circled data-points in Figs. 6B and D). Because of the complex nature of the interaction between Bt survival and R-allele functional dominance, it is essential to have good measures of these parameters before attempting to predict resistance evolution. However, as the counteracting effects approximately canceled out, the default values appear to provide a good indication of the pattern of resistance evolution.

Conclusions

This study has identified several consistently important parameters that determine the rate at which *H. zea* populations adapt to Bt crops in an agroecosystem like eastern North Carolina. Because the model output depends on uncertain values for important input parameters, it should not be interpreted as predictive of absolute time to resistance, but must be interpreted within the context of the particular set of parameter values used to obtain the output. For those parameters that are inherently variable or that cannot be controlled, continual monitoring should provide early warning of conditions that are likely to increase the risk of resistance. For those parameters that are in control of the biotechnology industry, farmers, and extension specialists, improved estimates of their values would greatly aid the assessment of resistance risks and improve our ability to manage those risks. Where possible, research and development should be focused on changing products and practices to make the parameter values more favorable.

The properties of the R-alleles themselves—functional dominance and initial frequency—are of prime importance. There is an urgent need to monitor for resistance in the field and to characterize the properties of resistance alleles in field populations to obtain useful estimates of these parameters. Recent field data for the *H. zea* population in eastern North Carolina indicate the R-allele frequency in 2000 could have been $\approx 4.3 \times 10^{-4}$ (Burd et al. 2001) and that the allele responsible may be incompletely dominant (i.e., $h = 0.5$) (Burd et al. 2000, 2001). It must be remembered when designing resistance management plans, however, that the properties of the R-alleles are likely to vary among environments and among Bt hybrids. Furthermore, different locations have different histories of Bt spray use and so are likely to have different initial R-allele frequencies.

The relative survival of susceptible insects on Bt and non-Bt hosts determines the selection pressure in each generation: higher relative survival on Bt slows resistance evolution. As new events or traits become available, they should be assessed on their own merits from an IRM standpoint, as the crop-insect interaction is very important in affecting resistance evolution (one size may not fit all).

Environmental factors are important. Parameters that drive the insect population dynamics affect resistance evolution through density-dependent selection. Seasonal weather patterns alter the timing of the start and end of the growing season, affecting the success of diapause induction of each genotype.

Agricultural considerations are also important. The proportions of each crop planted to Bt varieties drive resistance evolution. Furthermore, the relative proportions of total corn and total cotton acreages affect the rate: more cotton hastens resistance evolution. A companion paper showed that the spatial distribution of transgenic and nontransgenic plantings can affect the region-wide evolution of resistance, and, more drastically, the resistance levels in local sub-populations (Storer et al. 2003). Thus, risks for resistance differ among locations and are likely to change through time. This highlights the importance of considering spatial aspects of crop deployment when attempting to manage resistance evolution.

The larval threshold that triggers insecticidal sprays in Bt and non-Bt cotton crops affects the rate of evolution. As the pest complexes in Bt cotton shift away from Heliothines, to tarnished plant bugs for example (Hardee and Bryan 1997), spraying practices and the chemicals used may change. Spraying patterns in both Bt and non-Bt fields need to be closely observed to receive early warnings of increased resistance risks.

Full validation of the model as a predictive tool will be hard, if not impossible, to achieve because controlled, region-wide, long-term field experiments will be impossible. The resources required to fully validate the model by accurately tracking not only gene frequencies, but also insect population ecology through time and space during resistance evolution are likely to be too extensive to be practical. Even if reality were to coincide with the computer predictions, it would be impossible to attribute with certainty such agreement to the same processes, because different sets of assumptions can produce similar results.

With this understanding of the parameter sensitivities, we can better understand the forces and interactions that are involved in resistance evolution, pointing to aspects of biology, genetics, and operations that are most critical, and thus improving our ability to slow resistance. The model can be used to find those IRM strategies that are likely to be successful over a wide range of conditions. The model sensitivities have illustrated where further field data are required to improve our confidence in the suitability of the plans. Together, the field data and model runs can provide the necessary scientific basis for a resistance management strategy that is truly robust, flexible, and responsive to the needs of farmers.

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