Using a Generational Time-Step Model to Simulate Dynamics of Adaptation to Transgenic Corn and Crop Rotation by Western Corn Rootworm (Coleoptera: Chrysomelidae)

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ABSTRACT We expanded a simulation model of the population dynamics and genetics of the western corn rootworm for a landscape of corn, soybean, and other crops to study the simultaneous development of resistance to both crop rotation and transgenic corn. Transgenic corn effective against corn rootworm was recently approved in 2003 and may be a very effective new technology for control of western corn rootworm in areas with or without the rotation-resistant variant. In simulations of areas with rotation-resistant populations, planting transgenic corn to only rotated cornfields was a robust strategy to prevent resistance to both traits. In these areas, planting transgenic corn to only continuous fields was not an effective strategy for preventing adaptation to crop rotation or transgenic corn. In areas without rotation-resistant phenotypes, gene expression of the allele for resistance to transgenic corn was the most important factor affecting the development of resistance to transgenic corn. If the allele for resistance to transgenic corn is recessive, resistance can be delayed longer than 15 yr, but if the resistant allele is dominant then resistance usually developed within 15 yr. In a sensitivity analysis, among the parameters investigated, initial allele frequency and density dependence were the two most important factors affecting the evolution of resistance. We compared the results of this simulation model with a more complicated model and results between the two were similar. This indicates that results from a simpler model with a generational time-step can compare favorably with a more complex model with a daily time-step.

KEY WORDS *Diabrotica virgifera virgifera*, simulation model, insect resistance management, crop rotation, transgenic corn

FOR MANY YEARS, CORN PRODUCERS throughout the Corn Belt have managed the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, by practicing crop rotation, a strategy of planting corn, *Zea mays* L., and a nonhost crop, such as soybean, *Glycine max* L., in alternate years. This has historically been an effective approach because eggs laid in rotated cornfields hatch the following year in the nonhost crop and the larvae will be unable to survive. However, since 1995, reports of serious larval injury to first-year corn in Illinois and Indiana have increased, and growers who had successfully used crop rotation for corn rootworm management have suffered serious crop losses (Levine et al. 2002). As the effectiveness of crop rotation for managing this pest has begun to fail in many areas of the midwestern United States, new management strategies are needed to control rotation-resistant populations. Transgenic corn effective against corn rootworm was recently approved in 2003 by U.S Environmental Protection Agency and may be a very valuable new technology for control of western corn rootworm in areas with and without the rotation-resistant variant.

A model by Onstad et al. (2003) indicated that planting transgenic corn to rotated cornfields may be the most effective strategy for managing rotation-resistant corn rootworm populations from both a biological and economic perspective. However, this model did not consider resistance to transgenic corn and assumed a constant rate of mortality for all larvae exposed to the transgenic crop. For transgenic corn to be a sustainable management strategy, measures are required to slow the evolution of resistance within the target insect population. For ≈10 yr, scientists have been focusing on the potential for the evolution of insect resistance to transgenic crops (Caprio 1994; Roush 1997; Onstad and Gould 1998a, b; Tabashnik et al. 1998; Onstad and Guse 1999; Peck et al. 1999; Davis and Onstad 2000; Carrière and Tabashnik 2001; Carrière et al. 2001a, b, 2002, 2003; Carpenter et al. 2002; Ives and Andow 2002; Storer 2003; Storer et al. 2003a, b). Much of the emphasis has focused on the high-dose/refuge strategy. With this strategy, the pest management industry attempts to create plants that can express toxins at doses high enough to kill most, if

The ideas expressed in this article may not represent those of the USDA.

not all, heterozygotes (SR) that may be partially resistant to the toxin at lower doses, where S and R refer to the alleles for susceptibility and resistance to the transgenic toxin, respectively (Gould 1998, Caprio 2001). The goal is functional dominance of susceptibility to the toxin. The refuge is used to produce susceptible homozygotes (SS) that will disperse and mate with any surviving resistant individuals, resulting in only SS or SR offspring that can be killed by the transgenic crop (Shelton et al. 2000, Tang et al. 2001).

Currently, no models exist that have focused on the simultaneous development of resistance to both crop rotation and transgenic crops. Onstad et al. (2003) created a deterministic model with a time-step of one generation that focused on the development of resistance to crop rotation and the impact of transgenic corn as a management strategy, but they did not consider many of the complexities involved with transgenic crops. Onstad et al. (2001a) created a deterministic model with a daily time-step that simulated the dynamics of adaptation to transgenic corn by western corn rootworm. This model showed that resistance to transgenic crops can be significantly delayed if the high-dose/refuge strategy is used effectively. However, this model assumed that transgenic crops were only deployed in continuous cornfields and did not analyze the impact of rotation-resistant phenotypes. Similarly, Storer (2003) created a spatially explicit stochastic model with a daily time-step that simulated adaptation to transgenic maize but did not consider the influence of rotation-resistant phenotypes. In this and a companion article, Crowder et al. (2005), we will expand both deterministic models by Onstad et al. (2001a, 2003) to evaluate the risk of resistance to both transgenic crops and crop rotation in landscapes with and without rotation-resistant phenotypes.

In this article, we will attempt to identify the most effective deployment strategies for transgenic crops to prevent both rotation-resistance and resistance to transgenic corn. Our analysis is focused on several regions. First, we investigated the evolution of resistance to crop rotation in a region without transgenic corn. These results were compared with the model of Onstad et al. (2003). Second, we simulated the use of transgenic corn in areas with rotation-resistant phenotypes. In these areas, we analyzed the use of transgenic corn in rotated cornfields, continuous cornfields, or both, to study the most effective strategies to slow resistance to both crop rotation and transgenic corn. We also investigated whether resistance to crop rotation affected resistance to transgenic corn and vice versa. Third, we investigated the use of transgenic corn in areas without rotation-resistant phenotypes. These results were compared with those of Onstad et al. (2001a) and Storer (2003). For each set of simulations, we investigated the influence of toxin dose in the crop, gene expression of both traits, and different refuge strategies on the evolution of resistance to both crop rotation and transgenic corn. Fourth, we conducted an extensive sensitivity analysis on the model.

Furthermore, we will compare results between the two improved models to help identify how different modeling assumptions affect the results. If simpler models compare favorably with more complicated ones, it may indicate that some or all of the complexity dealing with adult survival, adult dispersal, mating, and oviposition was not as significant as other factors, such as gene expression, and may have only added uncertainty and complications.

Materials and Methods

In this section, we describe the development of the generational model and its analysis. First, we describe observations and literature that support the ecological framework and biological processes used to create the model. The details and justifications for functions can be found in Onstad et al. (2003) unless otherwise noted. Second, we describe the ecological equations used to develop the model. Third, we discuss the standard simulation conditions for areas with or without rotation-resistant phenotypes. Then, we describe the sensitivity analyses that were performed on the model.

Population Genetics. We assumed a simple population genetics model of *D. virgifera virgifera* in a landscape of corn and noncorn crops to explain the development of resistance to both crop rotation and transgenic corn. We assume this is an autosomal, two-locus, two-allele per locus, diploid genetic system. Despite a lack of empirical evidence on the population genetics of *D. virgifera virgifera*, this simple genetic system was chosen because it is comparable with several natural systems described by Onstad et al. (2001b), and has been previously used in models that simulated the development of resistance by western corn rootworm to crop rotation (Onstad et al. 2001b, 2003) and transgenic corn (Onstad et al. 2001a, Storer 2003).

The allele for susceptibility to transgenic corn is S; the allele for resistance to transgenic corn is R. With regard to crop rotation, we defined the X allele for no movement out of corn (wild type) and the Y allele for the tendency to move to all patches (rotation-resistant). We studied nine types of gene expression: Y as recessive with R as recessive, partially recessive, or dominant; Y as additive with R as recessive, partially recessive, or dominant; and Y as dominant with R as recessive, partially recessive, or dominant. We assume that mutations do not occur after the start of the simulations.

Model Landscape. The region consists of 100 ha of cropland consisting of up to four crops and a maximum of six fields. The four crops are: corn grown in the same location each year (continuous corn), C_c; corn that follows a soybean field in a 2-yr rotation of corn and soybean (rotated corn), C_r; soybean that precedes corn in a 2-yr rotation of corn and soybean, Soy; and the extra noncorn, Ex. We assume that Ex is any patch that is not planted to corn or soybean and is not rotated to corn. A proportion of both corn patches can be planted to a transgenic cultivar in a block configuration. The proportion of the continuous and rotated cornfields that are planted to a transgenic cultivar is

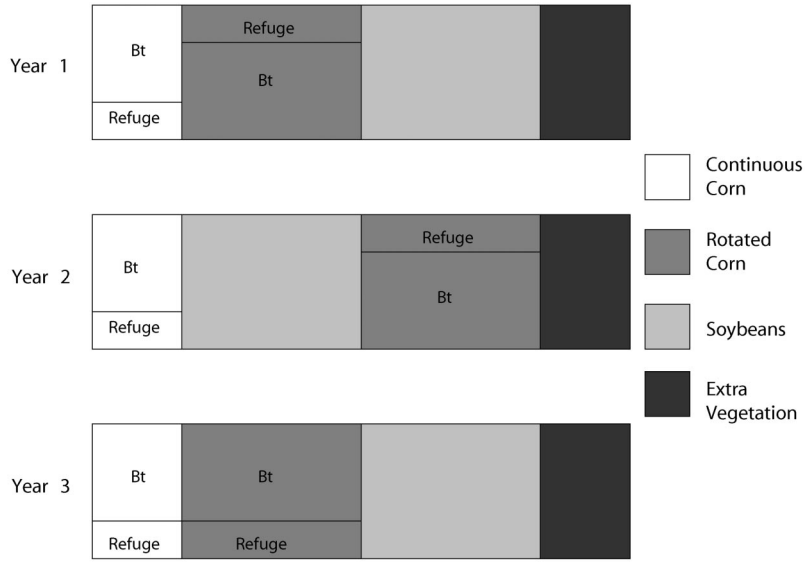

Fig. 1. Typical model landscape over a 3-yr time horizon with four crops and transgenic corn planted to both continuous and rotated cornfields.

Tc and Tr, respectively. The proportion of the landscape planted to the transgenic continuous and rotated cornfields is Tc × Cc and Tr × Cr, respectively. The proportion of the landscape planted to the refuge continuous and rotated cornfields is (1 × Tc) × Cc and (1 − Tr) × Cr, respectively. In cases where Tc or Tr > 0, we studied refuge sizes occupying 5, 10, 20, and 30% of the field. Figure 1 shows a hypothetical model landscape over a time horizon of 3 yr for a landscape with transgenic corn planted to both the continuous and rotated cornfields. In simulations with transgenic corn in the continuous cornfield, we assume that the transgenic block remains at the same site in successive years (Fig. 1). In simulations with transgenic corn planted to the rotated cornfield, the location of the refuge and transgenic blocks vary from year to year and are randomly determined due to the rotation of corn and soybean (Fig. 1). The location of the refuge within the rotated cornfield does not affect the evolution of resistance because we assume that beetles emerging in rotated cornfields hatch from eggs distributed uniformly throughout the soybean field the previous year.

Movement. Table 1 shows the movement parameters for the three possible genotypes at the locus for resistance to crop rotation. Regardless of gene expression, normal (−**XX**) individuals move from the natal corn patch and distribute themselves (and their eggs) across all corn patches according to their relative proportional areas, where − indicates the alleles for susceptibility to transgenic corn. Likewise, regardless of gene expression, rotation-resistant (−**YY**) individuals move into all patches according to their proportional representation in the region. With **Y** recessive, heterozygotes (−**XY**) disperse and lay eggs across all corn patches in the region; with **Y** dominant, heterozygotes disperse into all patches. In the additive case, half of the heterozygotes (−**XY**) disperse only into corn, whereas the other half disperse into all fields (Onstad et al. 2003) (Table 1). The alleles for resistance to transgenic corn do not affect movement.

Oviposition. We set the fecundity per individual to 220 viable eggs, which is half of the number per female, and ignore gender (Onstad et al. 2001a). The model of Onstad et al. (2001b) included a fecundity cost for rotation-resistant individuals due to feeding on non-

Table 1. Parameter values for three genotypes where the proportion of land planted to continuous corn, rotated corn, soybean, and extra vegetation are C_c, C_r, Soy, and E, respectively

Genotype[a]	Gene expression[b]	Probability of moving to C_c[c]	Probability of moving to Soy[c]
(−XX)		$C_c/(C_c + C_r)$	
(−YY)		C_c	S
(−XY)	X dominant	$C_c/(C_c + C_r)$	0
	Y dominant	C_c	Soy
	Additive	$0.5 \times (C_c + (C_c/(C_c + C_r))$	0.5 × Soy

The alleles for resistance to transgenic corn (denoted by dashes) do not affect these parameter values.
[a] Dashes indicate the alleles for resistance to transgenic corn.
[b] Gene expression does not affect the movement and oviposition of homozygotes.
[c] Probability of moving to either C_c or Soy is the same as the probability of oviposition in that field

corn tissues. In a sensitivity analysis, Onstad et al. (2003) tested the effect of this parameter by varying the value from 0 (no fecundity cost) to a maximum value of 1 − (Soy + Ex). Results showed that the model was not sensitive to this parameter. In addition, recent work by Mabry and Spencer (2003) showed that as long as beetles move around the landscape often enough to return to corn once every 2–3 d, fecundity and longevity are not reduced. Mabry and Spencer (2003) also concluded that corn tissues consumed by females the first week or so after emergence are much more important for later reproduction than various crop tissues consumed during the oviposition period. Therefore, we did not include reduced fecundity for rotation-resistant individuals in this model. The offspring of normal (−XX) individuals are divided across the corn patches according to their proportional areas, whereas the offspring of rotation-resistant individuals (−YY) are divided across all patches according to their proportional areas (Table 1). The placement of offspring of the heterozygotes (−XY) is determined by the gene expression at the locus for resistance to crop rotation (Table 1).

Toxin Mortality. Density independent toxin mortality incurred by larvae, Qtox, is dependent on the dose of the toxin, the genotype of the individual at the locus for resistance to transgenic corn, and the gene expression at this locus. We assume this mortality is applied at the same time as overwintering survival. We studied four doses of toxin based on values used in the model of Onstad et al. (2001a).

Homozygous-resistant individuals, (RR−), always have 100% survival to the transgenic cultivar regardless of dose (Qtox = 0). With R dominant, heterozygous (sR−) individuals also always have 100% survival to the transgenic crop. The survival of homozygous-susceptible individuals (SS−), or heterozygotes with R recessive (Sr−), is 0, 0.001, 0.05, and 0.20 with a theoretical high, practical high, medium, and low toxin dose, respectively. With R partially recessive, survival of the heterozygotes, (sr−), is 0, 0.01, 0.50, and 0.60 with a theoretical high, practical high, medium, and low toxin dose, respectively. Therefore, the functional dominance of the R allele is ≈0 (completely recessive), 0.01, 0.47, and 0.50 with a theoretical high, practical high, medium, and low dose, respectively. In cases where a proportion of both the continuous and rotated cornfields are planted to a transgenic cultivar, we assume that the dose used in each field is the same. The alleles for susceptibility to crop rotation (denoted by dashes), do not affect survival to the transgenic toxin.

Immature Survival. All larvae emerging from eggs in noncorn patches die. Offspring in continuous and rotated cornfields incur an overwintering mortality of 50% during the egg stage (Godfrey et al. 1995) and incur density-dependent mortality during the larval stage. Individuals emerging in fields planted to a transgenic cultivar incur density-independent mortality, Qtox, based on the dose of the toxin, the insect's S/R genotype, and the gene expression at this locus. The model of Onstad et al. (2003) used a density-dependent larval survival function fit to the data of Gray and Tollefson (1988). The density-dependent survival of larvae per stage is $0.21 \times \exp(-0.058\mathrm{EGG})$, where EGG is the density of eggs (in millions per hectare). The maximum larval survival based on this function is 21%. We assumed that density-dependent mortality occurs after mortality due to overwintering and toxin exposure.

Model Equations. The number of eggs $E_{i,p}(t+1)$ of genotype i in patch p for year t + 1 as a function of the number of adults $A_{i,p}$ in year t is

$$E_{i,p}(t+1) = 220 \times \sum_{j=1}^{9} \{P_{j,p} \times [A_{j,rc}(t) \times \sum_{k=1}^{9} w_{j,k}Q_{k,rc}(t) + A_{j,rr}(t) \times \sum_{k=1}^{9} w_{j,k}Q_{k,rr}(t) + A_{j,tc}(t) \times \sum_{k=1}^{9} w_{j,k}Q_{k,tc}(t) + A_{j,tr}(t) \times \sum_{k=1}^{9} w_{j,k}Q_{k,tr}(t)]\} \quad [1]$$

where P is the probability of genotype j moving to patch p. We assume that beetles mate randomly within the field of emergence and that the offspring will have an expected frequency distribution dependent upon the frequencies of each genotype emerging in the natal patch. Therefore, Q is the frequency of genotype k in natal patches rc, rr, tc, or tr (continuous corn refuge, rotated corn refuge, continuous transgenic corn, and rotated transgenic corn, respectively) that can reproduce the particular offspring genotype i when mated with genotype j. Each weight, w, equals the Mendelian proportion of all offspring that are genotype i when genotypes j and k mate.

To calculate the number of older larvae and adults, we must calculate density-dependent survival. First, we calculated the total number of larvae TL in each kind of corn habitat in the landscape f (where f is rc, rr, tc, or tr), surviving the winter and toxicity of transgenic corn roots (1 − Qtox).

$$TL_f = \sum_{i=1}^{9} E_{i,f}(t) \times 0.5 \times (1 - \mathrm{Qtox}) \quad [2]$$

The numbers of older larvae or adults in the four corn habitats f are

$$A_{i,rc}(t) = 0.5 \times E_{i,rc}(t) \times 0.21 \times \exp\{-0.058 \times [TL_{rc}/(100 \times (1 - Tc) \times C_c \times 10^6)]\} \quad [3]$$

$$A_{i,tc}(t) = 0.5 \times E_{i,tc}(t) \times (1 - \mathrm{Qtox}) \times 0.21 \times \exp\{-0.058 \times [TL_{tc}/(100 \times Tc \times C_c \times 10^6)]\} \quad [4]$$

$$A_{i,rr}(t) = 0.5 \times E_{i,rr}(t) \times 0.21 \times \exp\{-0.058 \times [TL_{rr}/(100 \times (1 - Tr) \times C_r \times 10^6)]\} \quad [5]$$

$$A_{i,tr}(t) = 0.5 \times E_{i,Tc}(t) \times (1 - Qtox) \times 0.21 \times \exp\{-0.058 \times [TL_{tr}/(100 \times Tr \times C_r \times 10^6)]\} \quad [6]$$

The number of adults emerging the next year is Ai,s (t) = Ai,e (t) = 0 in rotated soybean and in extra vegetation.

Male Dispersal. Crowder et al. (2005) and Onstad et al. (2001a) assumed that 25% of males could disperse out of the natal cornfield before mating the first or multiple times. In contrast, the model of Onstad et al. (2003) did not simulate male dispersal before mating and allowed males to mate only once. We expanded the model of Onstad et al. (2003) to test the effect of allowing a proportion of males to mate outside of the natal cornfield. To simulate this, we recalculated the genotype frequencies of males before mating in each field after dispersal. This was performed using our assumption that 50% of emerging adults are male. All females remain in the natal field to mate, whereas a proportion, PM, of the males move out of the natal field and into the other cornfields according to their proportional areas. The standard value for PM is 0.25. For example, the adjusted number of males in the continuous nontransgenic corn is

$$M_{j,rc}(t) = 0.5 \times M_{i,rc} \times (1 - PM) + 0.5 \times (M_{i,tc} + M_{i,rr} + M_{i,tr}) \times PM \times [P(f)/\sum_{k=1}^{3} P(k)] \quad [7]$$

where $M_{j,f}$ (t) is the adjusted number of males of genotype j in field f (rc, tc, rr, or tr), whereas $M_{i,f}$ (t) is the number of males of genotype i that emerged in field f. P(f) is the proportion of land planted to field f that males are dispersing into and P(k) is the proportion of land planted to each cornfield that is not the natal field f for individual $M_{i,f}$ (t). On the right-hand side of equation 7, the first product is the number of males that remain in field f. The other three products represent the number of males moving into f from the other cornfields.

The adjusted number of males after male dispersal is used to recalculate male genotype frequencies in each field before mating. In the model, these frequencies are calculated before equations 1–6 and are used in equation 1 to calculate eggs. However, we did not adjust population densities in any field due to male dispersal. The reason for this is a constraint of the model. Because the model doesn't simulate males and females separately, if population densities in each field are calculated after male movement there would be deviation from a 1:1 sex ratio, and our assumptions about fecundity per individual would not be valid.

Standard Conditions. The model is programmed in Visual Basic in Microsoft Excel 2002 (Microsoft 2002). The model has a time-step of 1 yr, a time horizon of 16 yr, and a spatial unit of 100 ha. The 16-yr time horizon includes the first year where the model is initialized without transgenic corn plus an additional 15 yr. The flowchart of the standard model is presented in Fig. 2. The initial number of adults is 50,000 per hectare of corn, which is the value used in the model of Onstad et al. (2001a). The initial adults are distributed proportionally to the areas of continuous and rotated corn.

In the standard simulations of areas with rotation-resistant phenotypes, the adults begin at Hardy–Weinberg equilibrium with initial R and Y-allele frequencies of 10^{-4}. The rotation level, RL, is the sum of the proportional areas of rotated corn and soybean, which are always equal in the model (RL = Soy + C_r). In these simulations, the landscape is defined as RL = 0.85, Ex = 0.05, and C_c = 0.10. We first analyzed a 2-yr crop rotation of nontransgenic corn and soybean (Tc = Tr = 0). In simulations with transgenic corn, we studied several deployment strategies for transgenic crops: planting transgenic corn in only rotated corn (Tc = 0), planting transgenic corn in only continuous corn (Tr = 0), or planting equal proportions of continuous corn and rotated corn to a transgenic cultivar (Tc = Tr). Each strategy was analyzed to determine whether resistance to either crop rotation or transgenic corn developed within 15 yr with four toxin doses, three types of gene expression for both R and Y, and four refuge sizes.

In the standard simulations of areas without rotation-resistant phenotypes, the adults begin at Hardy–Weinberg equilibrium with an initial R- and Y-allele frequency of 10^{-4} and 0, respectively. We studied three levels of continuous corn for areas without rotation-resistant phenotypes: 20, 60, and 100%. The remainder of the landscape is divided equally among the rotated corn and soybean fields. With C_c = 0.20, 0.60, and 1.0, the amount of land planted to C_r and Soy is 0.40, 0.20, and 0.0, respectively. In the simulations without rotation resistance, we only studied the use of transgenic corn in continuous cornfields. In areas without rotation resistance, farmers should only plant transgenic corn to continuous cornfields where damage is most likely to occur. With any set of initial conditions (four toxin doses, three types of gene expression for R allele, and four refuge sizes), we determined whether resistance to transgenic corn developed within 15 yr.

In this article, we emphasize changes in the R- and Y-allele frequencies over the 15-yr time horizon after the first year when the model is initialized. Results presented are the number of years required for the resistance allele frequencies to increase from the initial value to 0.50. In some cases, we also present the frequencies of the alleles for resistance if they did not exceed 50% within 15 yr but resistance did occur. We also compare resistant allele frequencies after year 15 in the sensitivity analysis as well as the number of years for the resistant allele frequencies to exceed 50%.

Sensitivity Analysis. In a sensitivity analysis, we studied five factors. First, we studied the influence of initial allele frequency by raising the initial R- and Y-allele frequencies to 0.001 and 0.01. In areas with rotation-resistant phenotypes, we also tested an initial Y-allele frequency of 0.1 to represent areas where rotation resistance is already a severe problem. Second, we tested the effect of initial population size by raising the initial density to 500,000 adults per hectare

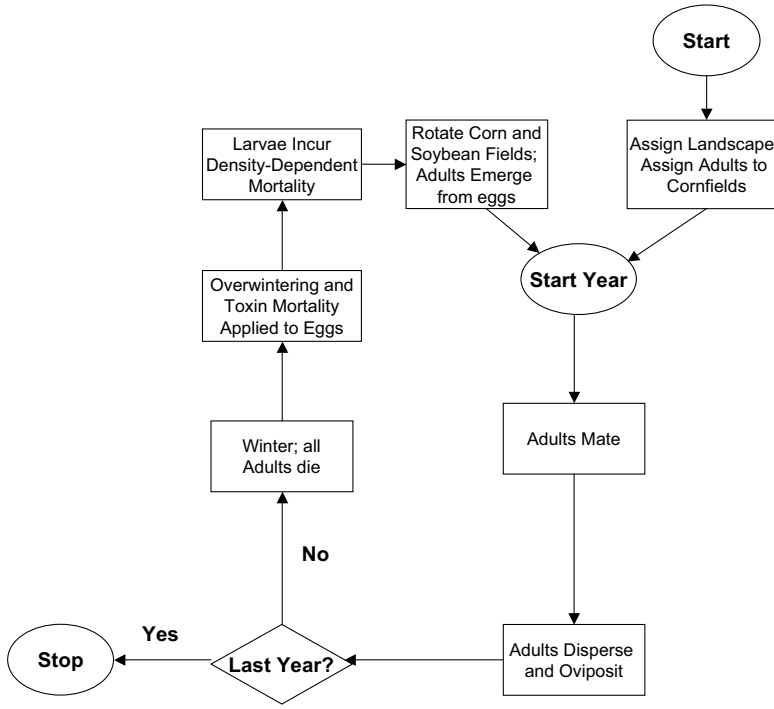

Fig. 2. Flowchart for the standard model.

and 1 million per hectare. Third, in simulations with rotation resistance, we tested whether the value of rotational level had a significant effect on the results by running the simulations with RL = 0.75 and RL = 0.95. The landscape is $C_c = 0.15$, Soy = $C_r = 0.375$, and Ex = 0.10 if RL = 0.75, or C_c = Ex = 0.025 and Soy = $C_r = 0.475$ if RL = 0.95.

Fourth, we simulated population dynamics with different versions of the density-dependent survival function. One was based on the model of Onstad et al. (2001a), $1/(1 + 2.42\text{EGG}^{0.7})$, which has a maximum larval survival of 100%. Using PROC NLIN in SAS (SAS Institute 2002), we fit another function to field data collected in three studies (Branson and Sutter 1985, Gray and Tollefson 1988, Elliott et al. 1989). This function had the form $1/(\text{A} + \text{B} \times \text{EGG}^{\text{C}})$, where A, B, and C are parameters. The best fit was obtained with values of 2.59, 1.29, and 0.88 for A, B, and C, respectively ($n = 20$, $r^2 = 0.89$). Therefore, the density-dependent survival of larvae by using this function is $1/(2.59 + 1.29\text{EGG}^{0.88})$, which has a maximum larval survival of 39%.

We also tested the model without density-dependent mortality but with an added density-independent mortality based on field data collected by Hibbard et al. (2004). The results of Hibbard et al. (2004) suggested that density-dependent mortality was negligible during establishment of western corn rootworm with viable egg levels of 100–3,200 per plant. Their study showed that density-independent establishment was between 2.5 and 5.7% when plants were sampled on the optimal date. For this model, we used the higher end of 5.0% for density-independent survival of larvae after overwintering and toxin mortality.

Finally, we studied the effect of lower fecundity by susceptible adults in transgenic corn. Wilson (2003) showed that beetles captured from transgenic treatments laid significantly fewer eggs than adults emerging from isoline (nontreated) fields. This 2-yr study showed that beetles emerging in transgenic corn produced nearly 50% fewer eggs than beetles in nontreated fields. Based on these data, we tested a lower fecundity of 50% for susceptible beetles emerging in transgenic fields. In these simulations, we tested only the R recessive and R dominant cases because we did not have any data on how the fecundity of (SR−) individuals would be affected in the partially recessive case. With R recessive, we lowered the fecundity of both (SS−) and (Sr−) beetles emerging in transgenic fields by 50%. With R dominant, only the fecundity of (SS–) beetles was lowered.

For the sensitivity analysis, in areas with rotation resistance we focused on the scenarios of planting transgenic corn to only rotated fields or continuous fields (Tc = 0 or Tr = 0). For simulations without rotation resistance, we performed the sensitivity analysis with all three values of continuous corn: 20, 60, and 100%. Unless otherwise noted, in the sensitivity analysis all variables were set to standard conditions except the variable being tested. In the sensitivity analysis, we studied a refuge size of 20% but did not consider refuge sizes of 5, 10, and 30%. When results are presented as a percent change compared with the stan-

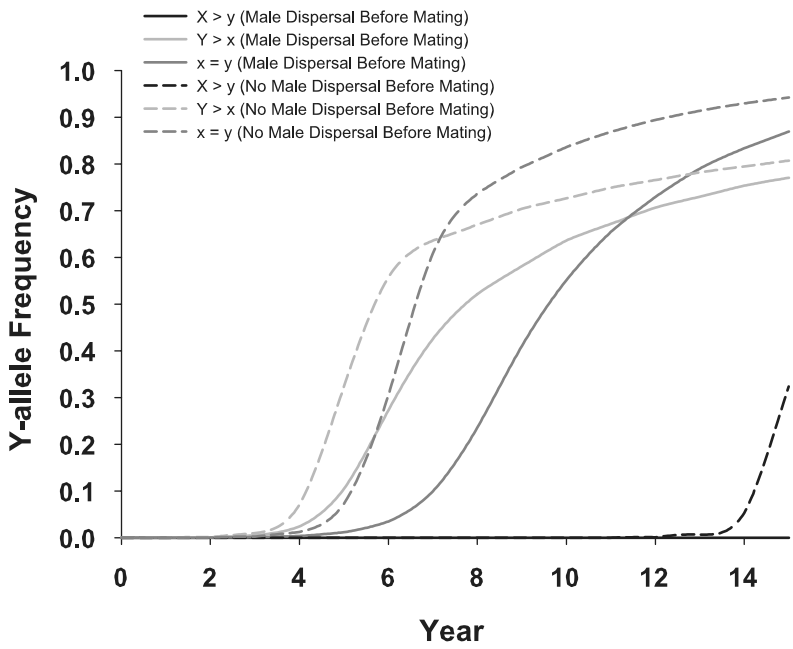

Fig. 3. Frequency of the allele for resistance to crop rotation, Y, over time in simulations with 85% rotation, no transgenic corn, and three types of gene expression with the current model that allowed 25% of males to disperse before mating or the model that did not allow males to disperse before mating (Onstad et al. 2003).

dard simulations, these percentages represent a multiplicative change.

Results

Areas with Rotation Resistance

Simulations without Transgenic Corn. Basic simulations of a 2-yr rotation of nontransgenic corn and soybean are shown in Figs. 3-4. We compared the results of our current model with results generated with the original model of Onstad et al. (2003) that did not allow male dispersal outside the natal field before mating. Results presented in this section differ slightly from Onstad et al. (2003) because we simulated their model with an initial population size of 50,000 adults per hectare rather than with their value of 1 million adults per hectare. As expected with greater gene flow, the Y-allele frequency increased at a slower rate with the current model compared with the original model with any Y-allele expression, especially with Y recessive. With Y recessive, the Y-allele frequency did not increase from the initial value of 0.0001 over 15 yr with the current model. This result differs from the original model, where the Y-allele frequency reached 0.32 within 15 yr with Y recessive (Fig. 3). Thus, more mating of susceptible males with resistant females delayed the evolution of rotation-resistance.

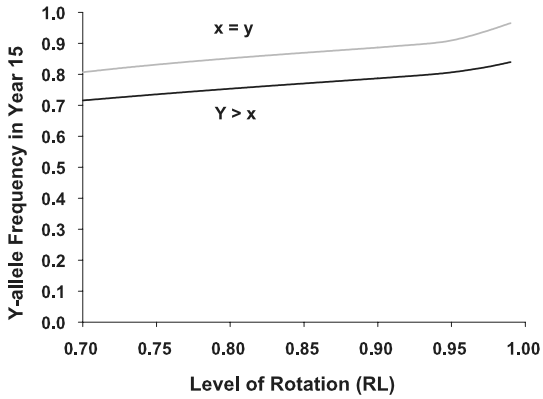

Fig. 4. Y-allele frequency in year 15 as a function of the level of rotation with Y additive (x = y), or Y dominant (Y > x).

A dominant Y expression permits the Y-allele frequency to increase the fastest initially, but after several years, the Y-allele frequency is actually greater when resistance is additive. These results are similar to those derived by Onstad et al. (2003). The percentage of eggs that are laid by heterozygotes in a field that will be planted to corn the following year, either continuous corn or soybean, ultimately determines the relative success of the Y-allele. With RL = 0.85, 52.5% of eggs laid by −YY individuals end up in a field that will be planted to corn the following year. With Y recessive, additive, and dominant, 19.0, 35.8, and 52.5%, respectively, of eggs laid by −XY individuals end up in a field that will be planted to corn the following year. Thus, the difference is greater with Y recessive or additive compared with Y dominant, resulting in the X-allele being successfully carried and passed on by heterozygotes to a greater extent when Y is dominant (Onstad et al. 2003).

Figure 4 indicates how the level of rotation, RL, influences the Y-allele frequency after 15 yr. With Y recessive, the Y-allele frequency never increased >0.0001 within 15 yr with any RL < 0.99, and this case is not shown in Fig. 4. With Y additive and dominant, the level of rotation in the landscape did affect the evolution of resistance to crop rotation. With Y additive and dominant, the Y-allele frequency reached ≈0.8 and 0.7, respectively, after 15 yr with an RL of 0.7 and increased up to 0.97 and 0.84, respectively, with an RL of 0.99 (Fig. 4). With any RL, the Y additive scenario resulted in the highest Y-allele frequency after 15 yr, followed by the Y dominant and recessive cases.

Simulations with Transgenic Corn Planted only to the Rotated Cornfield. Planting transgenic corn to only rotated cornfields was a robust strategy to prevent both rotation resistance and resistance to transgenic corn. For all simulations with 5–30% refuge in the rotated cornfield and no transgenic corn planted to the continuous field, the R-allele frequency never exceeded 0.002 over 15 yr. Similarly, the Y-allele frequency never exceeded 50% with any combination of refuge size, toxin dose, and R-allele expression. However, with Y dominant and a refuge size of 30%, the Y-allele frequency reached 0.40–0.45 after 15 yr with any dose. In all other cases, the Y-allele frequency did not exceed 0.04 over 15 yr.

Simulations with Transgenic Corn Planted only to the Continuous Cornfield. In simulations with transgenic corn planted to only continuous cornfields, gene expression and refuge size affected the evolution of resistance to both traits. With Y recessive, the Y-allele frequency never exceeded the initial value of 0.0001 within 15 yr with any simulated conditions. Table 2 shows the results with Y additive. With Y additive, the Y-allele frequency exceeded 50% within 6–10 yr. In every case, decreasing the toxin dose resulted in slower evolution of resistance. Likewise, increasing the refuge size in the continuous cornfield slowed the development of resistance. Rotation resistance developed more slowly with R dominant compared with the R recessive and partially recessive cases (Table 2). With Y dominant, the results were similar to the Y additive case, as the Y-allele frequency exceeded 50% within 5–9 yr. Thus, planting transgenic corn only in continuous cornfields was an inferior strategy for delaying rotation resistance compared with planting transgenic corn in rotated cornfields.

Planting transgenic corn to continuous cornfields was also not a robust strategy to prevent resistance to transgenic corn compared with planting transgenic corn to rotated cornfields. With R recessive, the R-allele frequency never exceeded the initial value of 0.0001 during the 15-yr simulation period. Similarly, with R partially recessive and a theoretical or practical high dose, the R-allele frequency never exceeded 0.0002 within 15 yr. However, with R partially recessive and a medium dose, the R-allele frequency increased above 0.24 after 15 yr in every case. The shortest time for the R-allele frequency to reach 50% with a medium dose and R partially recessive was 6 yr.

Table 2. Year in which the allele for resistance to crop rotation, Y, exceeded 50% with Y additive, four toxin doses, and four refuge sizes in areas with rotation-resistant phenotypes and varying gene expression for the allele for resistance to transgenic corn, R

Proportion of continuous cornfield planted to refuge[a]	Toxin dose			
	Theoretical high	Practical high	Medium	Low
	R recessive			
0.05	6 (0.97)	6 (0.97)	7 (0.94)	10 (0.83)
0.10	7 (0.95)	7 (0.95)	8 (0.92)	10 (0.82)
0.20	9 (0.92)	9 (0.92)	9 (0.89)	10 (0.82)
0.30	10 (0.90)	10 (0.90)	10 (0.87)	10 (0.83)
	R partially recessive			
0.05	6 (0.97)	6 (0.97)	7 (0.86)	10 (0.81)
0.10	7 (0.95)	7 (0.95)	8 (0.85)	10 (0.81)
0.20	9 (0.92)	9 (0.92)	9 (0.83)	10 (0.82)
0.30	10 (0.90)	10 (0.90)	10 (0.84)	10 (0.82)
	R dominant			
0.05	8 (0.90)	9 (0.89)	10 (0.87)	10 (0.84)
0.10	9 (0.88)	9 (0.88)	10 (0.86)	10 (0.84)
0.20	10 (0.86)	10 (0.86)	10 (0.85)	10 (0.83)
0.30	10 (0.83)	10 (0.83)	10 (0.84)	10 (0.83)

The Y-allele frequency after 15 yr is shown in parentheses.

[a] Proportion of continuous cornfield planted to transgenic cultivar is 1 − proportion refuge.

Evolution of resistance to transgenic corn occurred slower with a low dose compared with a medium dose, because the shortest time for the R-allele frequency to reach 50% was 10 yr.

Table 3 shows the results with R dominant and three types of gene expression for Y. With R dominant and Y recessive, the R-allele frequency exceeded 50% within 4–11 yr. With Y additive and dominant, the results were variable depending on toxin dose, refuge size, and Y-allele expression. In every case, resistance evolved more rapidly with greater toxin doses and decreased refuge size. Although resistance developed more quickly with Y additive compared with Y dominant in some cases and vice versa, the Y-allele frequency in year 15 was similar with any dose when comparing the Y additive and Y dominant cases (Table 3).

Simulations with Transgenic Corn Planted to Both Cornfields. In areas with rotation-resistant phenotypes, planting transgenic corn to both cornfields was always less effective at preventing resistance to both traits compared with planting transgenic corn to only rotated cornfields. For example, with a medium or low dose, the Y-allele frequency always exceeded 50% within 15 yr with any simulated conditions. Likewise, with Y dominant, the Y-allele frequency exceeded 50% within 15 yr in every case. With Y additive, the Y-allele frequency did not always exceed 50% within 15 yr, but reached as high as 0.64 with a low dose. In each case resistance to crop rotation evolved most rapidly with a low dose and slowest with a theoretical or practical high dose.

With R recessive, the R-allele frequency never exceeded the initial value of 0.0001. With R dominant, the R-allele frequency always exceeded 50% within

Table 3. Year in which the allele for resistance to transgenic corn, R, exceeded 50% with R dominant, four toxin doses, and four refuge sizes in areas with rotation-resistant phenotypes, transgenic corn planted to 80% of the continuous cornfield, and varying gene expression for the allele for resistance to crop rotation, Y

Proportion of continuous cornfield planted to refuge[a]	Toxin dose			
	Theoretical high	Practical high	Medium	Low
		Y recessive		
0.05	4 (0.92)	4 (0.92)	5 (0.91)	8 (0.87)
0.10	5 (0.91)	5 (0.91)	6 (0.90)	8 (0.85)
0.20	7 (0.88)	7 (0.88)	7 (0.88)	9 (0.82)
0.30	8 (0.84)	9 (0.85)	9 (0.84)	11 (0.77)
		Y additive		
0.05	6 (0.60)	6 (0.62)	6 (0.62)	>15 (0.42)
0.10	13 (0.55)	8 (0.56)	6 (0.57)	>15 (0.38)
0.20	>15 (0.45)	>15 (0.46)	>15 (0.46)	>15 (0.30)
0.30	>15 (0.38)	>15 (0.38)	>15 (0.38)	>15 (0.24)
		Y dominant		
0.05	13 (0.57)	13 (0.57)	14 (0.54)	>15 (0.39)
0.10	14 (0.54)	14 (0.55)	15 (0.52)	>15 (0.36)
0.20	>15 (0.50)	>15 (0.50)	>15 (0.47)	>15 (0.30)
0.30	>15 (0.45)	>15 (0.45)	>15 (0.42)	>15 (0.23)

The R-allele frequency in year 15 is shown in parentheses.

[a] Proportion of continuous cornfield planted to transgenic cultivar is 1 − proportion refuge.

15 yr, and the R-allele frequency increased above 0.7 after 15 yr in every case. With R partially recessive, there were several cases where resistance to transgenic corn did not evolve with a theoretical or practical high dose. However, in >70% of the simulations with R partially recessive, the R-allele frequency increased above 0.7 within the 15-yr simulation period.

Areas without Rotation-Resistance

Allele expression was the most important factor affecting the evolution of the R-allele in simulations of areas without rotation-resistant phenotypes and various levels of continuous corn. With R recessive, resistance to transgenic corn never evolved. With R dominant, resistance to transgenic corn always occurred in <10 yr. With R partially recessive, then evolution of resistance depended on the toxin dose expressed in the transgenic corn. All of these results matched those of Onstad et al. (2001a).

With R dominant and any proportion of land planted to continuous corn (20, 60, and 100%), the R-allele frequency exceeded 50% within 4–9 yr with any refuge size and toxin dose. The time for the R-allele frequency to reach 50% did not change by >1 yr when comparing simulations run with a theoretical high, practical high, and medium toxin dose, but increased up to 3 yr with a low dose. Changing the refuge size from 5 to 30% did not affect the time for the R-allele frequency to reach 50% by >1 yr with R dominant.

If R is recessive, the R-allele frequency never exceeded the initial value of 0.0001 within 15 yr. With R partially recessive and any amount of continuous corn, the R-allele frequency never exceeded 0.0001 and 0.002 within 15 yr with a theoretical high and practical high dose, respectively. With R partially recessive and 60 or 100% continuous corn, the R-allele frequency exceeded 50% in 6–7 and 10 yr with a medium and low dose, respectively, with any refuge size. The evolution of resistance occurred slightly slower with 20% continuous corn in the landscape, as the R-allele frequency reached 50% in 6–9 and 10–12 yr with a medium and low dose, respectively, with any refuge size. In every case where a range is presented, increasing the size of the refuge increased the number of years for resistance to evolve to transgenic corn. Again, these results match those of Onstad et al. (2001a) (Table 3).

Sensitivity Analysis

Areas with Rotation Resistance. *Simulations without Transgenic Corn.* In simulations of a 2-yr rotation of nontransgenic corn and soybean, changes in density-dependent survival, initial allele frequency, and initial population size affected the results. The results were more sensitive with Y additive or dominant compared with the Y recessive case. With Y recessive, only raising the initial Y-allele frequency to 0.1 affected the results, because the Y-allele frequency exceeded 50% within 5 yr and reached 0.998 after 15 yr, compared with 0.0001 under standard conditions. With Y recessive, changing the density-dependent survival function or initial population size did not affect the results, as the Y-allele frequency did not exceed 0.0001 within 15 yr.

With Y additive and an initial population size of 500,000 and 1 million adults per hectare, the Y-allele frequency exceeded 50% within 9 and 7 yr, respectively, compared with 10 yr under standard conditions. With Y dominant, raising the initial population size to 500,000 and 1 million adults per hectare resulted in the Y-allele frequency exceeding 50% within 7 and 6 yr, respectively, compared with 8 yr under standard conditions.

With Y additive, raising the initial Y-allele frequency to 0.001, 0.01, and 0.1 shortened the time for the Y-allele frequency to exceed 50% to 8, 6, and 4 yr, respectively, compared with 10 yr under standard conditions. With Y dominant, raising the initial Y-allele frequency to 0.001, 0.01, and 0.1 shortened the time for the Y-allele frequency to exceed 50% to 7, 6, and 4 yr, respectively, compared with 8 yr under standard conditions.

Figure 5 shows the effect of density-dependent survival on the results with Y additive and dominant. With Y additive or dominant, with a density-dependent function that allowed 39 and 100% maximum survival, Y-allele frequency exceeded 50% up to 3 yr faster compared with the standard simulations. With the density-independent function, the Y-allele frequency did not exceed 50% within 15 yr, reaching a value of 0.006 and 0.18 with Y additive and dominant, respectively (Fig. 5). This represented a 99 and 77% decrease

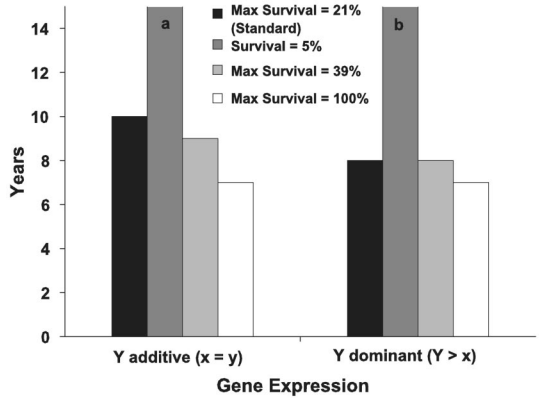

Fig. 5. Year in which the Y-allele frequency exceeded 50% with four types of density-dependent survival with a 2-yr rotation of nontransgenic corn and soybean (R = 0.85) and Y additive (x = y) or Y dominant (Y > x). Bars marked with letters indicate that the Y-allele frequency did not exceed 50% within 15 yr. The Y-allele frequency reached 0.006 and 0.18 for bars a and b, respectively.

in allele frequency compared with the standard simulations, where the Y-allele frequency reached 0.88 and 0.77 after 15 yr with Y additive and dominant, respectively.

Simulations with Transgenic Corn Planted to only the Rotated Cornfield. In simulations with transgenic corn planted only to the rotated cornfield with 20% refuge, density-dependent survival and initial allele frequency had the greatest effect on the results. Changing the initial population size or adding a fecundity cost for susceptible beetles emerging in transgenic corn had the least effect on the results. Typically, changes in the simulation conditions had a greater effect on the evolution of resistance to crop rotation compared with the evolution of resistance to transgenic corn. In this section, we focus on changes in the simulation conditions that affected the time for the resistant allele frequencies to reach 50%. We also present results where the allele frequency after 15 yr differed from the standard by a large percentage.

With either a 25 or 50% fecundity cost, the final R- and Y-allele frequencies after 15 yr did not differ from the standard simulations. Increasing the initial population size to 500,000 or 1 million beetles per hectare affected the evolution of resistance to crop rotation only in the following cases. With Y dominant and a medium or greater dose, raising the initial population size to 500,000 or 1 million beetles per hectare resulted in the Y-allele frequency reaching 0.04–0.06 or 0.07–0.09 after 15 yr, respectively, compared with 0.02 under standard conditions. With a low dose and Y dominant, raising the initial population size resulted in the Y-allele frequency reaching 0.11 and 0.17, respectively, compared with 0.04 with standard conditions. With either increased population size, the R-allele frequency after 15 yr did not differ from the standard simulations by >0.001.

Changing the rotational level to 75 or 95% affected the evolution of resistance to crop rotation in certain cases but did not greatly affect the evolution of resistance to transgenic corn. With RL = 0.75 or 0.95, the R-allele frequency after 15 yr did not differ by >0.002 compared with the standard simulations. Changing the rotational level in the landscape did affect the Y-allele frequency after 15 yr with Y dominant. With RL = 0.75 and Y dominant, the Y-allele frequency after 15 yr decreased by 50% compared with the standard simulations with any toxin dose. With RL = 0.95, the Y-allele frequency after 15 yr reached 0.08–0.15 with any dose and Y dominant, representing a 3.8–5.3-fold increase compared with the standard simulations. In every case, the greatest percentage change occurred with a theoretical high dose and the least with a low dose.

With the density-independent survival function, the R- or Y-allele frequencies never exceeded 0.0001 within 15 yr in any case. This represented a decrease in the rate of evolution of resistance to both crop rotation and transgenic corn compared with the standard simulations. Likewise, with a density-dependent function that allowed 39% maximum survival, neither the R- or Y-allele frequency exceeded 50% within 15 yr in any case. However, with Y additive and the 39% maximum survival function, the Y-allele frequency reached 0.04–0.08 after 15 yr with any toxin dose, representing an ≈40 fold increase in allele frequency compared with the standard simulations. With Y dominant and the 39% maximum survival function, the Y-allele frequency reached 0.32–0.42 after 15 yr with any dose, representing a 10.5–21.3-fold increase compared with the standard simulations.

A density-dependent function that allowed 100% maximum survival affected the rate of evolution of resistance to both crop rotation and transgenic corn in the following cases. With Y additive and a medium or greater dose, the Y-allele frequency reached 0.37–0.49 after 15 yr, representing a 370–435-fold increase over the standard simulations. With Y dominant and a medium or greater dose, the Y-allele frequency reached 0.39–0.50 after 15 yr, exceeding 50% in year 15 in some cases, a 9.2–19.5-fold increase in allele frequency compared with the standard simulations. With a low dose and Y additive and dominant, the Y-allele frequency reached 0.49 and 0.50 after 15 yr. This represented a 245- or 12.5-fold increase, respectively, in allele frequency compared with the standard simulations. With R dominant, and a theoretical or practical high dose and Y additive and dominant, the R-allele frequency after 15 yr reached 0.09 and 0.32, respectively, an ≈900- or 3,200-fold increase, respectively, in allele frequency compared with the standard simulations where the R-allele frequency never exceeded 0.0001. With R dominant, a medium dose, and Y additive and dominant, the R-allele frequency after 15 yr reached 0.02 and 0.13, respectively, a 170- or 1,290-fold increase, respectively, compared with the standard simulations.

In simulations with transgenic corn planted to only the rotated cornfield with 20% refuge, raising the initial R-allele frequency to 0.001 or 0.01 did not result in

R-allele frequency reaching 50% within 15 yr. In most cases, the R-allele frequency never exceeded the initial value of 0.001 or 0.01. Raising the initial Y-allele frequency to 0.001 affected the Y-allele frequency after 15 yr in the following cases. With Y dominant, any dose, and an initial Y-allele frequency of 0.001, the Y-allele frequency after 15 yr reached 0.25–0.36, an ≈9–12.5-fold increase compared with the standard simulations. With an initial Y-allele frequency of 0.01, the Y-allele frequency exceeded 50% within 15 and 13 yr with Y dominant and a medium and low toxin dose, respectively. With the greater doses, the Y-allele frequency reached 0.49 after 15 yr with Y dominant. With an initial allele frequency of 0.01 and Y additive, the Y-allele frequency reached 0.27–0.38 after 15 yr with any dose, a 1,900–2,700-fold increase compared with the standard simulations. The greatest percentage changes occurred with a theoretical high dose and the least with a low dose.

With an initial allele frequency of 0.1, the Y-allele frequency exceeded 50% within 9–11 yr with any dose and Y additive or dominant. In every case, evolution of resistance occurred most rapidly with a low dose. Decreasing the dose in rotated cornfields increases the relative survival of rotation-resistant beetles and results in faster evolution of resistance to crop rotation. With an initial allele frequency of 0.1 and Y recessive, the Y-allele frequency reached 0.25–0.36 with any dose. Further analysis of initial allele frequency indicated that the evolution of the Y-allele can be prevented by planting transgenic corn to rotated cornfields only if the initial Y-allele frequency is below 0.08, 0.01, and 0.002 for the Y recessive, additive, and dominant cases, respectively.

Simulations with Transgenic Corn Planted to only the Continuous Cornfield. In simulations with transgenic corn planted only to the continuous cornfield with 20% refuge, increasing the initial population size to 500,000 or 1 million adults per hectare affected the number of years for the R- and Y-allele frequencies to reach 50%. With these increased initial population sizes, the number of years for both the R- and Y-allele frequency to reach 50% decreased by a maximum of 2 and 4 yr, respectively, compared with the standard simulations.

Imposing a fecundity cost of 25 or 50% for susceptible beetles emerging in transgenic fields increased the time for the R-allele frequency to reach 50% by up to 1 yr. Imposing these fecundity costs decreased the for the Y-allele frequency to reach 50% by up to 1 yr. In all cases the R- or Y-allele frequency after 15 yr did not differ by >0.04 compared with the standard simulations.

With RL = 0.75, the time for the Y-allele frequency to exceed 50% never changed by >1 yr compared with the standard simulations. The greatest percentage change in allele frequency after 15 yr occurred with R and Y dominant and a medium dose, where the Y-allele frequency reached 0.68, a 6% decrease compared with the standard simulations. With RL = 0.75, in most cases results of evolution of resistance to transgenic corn were similar compared with the standard simulations, as the number of years for the R-allele frequency to reach 50% did not change by >2 yr. However, with R dominant and Y additive, the Y-allele frequency exceeded 50% in 7 yr with a theoretical high, practical high, and medium dose with RL = 0.75. This represented a decrease in time compared with the standard simulations where the R-allele frequency reached 0.45–0.46 after 15 yr.

With RL = 0.95, the time for the Y-allele frequency to reach 50% decreased by up to 3 yr compared with the standard simulations. The greatest percentage change in the Y-allele frequency after 15 yr occurred with Y additive and R partially recessive and a medium or low dose, where the Y-allele frequency in year 15 increased by 13–14% compared with the standard simulations. With RL = 0.95, the time for the R-allele frequency to reach 50% increased by up to 2 yr compared with the standard model. The greatest percentage change in the R-allele frequency occurred with R dominant and Y additive or dominant, where the R-allele frequency in year 15 decreased by ≈90–99% with any dose compared with the standard simulations. In every case, the greatest percentage change occurred with a low dose and the smallest change occurred with a theoretical high dose.

With the density-independent survival function, in most cases the time for the Y-allele frequency to reach 50% never increased by >2 yr and the Y-allele frequency in year 15 did not change by >8% compared with the standard simulations. However, with Y additive, R dominant, and any dose, the Y-allele frequency in year 15 decreased by 14–57% compared with the standard simulations. With the density-dependent functions that allowed 39 and 100% maximum survival, the time for the Y-allele frequency to reach 50% decreased by up to 2 and 4 yr, respectively, with Y additive or dominant and any dose.

With any function for density-dependent or -independent survival, the R-allele frequency did not exceed 0.0001 within 15 yr with R recessive, similar to results of the standard simulations. With R partially recessive and Y recessive, the time for the R-allele frequency to exceed 50% increased by up to 2 yr with the density-independent function and decreased by up to 2 and 3 yr with the density-dependent functions that allowed 39 and 100% maximum survival, respectively. With either the density-independent function or the 39 or 100% maximum survival functions, the R-allele frequency in year 15 never changed by >0.03 compared with the standard simulations with R partially recessive and Y additive and dominant.

Table 4 shows the influence of density dependence on the evolution of the R-allele with R dominant. The density-independent mortality function that allowed 5% of beetles to survive increased the time for the R-allele frequency to reach 50% with Y recessive or dominant but decreased the number of years for resistance to evolve with Y additive. The density-dependent function that allowed 100% maximum survival slowed the development of resistance compared with the standard with Y additive or dominant but increased the rate of resistance with Y recessive. The

Table 4. Year in which the frequency of the allele for resistance to transgenic corn, R, exceeded 50% with R dominant, four toxin doses, and four density-dependent functions in areas with rotation-resistant phenotypes, transgenic corn planted to 80% of the continuous cornfield, and varying gene expression for the allele for resistance to crop rotation, Y

Density dependence function	Toxin dose			
	Theoretical high	Practical high	Medium	Low
		Y recessive		
Standard (max. survival = 0.21)	7 (0.88)	7 (0.88)	7 (0.88)	9 (0.82)
Max. survival = 0.39	6 (0.90)	5 (0.90)	6 (0.89)	9 (0.83)
Max. survival = 1.0	4 (0.91)	4 (0.91)	5 (0.89)	9 (0.83)
Survival = 0.05	8 (0.87)	8 (0.87)	8 (0.86)	11 (0.78)
		Y additive		
Standard (max. survival = 0.21)	>15 (0.45)	>15 (0.46)	>15 (0.46)	>15 (0.30)
Max. survival = 0.39	>15 (0.46)	>15 (0.47)	>15 (0.43)	>15 (0.19)
Max. survival = 1.0	>15 (0.46)	>15 (0.46)	>15 (0.36)	>15 (0.08)
Survival = 0.05	13 (0.56)	12 (0.58)	9 (0.74)	12 (0.65)
		Y dominant		
Standard (max. survival = 0.21)	>15 (0.50)	>15 (0.50)	>15(0.47)	>15 (0.30)
Max. survival = 0.39	>15 (0.39)	>15 (0.39)	>15 (0.35)	>15 (0.09)
Max. survival = 1.0	>15 (0.40)	>15 (0.41)	>15 (0.31)	>15 (0.04)
Survival = 0.05	>15 (0.06)	>15 (0.06)	>15 (0.11)	>15 (0.06)

The R-allele frequency in year 15 for each set of conditions is shown in parentheses.

density-dependent function that allowed 39% maximum survival slowed the development of resistance compared with the standard with Y dominant but increased the rate of resistance with Y recessive (Table 4).

Changing the initial R-allele frequency to 0.001 or 0.01 affected the rate of evolution of resistance to transgenic corn but generally did not affect resistance to crop rotation. With either increased initial R-allele frequency, the time for the Y-allele frequency to exceed 50% did not change by >1 yr and the Y-allele frequency after 15 yr did not change by >0.02. With R recessive and an initial R-allele frequency of 0.001 or 0.01, the R-allele frequency in year 15 did not differ by >0.001 or 0.03 compared with the standard simulations. With R partially recessive or dominant, changing the initial R-allele frequency to 0.001 or 0.01 shortened the time for the R-allele frequency to reach 50% by multiple years in nearly every case. The greatest change occurred with R dominant, a medium dose, and an initial allele frequency of 0.01, where the R-allele frequency exceeded 50% within 4 yr with Y additive or dominant. This is comparable with the standard simulations where the R-allele frequency did not exceed 50% within 15 yr under these conditions. This case also represented the greatest percentage change in allele frequency after 15 yr compared with the standard simulations, 11%.

Changing the initial Y-allele frequency to 0.001 or 0.01 never changed the time for the R-allele frequency to reach 50% by >1 yr. Increasing the initial Y-allele frequency to 0.001 did not affect the evolution of resistance to crop rotation with Y recessive but decreased the time for the Y-allele frequency to reach 50% compared with the standard simulations by 1–2 yr in every case with Y additive or dominant. With an initial Y-allele frequency of 0.01, the Y-allele frequency reached 0.02 within 15 yr with Y recessive in any case, compared with the standard simulations where the Y-allele frequency never exceeded 0.0001 within 15 yr with Y recessive. With an initial Y-allele frequency of 0.01 and Y additive or dominant, the time for the Y-allele frequency to reach 50% decreased by 3–4 yr compared with the standard simulations in every case.

With an initial Y-allele frequency of 0.1, there were several cases when the evolution of resistance to transgenic corn occurred less rapidly compared with the standard simulations. With R partially recessive and Y recessive, the R-allele frequency reached 0.26 and 0.02 with a medium and low dose, respectively. This is comparable with the standard simulations where the R-allele frequency exceeded 50% within 10 and 13 yr, respectively, under these conditions, reaching 0.97 and 0.78 after 15 yr, respectively. With R dominant and Y recessive, the Y-allele frequency reached 0.32–0.54 after 15 yr with any dose and an initial Y-allele frequency of 0.1, a 35–60% decrease in allele frequency compared with the standard simulations. With an initial Y-allele frequency of 0.1, the Y-allele frequency exceeded 50% within 3–4 yr in every case.

Areas without Rotation Resistance. In areas without rotation resistance and 20% refuge in the continuous corn, the results of the sensitivity analysis were unaffected by the amount of land planted to continuous corn (20, 60, and 100%). Raising the initial population size to 500,000 or 1 million adults per hectare decreased the time for the R-allele frequency to exceed 50% by 1–2 yr compared with the standard simulations. Imposing a fecundity cost of 25 or 50% for susceptible beetles emerging in transgenic corn did not change the time for the R-allele frequency to exceed 50% by >1 yr. In addition, raising the initial population size and imposing a fecundity cost never changed the R-allele frequency by >10 and 3%, respectively, compared with the standard simulations.

Raising the initial R-allele frequency to 0.001 shortened the time for the R-allele frequency to reach 50%

Table 5. Year in which the frequency of the allele for resistance to transgenic corn, R, exceeded 50% with R partially recessive or dominant, four toxin doses, and four density-dependent functions in areas without rotation-resistant phenotypes and 60% continuous corn, transgenic corn planted to 80% of the continuous cornfield, and varying gene expression for the allele for resistance to crop rotation, Y

Density dependence function	Toxin dose			
	Theoretical high	Practical high	Medium	Low
		R partially recessive		
Standard (max. survival = 0.21)	>15 (0.0001)	>15 (0.0002)	7 (0.99)	10 (0.94)
Max. survival = 0.39	>15 (0.0001)	>15 (0.0007)	7 (0.99)	11 (0.88)
Max. survival = 1.0	>15 (0.0001)	>15 (0.05)	6 (0.99)	12 (0.86)
Survival = 0.05	>15 (0.0001)	>15 (0.0002)	12 (0.89)	>15 (0.44)
		R dominant		
Standard (max. survival = 0.21)	5 (0.90)	5 (0.89)	5 (0.89)	8 (0.84)
Max. survival = 0.39	5 (0.90)	5 (0.90)	5 (0.89)	8 (0.83)
Max survival = 1.0	4 (0.91)	4 (0.91)	5 (0.89)	9 (0.83)
Survival = 0.05	8 (0.87)	8 (0.87)	8 (0.86)	11 (0.78)

The R-allele frequency in year 15 for each set of conditions is shown in parentheses.

by 1–2 yr. In areas with 60% continuous corn, an initial R-allele frequency of 0.01 and R recessive, the R-allele frequency reached 50% within 14 yr with a medium or greater dose. This result is comparable with the standard simulations where the R-allele frequency never exceeded the initial value of 0.0001 within 15 yr with any dose and R recessive. Only with a low dose did the R-allele frequency not exceed 50% within 15 yr with an initial R-allele frequency of 0.01, reaching a value of 0.02 after 15 yr. With R partially recessive and an initial R-allele frequency of 0.01, the R-allele frequency exceeded 50% within 5 and 6 yr with a medium and low dose, respectively. This result is comparable with 9 and 11 yr, respectively, under standard conditions. With R dominant, any dose, and an initial R-allele frequency of 0.01, the time for the R-allele frequency to reach 50% was shortened by 2–3 yr compared with the standard simulations. The changes in time for the R-allele frequency to exceed 50% were similar in areas with 20 or 100% continuous corn.

Table 5 shows how changes in the density-dependent survival functions affected the evolution of resistance to transgenic corn with R partially recessive or dominant in areas with 60% continuous corn. Changes in density-dependent survival did not affect the evolution of resistance to transgenic corn with R recessive. The percentage of change in allele frequency after 15 yr and the change in time for the R-allele frequency to reach 50% were typically greater with R partially recessive compared with the R dominant case. The greatest percent changes in allele frequency after 15 yr occurred with a low dose and the least with a theoretical high dose. The greatest change in the time for the R-allele frequency to reach 50% occurred with a medium or low dose and R partially recessive. The results presented in this section were comparable with simulations of areas with 20 or 100% continuous corn.

Discussion

In simulations of a 2-yr rotation of nontransgenic corn and soybean, resistance to crop rotation evolved rapidly with Y additive or dominant (Fig. 3). These results are similar to those of Onstad et al. (2001b, 2003). Only with Y recessive could rotation resistance be delayed longer than 15 yr, even with 99% of the landscape in rotation between corn and soybean. This differed from the results of Onstad et al. (2003), who showed that resistance to crop rotation could develop with Y recessive within 15 yr. Allowing some males to move outside the natal field before mating results in greater gene flow between continuous and rotated cornfields, as the number of matings between susceptible males and resistant females increases. This slows the evolution of resistance to crop rotation. Western corn rootworm invaded east central Illinois between 1968 and 1970 (Metcalf 1983), ≈16 yr before the first observation of resistance to crop rotation in Ford County, IL, during the 1980s (Onstad et al. 1999). Therefore, results of the current model suggest the allele for resistance to crop rotation is not recessive but either additive or dominant. Although this result is directly linked with our assumptions about the nature of the genetic system conferring resistance to crop rotation, this finding may help reduce uncertainty in future studies of the rotation-resistant variant.

In simulations of a 2-yr rotation of nontransgenic corn and soybean, density-dependent survival had the greatest impact on the evolution of resistance. Functions that allowed 39 or 100% survival decreased the number of years for the Y-allele frequency to reach 50%. If the survival of beetles is density-independent, results indicate that a 2-yr rotation of nontransgenic corn and soybean may be sufficient to prevent rotation resistance if Y is recessive or additive.

The results of the model indicate that planting transgenic corn to only rotated cornfields may potentially be the most effective management strategy to prevent resistance to both crop rotation and transgenic corn in areas with rotation-resistant phenotypes. With 5–20% refuge planted to only the rotated cornfield and no transgenic corn in the continuous cornfield, resistance to both crop rotation and transgenic corn never evolved within 15 yr. Planting transgenic corn to rotated cornfields reduces the population density of "variant" beetles in rotated cornfields and effectively

prevents rotation resistance from evolving. In turn, the small population densities in the rotated field prevent resistance to transgenic corn from evolving. These results are comparable with Onstad et al. (2003), who showed that planting a transgenic cultivar that killed 90% of all insects in a rotated cornfield prevented the evolution of resistance to crop rotation.

In areas with rotation-resistant phenotypes, planting transgenic corn to continuous cornfields was not an effective strategy to prevent resistance to either transgenic corn or crop rotation. Planting transgenic corn to only continuous cornfields resulted in faster evolution of the Y-allele compared with simulations of a 2-yr rotation of nontransgenic corn and soybean. In simulations of areas with rotation-resistant phenotypes and transgenic corn planted to only the continuous cornfield, gene expression, toxin dose, and refuge size affected the evolution of resistance to both traits. With Y recessive, resistance to crop rotation never developed within 15 yr but with Y additive or dominant resistance to crop rotation evolved within 5–10 yr. In every case, resistance to crop rotation occurred more rapidly as toxin dose increased and refuge size in the continuous cornfield decreased. Planting transgenic corn to only continuous cornfields prevented the evolution of resistance to transgenic corn with R recessive but was not always an effective strategy with R partially recessive or dominant. These results indicate that planting transgenic corn to continuous cornfields is an inferior strategy compared with planting transgenic corn in rotated cornfields in areas with rotation-resistant phenotypes.

In areas without rotation-resistant phenotypes, gene expression of the R-allele was the most important factor affecting the evolution of resistance. With any proportion of the landscape planted to continuous corn, resistance to transgenic corn never developed within 15 yr with R recessive. With R dominant, resistance always developed within 4–9 yr. With R partially recessive, the refuge size and toxin dose were the most important factors affecting the evolution of resistance. All of these results match those of Onstad et al. (2001a). In all cases, smaller refuge sizes increased the rate of evolution of resistance to transgenic corn. A similar result was obtained by both Onstad et al. (2001a) and Storer (2003). Similar to the daily time-step model of Crowder et al. (2005) results were generally robust when comparing the results of simulations with 20, 60, and 100% continuous corn. Therefore, farmers in areas without rotation-resistant phenotypes can develop management strategies for preventing resistance to transgenic corn without placing too much concern on the rotational level of their particular landscape.

Similar to results from the daily model in our companion article (Crowder et al. 2005), greater doses of toxin were generally not as effective at preventing rotation resistance compared with a medium or low dose. With regard to transgenic corn, a medium dose may be the most risky. In general, resistance to transgenic corn evolved more rapidly with a medium dose than with a theoretical high, practical high, and low dose. A similar result was derived by Onstad et al. (2001a) and Storer (2003). Onstad et al. (2001a) indicated that an insecticidal protein dose that allowed 5 or 10% survival of susceptible beetles resulted in the maximum rate of adaptation to transgenic corn, whereas Storer (2003) showed that a dose that allowed 10% survival of susceptible beetles resulted in the maximum rate of adaptation. These results are similar to our medium dose that allowed 5% survival of susceptible beetles. With greater doses, mortality of heterozygous insects keeps the resistance allele from increasing at a rapid rate, whereas at lower doses, the significant survival of susceptible beetles acts to slightly slow the rate of evolution of resistance to transgenic corn (Storer 2003).

Similar to the daily model (Crowder et al. 2005), results of the sensitivity analysis indicate that initial allele frequency and density dependence are the two most important factors affecting the evolution of resistance to both traits. Results of this model support the observations of Onstad et al. (2003) that it is difficult, if not impossible, to halt the evolution of rotation resistance once the resistance allele frequency reaches ≈1%. Thus, due to the uncertainty in the timing of invasion by the rotation-resistant variant and initial allele frequency, corn producers may need to develop preventative management strategies by using transgenic corn to reduce their risk in the future.

Results were similar when comparing our standard density-dependent function and the density-independent function that allowed 5% survival of immatures, except in simulations of a 2-yr rotation of nontransgenic corn and soybean, where a density-independent survival function slowed the evolution of resistance to crop rotation. With these functions, population densities of both susceptible and resistant insects tended to be smaller than with the functions that allowed greater maximum survival and fluctuated by as much as 90% from year to year. Using a density-dependent function that allowed 39 or 100% maximum survival resulted in faster evolution of both traits. In general, these functions resulted in larger and more stable populations in all fields compared with the other functions. Both of these factors could have influenced the evolution of resistance.

The application of these results is limited by several assumptions in the model. First, we assumed that a very simple genetic system is responsible for evolution of resistance to both crop rotation and transgenic corn. Second, we assumed that all farms are the same in a homogeneous region or that areawide pest management is occurring. Third, we only considered the use of transgenic corn in a block configuration. Onstad et al. (2001a) and Storer (2003) showed that different refuge deployment strategies in continuous cornfields could significantly change the rate of adaptation to transgenic corn. Changes in these assumptions could produce other outcomes.

To determine how robust our conclusions are, we compared the major results of the generational model to those of the daily model (Crowder et al. 2005). The results presented represent standard simulations of

Table 6. Year in which the frequency of the allele for resistance to transgenic corn, R, exceeded 50% with two simulation models, the generational model and the daily model from a companion article (Crowder et al. 2005), with varying landscapes and gene expression

Landscape	Generational model		Daily model	
	Recessive R	Dominant R	Recessive R	Dominant R
Only continuous corn, no rotation-resistance	>15	4–8	>15	4–14
Bt in continuous corn, 40–80% rotated fields, no rotation resistance	>15	4–9	>15	4–14
Bt in rotated corn, 85% rotated fields, rotation resistance	>15	>15	>15	>15

The range of numbers presented represents simulations with all combinations of Y-allele expression, toxin dose, and refuge size.

both models; we did not attempt to adjust either model to make them more similar. In general, resistance to both crop rotation and transgenic corn evolved slightly faster with the generational model compared with the daily model. With both models, results indicated that planting transgenic corn to only rotated cornfields was a robust strategy to prevent resistance to crop rotation. With any Y-allele expression, the Y-allele frequency did not exceed 50% within 15 yr in simulations with the daily model and transgenic corn planted to only rotated fields. Results were similar with the generational model, except in the case with 30% refuge in the rotated cornfield and Y dominant, where the Y-allele frequency exceeded 50% in 15 yr with a low dose and reached 0.46–0.48 with a medium or greater dose.

Table 6 compares the results of adaptation to transgenic corn generated with the generational model and the daily model (Crowder et al. 2005). In general, the results between the two models with regard to resistance to transgenic corn were robust. The only difference between the two models was that resistance to transgenic corn evolved slightly slower with the daily time-step model compared with the generational model (Table 6). The differences between simulations of areas without rotation-resistant phenotypes with either model increased as the size of the refuge increased in the continuous cornfield.

One major difference between the results of the sensitivity analyses performed on the two models was in simulations of areas with rotation resistance and transgenic corn planted to only rotated cornfields. With the daily model (Crowder et al. 2005), planting transgenic corn to rotated cornfields was an effective strategy to reverse the evolution of resistance to crop rotation even at high initial allele frequencies ($Y > 0.9$). However, results of the generational model indicate that transgenic corn may not be effective at preventing rotation resistance with initial allele frequencies >0.01. Because the results of both models were similar in most cases (Table 6), it may be assumed that the complexity involving dispersal, mating, emergence, and oviposition needed to develop the daily model did not affect the results as significantly as other assumptions about gene expression, refuge size, and toxin dose. Comparing the results of the two models indicates that future modeling work may only need to be performed on one of the two models because results between the two should be robust.

Further modeling efforts will focus on how farmers can develop dynamic management strategies for using transgenic corn in areas with or without rotation-resistant phenotypes. Sampling soybean fields each summer may help farmers identify whether they face a problem from rotation-resistant beetles in the future and need to shift from planting transgenic corn in continuous cornfields to rotated cornfields. Because, in general, the results of both models were robust, farmers can potentially develop management strategies by using the results of either model. Despite the many uncertainties inherent to the system, both models indicate that transgenic corn may be the future of corn rootworm pest management.

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